



# Growth, ionic response, and gene expression of shoots and roots of perennial ryegrass under salinity stress

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## Abstract

Growth, ionic responses, and expression of candidate genes to salinity stress were examined in two perennial ryegrass accessions differing in salinity tolerance. The salinity tolerant (PI265349) and sensitive accessions (PI231595) were subjected to 75-mM NaCl for 14 days in a growth chamber. Across two accessions, salinity stress increased shoot dry weight and concentrations of malondialdehyde (MDA) and Na<sup>+</sup> in the shoots and roots, but decreased shoot Ca<sup>2+</sup> and root K<sup>+</sup> concentrations. Salinity stress also increased root expressions of *SOS1*, *PIP1*, and *TIP1*. Plant height and chlorophyll content were unaffected by salinity stress in the tolerant accession but significantly decreased in the sensitive accession. Shoot MDA content did not change in the tolerant accession but increased in the sensitive accession. A more dramatic increase in Na<sup>+</sup> was found in the roots of the sensitive accession. Relative to the control, salinity stress reduced expression of *SOS1*, *NHX1*, *PIP1*, and *TIP1* in the shoots but increased expression of these genes in the roots of the tolerant accession. Expression levels of *SOS1* increased in the roots and expression of *NHX1* increased in the shoots but decreased in the roots of the sensitive accession under salinity stress. A decline in *PIP1* expression in the shoots and dramatic increases in *TIP* expression in both shoots and roots were found in the sensitive accession under salinity stress. The results suggested maintenance of plant growth and leaf chlorophyll content, lesser Na<sup>+</sup> accumulation in the roots, and lower lipid peroxidation in the shoots which could be associated with salinity tolerance. The decreased expressions of *SOS1*, *NHX1*, and *TIP1* in the shoots, and increased expressions of *NHX1* and *PIP1* in the roots might also be related to salinity tolerance in perennial ryegrass.

**Keywords** *Lolium perenne* · Ion transport · Mechanism · Salt tolerance

## Introduction

Plants response to salinity stress involve numerous growth, and physiological and biochemical alterations (Munns and Tester 2008). At the whole-plant level, plant growth is sensitive to salinity stress. Salinity reduced length of shoots and roots, leaf dry weight, leaf and root water content, and plant

transpiration (Vysotskaya et al. 2010; Ruiz-Carrasco et al. 2011; Tang et al. 2013a). However, salinity effects on plant growth varied largely among or within a perennial grass species (Dai et al. 2008; Wang et al. 2011; Uddin et al. 2012; Tang et al. 2013a). Variations of plant growth under salinity stress depend on plant species, genotypes, and intensity and duration of a stress.

Salinity stress increases Na<sup>+</sup> level in the plants. The decrease in K<sup>+</sup> concentration under salinity stress often occurs along with Na<sup>+</sup> accumulation, which is considered an imbalance of ion homeostasis. The tolerant genotypes of grass species showed low level of Na<sup>+</sup>, high level of K<sup>+</sup>, and high K<sup>+</sup>/Na<sup>+</sup> ratio (Krishnan and Brown 2009; Hu et al. 2012b), but the results were not consistent. Salinity stress also interferes with uptake and accumulation of other nutrients such as Ca<sup>2+</sup> and Mg<sup>2+</sup>, and reductions in Ca<sup>2+</sup> and Mg<sup>2+</sup> concentration were greater in the salinity-sensitive cultivar of pea (*Pisum sativum* L.) (Ahmad et al. 2008). The addition of Ca<sup>2+</sup> considerably increased the uptake and

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transport capacity for  $K^+$  over  $Na^+$ , suggesting that  $Ca^{2+}$  could regulate  $K^+/Na^+$  homeostasis (Wu and Wang 2012). However, the effects of  $Ca^{2+}$  on the plants also varied with genotypes and  $NaCl$  concentrations (Cabot et al. 2009). Collectively, plant tolerance to salinity stress is associated with ionic stress tolerance (Munns and Tester 2008). Molecular mechanisms of ion transport may be essential for maintenance of ion homeostasis that contributes to salinity tolerance.

At the molecular level, candidate genes for ion accumulation and water movement are of interest in illustrating mechanisms of salinity tolerance. The salt overly sensitive (*SOS1*) gene, encoding a  $Na^+/H^+$  antiporter in plasma membrane, can play an important role in excluding  $Na^+$  and controlling long-distance transport of  $Na^+$  from the roots to shoots in *Arabidopsis thaliana* (Shi et al. 2002). Salinity stress increased the expression level of *SOS1*, which might be associated with salinity tolerance (Gao et al. 2016). Expressions of *CqSOS1* were differentially regulated in the shoots and roots as well as between the tolerant and sensitive genotypes of quinoa (*Chenopodium quinoa* Wild.) (Ruiz-Carrasco et al. 2011). Moreover, overexpression of *SOS1* enhanced salinity tolerance of the transgenic plants (Gao et al. 2016). *NHX1*, a vacuolar  $Na^+/H^+$  antiporter, controls  $Na^+$  movement across the tonoplast membrane into the vacuole (Munns and Tester 2008). Expression patterns of *ZjNHX1* varied with tissue types in zoysia grass (*Zoysia japonica* Steud.) under salinity stress (Du et al. 2010). Expression of *CqNHX1* also differed in shoots, roots, and genotypes (Ruiz-Carrasco et al. 2011). Overexpression of *NHX1* improved salinity tolerance in some plant species (Banjara et al. 2012; Sahoo et al. 2016). However, overexpression of *AtNHX1* alone did not improve salinity tolerance in transgenic *Arabidopsis* (Yang et al. 2009). The results indicate that gene coordination for controlling ion homeostasis can be vital for plant salinity tolerance.

Salinity causes osmotic stress and interferes in plant–water relations. Aquaporins regulate water movement across the plant plasma membrane (e.g., *PIP*) and vacuolar membrane (e.g., *TIP*) (Maurel et al. 2008). The salinity tolerant wild barley (*Hordeum spontaneum* L.) had a fast and persistent down-regulation of the *HtPIP2;4* gene, while the sensitive line showed a delayed down-regulation of the gene, consistent with inhibited transpiration and hydraulic conductivity caused by salinity (Vysotskaya et al. 2010). In cucumber (*Cucumis sativus* L.), the decreased hydraulic conductivity under salinity stress was related to a down-regulation of *CsPIP1;2* and *CsPIP2;4*, while the alterations of *CsPIP* expression were consistent with partial recovery of hydraulic conductivity of the roots after salinity treatment (Qian et al. 2015). The transcript levels of *FrPIP2;1* were suppressed in red fescue (*Festuca rubra* L.) subjected to 500 mM  $NaCl$  stress, but *PIP2;1* expression level was correlated with salinity tolerance (Diédhiou et al. 2009). In

addition, overexpression of aquaporin genes such as *PIP* and *TIP* increased salinity tolerance of plants (Hu et al. 2012b; Zhou et al. 2014; Wang et al. 2015). It appears that aquaporins play a role in salinity tolerance by mediating water transport of seedlings, although the regulatory mechanisms could vary with species, stress durations, and specific organs.

Perennial ryegrass (*Lolium perenne* L.) is a commonly used perennial grass for turf, forage, and soil stabilization. This species exhibited large natural variation of salinity responses (Tang et al. 2013b). However, whole-plant and molecular mechanisms of salinity tolerance are not well understood in this species, especially under low salinity stress. Thus, the experiment was designed to study growth, ionic responses, and expression of candidate genes to salinity stress in two perennial ryegrass accessions differing in salinity tolerance. The results would contribute to a better understanding of whole-plant physiological and molecular mechanisms of salinity tolerance in perennial grass species under suboptimal growing conditions.

## Materials and methods

### Plant materials

The salinity tolerant (PI265349) and sensitive accessions (PI231595) of perennial ryegrass were seeded in plastic pots (4-cm diameter, 9-cm deep) containing sand in a greenhouse at Purdue University, USA, on 7 April 2014. Plants were cut twice a week to a height of 5–6 cm and irrigated three times a week or whenever needed using a half-strength Hoagland solution ( $pH \approx 6.5$ , electrical conductivity  $\approx 1.2 \text{ dS m}^{-1}$ ). All the pots were well drained after irrigation. Grasses were grown at temperatures of 21/16 °C (day/night) with a photosynthetically active radiation (PAR) of  $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . On 23 June 2014, pots were placed in a growth chamber with temperatures of 20/15 °C (day/night) and PAR of  $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . The grasses were irrigated daily with a half-strength Hoagland solution and mowed twice a week for 7 days in the growth chamber before initiation of salinity stress.

### Salinity treatment

Plants were irrigated daily with a half-strength Hoagland solution (non-stress control) or with 75-mM  $NaCl$  in a half-strength Hoagland solution ( $EC \approx 8.5 \text{ dS m}^{-1}$ ). Salt concentration was increased gradually by 25 mM every day. Plants were harvested after 14 days of 75-mM  $NaCl$  when two genotypes of perennial ryegrass visually showed differences in growth and color under salinity stress. Plants were not cut during salinity treatment.

## Measurements

Plant height (HT) was determined from the soil surface to the top of leaf blade. Shoot tissues were weighed and dried at 80 °C for 3 days, and then, the dry weight was measured. For chlorophyll (Chl) extraction, approximately 50 mg of randomly selected fresh leaves were soaked in 15-mL dimethyl sulfoxide in dark for 48 h. Subsequently, the absorbance of Chl extract was read at 665, 649, and 480 nm, and Chl was calculated according to the method of Wellburn (1994). Roots were harvested, and the fresh and dried tissues were saved for analyses.

Dry shoot and root samples were ground into a fine powder with liquid nitrogen. For extraction of ions, we followed the method described by Tang et al. (2013a). Briefly, a 50-mg fine powder was mixed with 3 mL of 18 M H<sub>2</sub>SO<sub>4</sub> in a digestion tube and then heated at 200 °C for 30 min. After the tubes cooled down to the room temperature, a 3-mL 30% H<sub>2</sub>O<sub>2</sub> was added and tubes were heated again for 30 min until the mixture became transparent and clear. Distilled water was added to the extraction to bring the volume to 50 mL. A 2-mL aliquot of extraction was diluted to 15 mL with distilled water. The Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> concentrations were determined using a plasma atomic emission spectrometer (ICP 9820; Shimadzu, Columbia, MD, USA).

The malondialdehyde (MDA) is indicator of lipid peroxidation (Dhindsa et al. 1981). We measured MDA concentration according to the method described by Liu and Jiang (2015). Approximately 50 mg frozen leaf or root tissues were ground into fine powder with liquid nitrogen. A 1 mL of extraction buffer (pH ≈ 7.8) containing 50-mM potassium phosphate, 1% polyvinylpyrrolidone, and 1 mM ethylenediaminetetraacetic acid was added to the powder. After centrifuging at 15,000×g for 30 min at 4 °C, a 0.5 mL of the supernatant was mixed with a 2 mL of trichloroacetic acid (20%) containing thiobarbituric acid (5%). The tube was heated at 95 °C for 30 min, immediately cooled in ice, and centrifuged at 10,000×g for 10 min. The MDA concentration was calculated using the absorbance at 532 and 600 nm, and an extinction coefficient of 155 mm<sup>-1</sup>cm<sup>-1</sup> (Health and Packer 1968).

Gene expression was examined using the real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) procedure. Briefly, total RNA was extracted with a Direct-zol<sup>TM</sup> RNA MiniPrep Kit (Zymo Research Corporation, Irvine, CA, USA). About 0.5–1 μg of extracted RNA was mixed with an iScript<sup>TM</sup> cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) to make cDNA under 25 °C for 5 min, 42 °C for 30 min, and 85 °C for 5 min. Quantitative RT-PCR was performed using a CFX96 Touch<sup>TM</sup> Real-Time PCR System with an iTaq<sup>TM</sup> Universal SYBR® Green Kit (Bio-Rad, Hercules, CA, USA), with a reaction at 95 °C for 3 min followed by 40

amplification cycles of 10 s at 95 °C and 1 min at 60 °C. All primers were designed using Primer Primer 5 (Lalitha 2004) from perennial ryegrass sequences as follows: for *SOS1* (AY987046.1), forward 5'-ATTATTACAGAA TCGGTGGT-3' and reverse 5'-AATCCTCGCAATGAC TAG-3'; for *NHX1* (AY987047.1), forward 5'-CCCAAT GAAATCAGTCGC-3', and reverse 5'-CCAAATAATAAC TTGCTGCCTA-3'; for *PIP1* (genomic sequence), forward 5'-TGTGCTCCCTGTTGTAGATGA-3' and reverse 5'-CCACTGCCTATTGGGTTTCG-3'; for *TIP1* (genomic sequence), forward 5'-TGAGGCAGTTTATTAGTTTC-3' and reverse 5'-CAGCAGATGATCGGTTTG-3'. Perennial ryegrass elongation factor 1-alpha (EU168438), forward 5'-GGCTGATTGTGCTGTGCTTA-3', and reverse 5'-CTC ACTCCAAGGGTCAAAGC-3' were used as a housekeeping gene for internal control. The relative gene expression level between the control and salinity stress treatment was calculated using the method of 2<sup>-ΔΔCT</sup> (Livak and Schmittgen 2001), with three biological and three technical replicates for each treatment.

## Experimental design and data analysis

The experimental was a split plot design, with main plot of salinity treatment (control and 75 mM NaCl) and subplot of accessions. Three pots representing three replicates were randomly assigned into each treatment. Analysis of variance was conducted to indicate effects of accessions, treatments, and accessions × treatments using SAS (version 9.1; SAS Institute, Cary, NC, USA). Least square means were tested in the mixed model at significance level of 0.05.

## Results and discussion

### Analysis of variance

Across the two cultivars, significant treatment effects were found for shoot dry weight (DW), concentrations of shoot and root malondialdehyde (MDA) and Na<sup>+</sup>, root K<sup>+</sup> concentration, shoot Ca<sup>2+</sup> concentration, expression of root *SOS1*, and expressions of *PIP1* and *TIP1* in the shoots and roots (Table 1).

The cultivar effects were observed for shoot chlorophyll content (Chl), plant height (HT), shoot and root DW, shoot MDA, shoot K<sup>+</sup>, root Na<sup>+</sup>, shoot *SOS1*, and expressions of *NHX1*, *PIP1*, and *TIP1* in the shoots and roots (Table 1). Significant treatment by cultivar interactions was shown for Chl, HT, shoot MDA, root Na<sup>+</sup>, shoot *SOS1*, and *NHX1*, *PIP1*, and *TIP1* in the shoots and roots (Table 1).

### Treatment effects on traits across two cultivars

Shoot DW, MDA, and Na<sup>+</sup> concentrations increased 33.1, 19.6%, and sevenfold, and shoot Ca<sup>2+</sup> was reduced 21.7% under 75-mM NaCl compared with the control, respectively (Table 2). In addition, for shoots, salinity stress significantly increased transcript level of *PIP1* but decreased expression of *TIP1* (Table 2). Root MDA concentration increased 8.9% and root Na<sup>+</sup> concentration dramatically increased 11.8-fold, but root K<sup>+</sup> decreased 41.9% under salinity stress, compared to the control (Table 2).

Expressions of root *SOS1*, *PIP1*, and *TIP1* increased under salinity stress, especially for *TIP1* (Table 2).

### Cultivar differences in traits across treatments

Across the control and salinity treatments, the tolerant accession of PI265349 had lower Chl and shoot MDA but higher shoot DW and K<sup>+</sup> than that of the sensitive accession of PI231595 (Table 3). The tolerant accession also had lower expression levels of *SOS1*, *NHX1*, and *TIP1* and higher level of *PIP1* than the sensitive accession (Table 3). In the roots, higher DW and lower Na<sup>+</sup> were observed in the tolerant

**Table 1** Analysis of variance of leaf chlorophyll content (Chl), plant height (HT), shoot and root dry weight (DW), shoot and root concentrations of malondialdehyde (MDA), K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>, and

relative expression levels of *SOS1*, *NHX1*, *PIP1*, and *TIP1* in perennial ryegrass accessions of PI265349 and PI231595 under the non-salinity control and 75-mM NaCl treatment

	Chl	HT	DW	MDA	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	<i>SOS1</i>	<i>NHX1</i>	<i>PIP1</i>	<i>TIP1</i>
Shoot												
Treatment (T)	ns	ns	***	**	ns	***	*	ns	ns	ns	*	***
Accession (A)	**	*	***	***	**	ns	ns	ns	**	***	*	***
T×A	***	***	ns	**	ns	ns	ns	ns	**	***	*	***
Root												
Treatment (T)	–	–	ns	**	***	***	ns	ns	**	ns	**	**
Accession (A)	–	–	**	ns	ns	***	ns	ns	ns	***	**	***
T×A	–	–	ns	ns	ns	***	ns	ns	ns	***	**	***

ns Not significant

\**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001, respectively

**Table 2** Treatment differences in shoot dry weight (DW), shoot and root concentrations of malondialdehyde (MDA), K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and relative expression levels of *SOS1*, *PIP1*, and *TIP1* across perennial ryegrass accessions of PI265349 and PI231595

Tissue	Treatment	DW (g)	MDA (nmol g <sup>-1</sup> FW)	K <sup>+</sup> (mg g <sup>-1</sup> DW)	Na <sup>+</sup> (mg g <sup>-1</sup> DW)	Ca <sup>2+</sup> (mg g <sup>-1</sup> DW)	<i>SOS1</i>	<i>PIP1</i>	<i>TIP1</i>
Shoot	Control	1.21b	13.8b	43.0	3.08b	14.3a	1.08	0.98a	1.23b
	75 mM NaCl	1.61a	16.5a	35.7	21.6a	11.2b	0.82	0.37b	19.8a
Root	Control	0.48	18.0b	26.5a	1.75b	11.2	1.01b	1.01b	0.92b
	75 mM NaCl	0.47	19.6a	15.4b	20.6a	8.74	1.45a	2.56a	28.3a

Mean values without letter followed for a given tissue comparison between treatment were not significantly different at *P* < 0.05

**Table 3** Differences in leaf chlorophyll content (Chl), plant height (HT), shoot dry weight (DW), shoot and root concentrations of malondialdehyde (MDA), K<sup>+</sup>, Na<sup>+</sup>, and relative expression lev-

els of *SOS1*, *NHX1*, *PIP1*, and *TIP1* in the salinity tolerant perennial ryegrass accession of PI265349 and the sensitive accession of PI231595

Tissue	Accession	Chl (mg g <sup>-1</sup> FW)	DW (g)	MDA (nmol g <sup>-1</sup> FW)	K <sup>+</sup> (mg g <sup>-1</sup> DW)	Na <sup>+</sup> (mg g <sup>-1</sup> DW)	<i>SOS1</i>	<i>NHX1</i>	<i>PIP1</i>	<i>TIP1</i>
Shoot	PI265349	1.46b	1.96a	12.4b	43.3a	12.3	0.84b	0.84b	0.71a	1.04b
	PI231595	1.76a	0.86b	17.9a	35.4b	12.34	1.06a	1.16a	0.54b	20.1a
Root	PI265349		0.52a	19.3	22.4	7.9b	1.28	1.35a	2.54a	2.53b
	PI231595		0.43b	18.3	19.5	14.5a	1.17	0.73b	1.03b	26.7a

Mean values without letter followed for a given tissue comparison between accession were not significantly different at *P* < 0.05

accession than in the sensitive accession (Table 3). In addition, higher expression levels of *NHX1* and *PIP1* and lower level of *TIP1* were found in the tolerant accession than in the sensitive accession (Table 3).

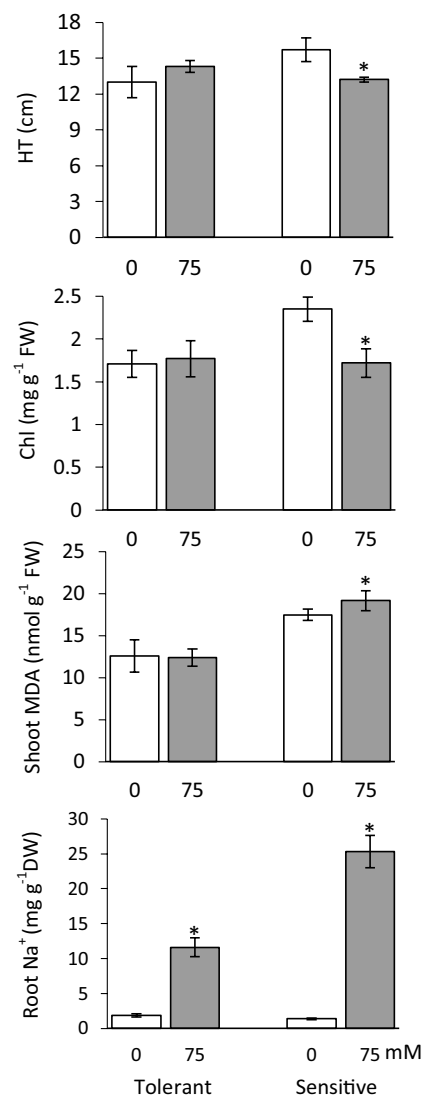
### Growth and physiological responses of individual cultivars to treatments

Plant height (HT) and Chl content were unaffected by 75-mM NaCl in the tolerant accession of PI265349, but significantly decreased 15.9 and 23.6% for the sensitive accession of PI231959, respectively, compared to the non-salinity control (Fig. 1). Salinity stress did not change MDA content in the shoots of the tolerant accession but increased 36.8% in the shoots of the sensitive accession (Fig. 1).

A reduction in HT in the sensitive accession suggested that plant growth of the sensitive accession of perennial ryegrass was inhibited by a relatively low NaCl concentration. However, the reduced HT was not consistent increase in SDW in the sensitive accession, suggesting that alterations of dry weight might not always be associated with salinity tolerance. A reduced or increased dry weight was found in plant species exposed to salinity stress including perennial grasses (Hu et al. 2012b; Uddin et al. 2012; Tang et al. 2013a; Yin et al. 2017). Under high salinity stress (~300-mM NaCl), HT was positively correlated with leaf dry weight among 56 perennial ryegrass accessions (Tang et al. 2013b). Genotypes, stress intensity, and duration may all cause variations of plant growth in different studies in perennial grasses.

Leaf Chl decreased or remained unchanged in perennial ryegrass under salinity stress, depending on salt concentration and plant materials (Hu et al. 2011; Tang et al. 2013a; Yin et al. 2017). Leaf Chl were unaffected in perennial ryegrass cultivar of Quick start II under 85- and 170-mM NaCl, but decreased under 255-mM NaCl (Hu et al. 2011). The decrease in Chl content in PI231955 indicated its sensitivity to salinity stress, which was consistent with its reduced HT and increased MDA in the shoots. MDA level is an indicator of lipid peroxidation. Increased leaf and root MDA content were observed in perennial grass and other plant species under salinity stress (Hu et al. 2011, 2012a; Chen and Ye 2014; Yin et al. 2017). The salinity tolerant perennial ryegrass cultivar accumulated lesser amount of MDA compared to the sensitive cultivar under 12 days of 250-mM NaCl (Hu et al. 2012a). The unchanged level of MDA in the tolerant accession and increased MDA in the sensitive accession in this study indicated that the degree of lipid peroxidation induced by salinity was more severe in the salinity-sensitive accession of perennial ryegrass.

Salinity increased root Na<sup>+</sup> concentrations for both accessions (Fig. 1). The Na<sup>+</sup> concentration of the roots increased 6.3-fold in the tolerant accession and 18.6-fold in the



**Fig. 1** Plant height (HT), leaf chlorophyll content (Chl), shoot malondialdehyde (MDA), and root Na<sup>+</sup> concentrations as affected by 14 days of 75-mM NaCl in the salinity tolerant accession of PI265349 and the sensitive accession of PI231955. Comparisons were made between the control (0-mM NaCl, open column) and 75-mM NaCl (shade column) for each accession. \**p* < 0.05

sensitive accession under salinity stress, compared with the control (Fig. 1). The low uptake of Na<sup>+</sup> and a higher K<sup>+</sup>/Na<sup>+</sup> ratio could be associated with salinity tolerance (Thalji and Shalaldehy 2007; Azadi et al. 2011; Hu et al. 2011; Tang et al. 2013a, b), but the results are not often consistent (Munns and James 2003; Yin et al. 2017). Compared with the sensitive accession, higher K<sup>+</sup> accumulation in the shoot and lower Na<sup>+</sup> accumulation in the roots were found in the tolerant accession (Table 3; Fig. 1), which may contribute to its better salinity tolerance.

Accumulation of Na<sup>+</sup> in the soil decreased Ca<sup>2+</sup> uptake and transport (Hu and Schmidhalter 1997). Meanwhile,



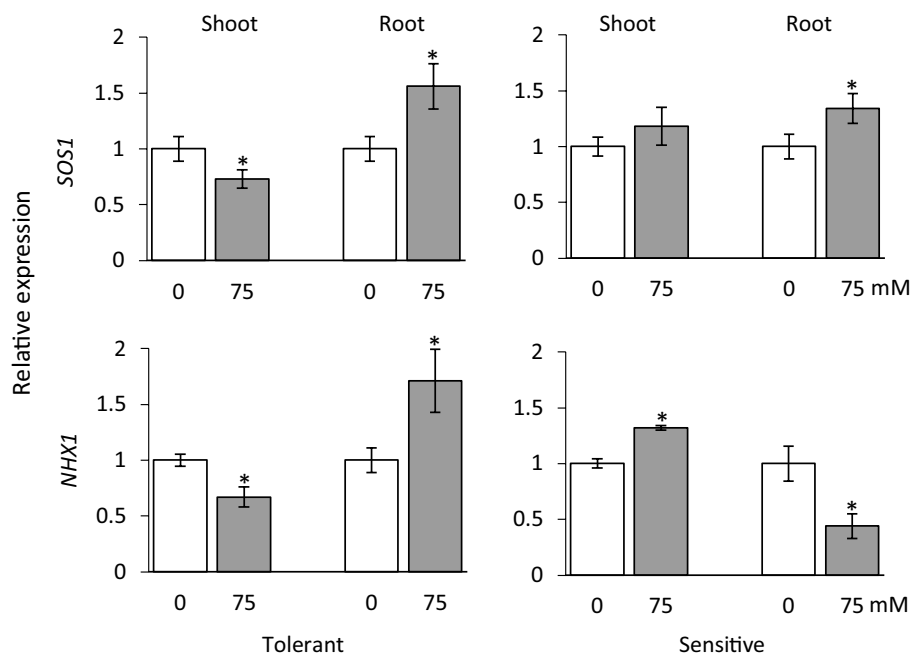
$\text{Ca}^{2+}$  highly competes with  $\text{Mg}^{2+}$  at the binding sites of root plasma membranes, which can interfere with both  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  uptake under salinity stress (Grattan and Grieve 1998). The reduced  $\text{Ca}^{2+}$  in the shoots under salinity stress could be due to a high level of  $\text{Na}^+$  accumulation (Table 2). The reductions in leaf  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations were found in two citrus stocks in response to increasing NaCl concentrations but not in other stocks (Ruiz et al. 1997). Collectively, the results suggested that the salinity effect of

$\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  varied with genotypes, but a relatively lower  $\text{Na}^+$  accumulation and higher levels of  $\text{Ca}^{2+}$  and  $\text{K}^+/\text{Na}^+$  could contribute to salinity tolerance in perennial ryegrass.

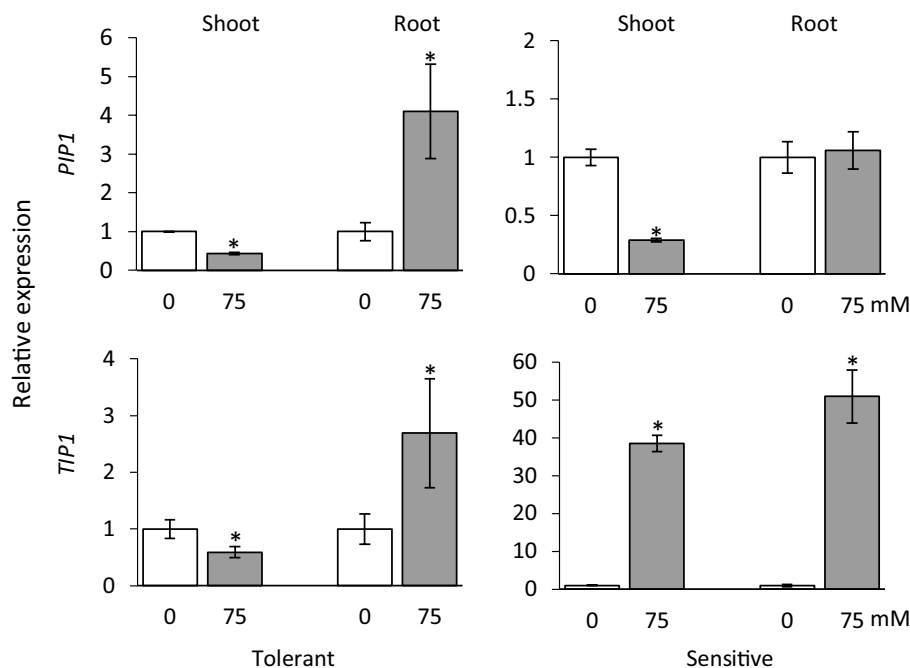
### Gene expression under salinity

The gene expression patterns were consistent in the shoots and roots in the tolerant accession of PI2653496, but not in the sensitive accession of PI231595 (Figs. 2, 3). Relative

**Fig. 2** Gene expression of *SOS1* and *NHX1* in the shoots and roots as affected by 14 days of 75-mM NaCl in the salinity tolerant accession of PI265349 and the sensitive accession of PI231595. Comparisons were made between the control (0-mM NaCl, open column) and 75-mM NaCl (shade column) for each accession. Data were normalized in comparison with their respective control. \* $p < 0.05$



**Fig. 3** Gene expression of *PIP1* and *TIP1* in the shoots and roots as affected by 14 days of 75-mM NaCl in the salinity tolerant accession of PI265349 and the sensitive accession of PI231595. Comparisons were made between the control (0-mM NaCl, open column) and 75-mM NaCl (shade column) for each accession. Data were normalized in comparison with their respective control. \* $p < 0.05$



to the non-stress control, the transcript abundance of *SOS1* and *NHX1* decreased in the shoots, but increased in the roots of the tolerant accession under 75-mM NaCl (Fig. 2). For the sensitive accession, the transcript levels of *SOS1* in the shoots were unaffected by salinity stress, but was reduced in the roots, while expression of *NHX1* increased in the shoots and decreased in the roots, relative to the control (Fig. 2). Expression patterns of *PIP1* and *TIP1* were similar to that of *SOS1* and *NHX1* in the roots of the tolerant accession exposed to 75-mM NaCl (Fig. 3). A decline in *PIP1* expression was found in the shoots but not in the roots, while dramatic increases in *TIP1* expressions were noted in both shoot and root tissues of the sensitive accession under salinity stress (Fig. 3).

The transport and compartment of Na<sup>+</sup> and K<sup>+</sup> between different tissues and subcellular locations may allow plants to adjust to a saline environment (Silva et al. 2015). Expression of *SOS1* gene encoding Na<sup>+</sup>/H<sup>+</sup> antiporter in plasma membrane and *NHX1* encoding vascular membrane Na<sup>+</sup>/H<sup>+</sup> antiporter may confer salinity tolerance (Wu et al. 2005; Gao et al. 2016). Variations in Na<sup>+</sup> accumulation in the shoots were associated with the expression level of *AtSOS1* in different *Arabidopsis* ecotypes (Jha et al. 2010). Higher expression levels of *SOS1* in the roots of both perennial ryegrass accessions were consistent with accumulation of Na<sup>+</sup> in the roots under salinity stress (Fig. 1). However, down-regulation of *SOS1* and *NHX1* in the shoots of the tolerant accession was not consistent with shoot Na<sup>+</sup> accumulation under salinity. Overall, our results in perennial ryegrass supported that expressions of *SOS1* and *NHX1* had tissue-specific patterns and were regulated differentially in the shoots and roots as well as between the tolerant and sensitive genotypes (Du et al. 2010; Ruiz-Carrasco et al. 2011). In addition, allelic variation of *NHX1* was strongly associated with leaf K<sup>+</sup>/Na<sup>+</sup> ratio in natural populations of perennial ryegrass (Tang et al. 2013b). In this study, reduced root K<sup>+</sup>/Na<sup>+</sup> along with the lesser expressed level of *NHX1* in the roots of the sensitive accession might suggest a role of this gene in salinity tolerance. However, other genes involved in K<sup>+</sup> transport could also be important for illustrating ion homeostasis under salinity stress.

Salinity stress influences plant water status. Aquaporins could affect salinity tolerance by mediating water transport across plant vacuolar and plasma membranes. Up- and down-regulations of *PIP1* and *TIP1* found in the shoots and roots of both accessions supported the differential expression patterns of *PIP* and *TIP* identified in other plant species under salinity stress (Diédhiou et al. 2009; Vysotskaya et al. 2010; Xu et al. 2013; Hove et al. 2015; Qian et al. 2015). The lowered PIP activity contributed to decreased water uptake capacity in the roots grown under salinity stress, but up-regulation of the *PIP1* gene enhanced salinity tolerance in transgenic *Nicotiana*

*tabacum* L. (Hu et al. 2012c). Our results also suggested that increased expression of *PIP1* and *TIP1* in the roots of the tolerant accession could contribute to salinity tolerance of this accession. Expression patterns of *PIP1* and *TIP1* were not consistent in the sensitive accession, suggesting differential roles of these genes in salinity response in the sensitive genotype of perennial ryegrass. In wheat (*Triticum aestivum* L.), the *TIP2;2* gene was down-regulated by salinity, and heterologous expression of *TaTIP2;2* compromised salinity and drought tolerance of *Arabidopsis thaliana* (Xu et al. 2013). The dramatic increases in *TIP1* in both shoots and roots of the sensitive accession indicated that *TIP1* might be a negative regulator of salinity tolerance in perennial ryegrass. This deserves further investigation.

In summary, maintenance of HT and Chl and absence of accumulation of MDA in the shoots were associated with salinity tolerance. Lesser increases of Na<sup>+</sup> in the roots contributed to salinity tolerance. The decreased expressions of *SOS1*, *NHX1*, and *TIP1* in the shoots and increased expressions of *NHX1* and *PIP1* in the roots might also be related to salinity tolerance in perennial ryegrass.

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**Author contribution statement** ML conducted the experiments, performed the statistical analysis of data and prepared the manuscript. XS performed ion analysis. YJ supervised the overall work and contributed to data interpretation and discussion as well as paper writing.

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