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Genotypic Variations in Plant Growth and Nutritional Elements of Perennial Ryegrass Accessions under Salinity Stress

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Abstract. **Perennial ryegrass (*Lolium perenne*) is a popular cool-season and forage grass around the world. Salinity stress may cause nutrient disorders that influence the growth and physiology of perennial ryegrass. The objective of this study was to identify the genotypic variations in growth traits and nutrient elements in relation to salinity tolerance in perennial ryegrass. Eight accessions of perennial ryegrass [PI265351 (Chile), PI418707 (Romania), PI303012 (UK), PI303033 (The Netherlands), PI545593 (Turkey), PI577264 (UK), PI610927 (Tunisia), and PI632590 (Morocco)] were subjected to 0 (control, no salinity) and 300 mM NaCl for 10 d in a greenhouse. Across accessions, salinity stress decreased plant height (HT), leaf fresh weight (LFW), leaf dry weight (LDW), leaf water concentration (LWC), and concentration of N, C, Ca^{2+}, Cu^{2+}, K^+, Mg^{2+}, and K^+/Na^+ ratio and increased Na^+ concentration. Negative correlations were found between C and Na^+, whereas positive correlations of K^+/Na^+ with C and N were found under salinity treatment. The principal component analysis (PCA) showed that the first, second, and third principal components explained 40.2%, 24.9%, and 13.4% variations of all traits, respectively. Based on loading values from PCA analysis, LWC, Na^+ concentration, and K^+/Na^+ ratio were chosen to evaluate salinity tolerance of accessions, and eight accessions were divided into the tolerant, moderate, and sensitive groups. The tolerant group had relatively higher LWC and K^+/Na^+ ratio and concentrations of C, P, and Fe^{2+} and lower Na^+ concentrations than the other two groups, especially the sensitive groups. The result suggested that lower Na^+ accumulation and higher K^+/Na^+ ratio and LWC were crucial strategies for achieving salinity tolerance of perennial ryegrass.**

At present, over one-third of cultivated lands are threatened by soil salinization (Rengasamy, 2010). Salinity affects plant growth by imposing both ionic and osmotic stresses. High Na^+ concentration disturbs plant ionic homeostasis and the plasma-lemma system, decreases plant growth, and even causes plant death (Munns and Tester, 2008). In addition, Na^+ competes with K^+ for uptake across the root cell, which caused a decline in the K^+/Na^+ ratio and nutrient deficiency or ion imbalances (Chen et al., 2015). Salinity-tolerant plants had the capacity to restrict the uptake of Na^+, sequester Na^+ into the vacuoles, and accumulate compatible molecules or compounds, such as proline, glycine betaine, and sugars, to maintain osmotic equilibrium (Thalji and Shalaldeh, 2007).

Nitrogen and P are the important nutrient elements for plant growth and development (Allen and Bryson, 2007; Sanchez, 2007). Salinity reduced organic matter and nitrogen accumulation in plants (Chen et al., 2015). High salinity prevented plant N uptake, which limited plant growth (Hu and Schmidhalter, 1997; Munns and Tester, 2008). Application of N could positively affect plant growth under salinity stress (Rengasamy, 2010). Phosphate availability is reduced in saline soils due to ionic strength effects that reduced the activity of phosphate, and thus salinity decreased the P concentration in plant tissue (Bloomfield et al., 2014). However, increased P accumulation was also found in the leaf sheath and roots of rice (*Oryza sativa*) exposed to salinity (Nemati et al., 2011). In addition, P added to saline soils did not necessarily increase salinity tolerance of some crop species, such as maize (*Zea mays*), carrot (*Daucus carota*), sugar beet (*Beta vulgaris*), and tomato (*Solanum lycopersicum*) (Champagnol, 1979). The results indicate a complex relationship between salinity and P nutrition of plants.

It is well-known that salinity stress increases Na^+ accumulation and decreases K^+ in plant tissues, including perennial grass species (Hu et al., 2012; Tang et al., 2013a, 2013b). However, shoot concentrations of Na^+ and K^+ were not consistently associated with the degree of salinity tolerance (Tang et al., 2013a). High Na^+ concentration in the soil decreased Ca^{2+} uptake and transport and inhibited plant growth (Hu and Schmidhalter, 1997). High Ca^{2+} concentration in plant tissue improved salinity tolerance by excluding Na^+ (Hawighorst, 2007; Hu and Schmidhalter, 2005). Ashraf and Fatima (1995) found that $CaSO_4$ treatment enhanced the germination of wheat

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(*Triticum aestivum*) under salinity stress and maintained K^+ concentration, suggesting a role of Ca^{2+} in salinity tolerance. In addition, Ca^{2+} is strongly competitive with Mg^{2+} at the binding sites of root plasma membranes, which can interfere with both Mg^{2+} and Ca^{2+} uptake under salinity stress (Grattan and Grieve, 1998). The reduced leaf Ca^{2+} and Mg^{2+} concentrations were more pronounced with increasing NaCl concentrations in sour orange (*Citrus aurantium*) and Carrizo citrange (*Citrus sinensis* × *Poncirus trifoliata*) but such changes in Ca^{2+} and Mg^{2+} levels were not consistently shown in Cleopatra mandarin (*Citrus reshni*) and alemow (*Citrus macrophylla*) (Ruiz et al., 1997). The results suggest that effects of salinity stress on Ca^{2+} and Mg^{2+} concentrations may depend on plant species or cultivars.

Salinity also affects micronutrient uptake in plants. For example, the concentration of Mn^{2+} increased under salinity stress in barley [*Hordeum vulgare* (Hassan et al., 1970a)] and rice (Verma and Neue, 1984), but decreased in maize (Hassan et al., 1970b), whereas Mn^{2+}, B, and Fe concentrations were unaffected by salinity in young leaves of wheat (Hu and Schmidhalter, 2001). No clear patterns of changing Mn^{2+} and Fe concentrations were observed in four Citrus stocks exposed to increasing NaCl as mentioned previously (Ruiz et al., 1997). These inconsistent results indicate that nutrient uptake and metabolism are complex and diverse in different plant species under saline environments.

Perennial ryegrass is a popular cool-season perennial grass species (Cornish et al., 1979). It was widely cultivated in temperate climates. Because of wide geographical distribution, significant natural variations of salinity tolerance existed in this species (Tang et al., 2013a, 2013b). However, alterations of nutrient elements to salinity stress are not fully understood in perennial ryegrass. Therefore, the experiment was designed to identify the genotypic variations in growth traits and nutrient elements in relation to salinity tolerance in perennial ryegrass. The results would provide insights into variability of plant growth and nutritional elements of perennial grass species under salinity stress.

Table 1. Experimental identification (ID) number, accession number (PI), origin, and collection status of perennial ryegrasses used in this experiment about plant growth and nutritional elements in relation to salinity tolerance.

ID no.	PI no.	Origin	Status
18	265351	Chile	Uncertain
29	418707	Romania	Wild
89	303012	UK	Cultivar
315	303033	Netherlands	Cultivar
451	545593	Turkey	Wild
466	577264	UK	Wild
556	610927	Tunisia	Wild
582	632590	Morocco	Wild

Materials and Method

Plant materials and growth conditions. Eight accessions of perennial ryegrass [PI265351 (Chile), PI418707 (Romania), PI303012 (UK), PI303033 (The Netherlands), PI545593 (Turkey), PI577264 (UK), PI610927 (Tunisia), and PI632590 (Morocco)] were selected as experimental materials, including five wild, two cultivars, and one uncertain, in accordance with the U.S. Department of Agriculture National Plant Germplasm (USDA-NAGS) classification (Table 1). These accessions have been preserved in greenhouses at Purdue University, West Lafayette, IN through tiller propagation since 2014 and were mature enough for the study. Five to six tillers for each accession were propagated in pots (10 cm diameter, 9 cm deep) containing sand and grown for 10 weeks from 18 Sept. to 30 Nov. 2015 in a greenhouse before exposing to salinity stress. Grasses were cut to 5–6 cm height once per week and irrigated twice per week with a 50-mL half-strength Hoagland solution with pH of 6.5, electrical conductivity (EC) of 1.4 $dS \cdot m^{-1}$ in a greenhouse. The average temperatures in the greenhouse were ≈18 ± 1.0/15 ± 0.5 °C (day/night), and the average *PAR* was ≈400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a 10 h light period of natural and artificial light.

Salinity treatment. Plants were treated either with half-Hoagland solution alone (control) or amended with 300 mM pure NaCl (≈25.0 $dS \cdot m^{-1}$). To avoid salinity shock to the grasses, salt concentration was increased gradually by 25 mM every day until the 100 mM and then increased with 50 mM daily from 100 to 300 mM (total 7 d after salinity stress initiation). Plants were then cut to 5–6 cm height and exposed to 300 mM NaCl for 10 d (21–30 Nov. 2015).

Growth and ion measurements. Plant height (HT), LFW, LDW, LWC, and concentrations of N, C, B, Ca^{2+}, Cu^{2+}, Fe, K^+, Mg^{2+}, Mn^{2+}, Mo^{2+}, Na^+, and P were measured at the end of salinity treatment.

After reaching 300 mM NaCl, plants were cut to 5–6 cm and HT1 for each individual pot was recorded. Plants were not cut during the salinity stress treatment. After 10 d under 300 mM NaCl, HT was measured again (HT2). Plant HT grown during the treatment phase was the difference between HT2 and HT1. All the leaves corresponding to this height in each pot were collected and measured as LFW. The LDW was determined after the leaf tissue was dried at 80 °C in an oven for 3 d. LWC was calculated as [(FW – DW)/FW] × 100.

For B, Ca^{2+}, Cu^{2+}, Fe, K^+, Mg^{2+}, Mn^{2+}, Mo^{2+}, Na^+, and P measurements, we followed the methods described by Tang et al. (2013a). Briefly, 50 mg dry powder of leaves was put into digestion tubes and 5 mL of 18 M H_2SO_4 was added to each tube and mixed on a vortex mixer for 5 s. Then, tubes were

Table 2. Analysis of variance of plant height (HT), leaf fresh weight (LFW), leaf dry weight (LDW), leaf water content (LWC), concentrations of N, C, P, K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe, B, Cu^{2+}, Mn^{2+}, and Mo^{2+} in eight perennial ryegrass accessions under the nonsalinity control and 300 mM NaCl treatment.

	HT	LFW	LDW	LWC	N	C	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Fe	B	Cu^{2+}	Mn^{2+}	Mo^{2+}
Treatment (T)	189.8***	487.2***	67.2***	67.8**	597.0***	102.7***	NS	365.8***	649.3***	15.4***	25.1**	NS	25.4***	11.4*	NS	51.7***
Accession (A)	3.0*	13.0***	2.9*	3.1*	7.4***	7.7***	NS	8.2***	5.9***	4.8**	NS	NS	7.0***	NS	6.6***	2.9*
T × A	NS	***	NS	NS	*	NS	NS	***	NS	NS	NS	NS	**	*	NS	NS

*, **, *** indicate significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; NS = not significant.

placed on the digestion block at 200 °C for 30 min and removed from the block and cooled for 10–15 min. After cooling, 5 mL of 30% H_2O_2 was slowly added to each tube and tubes were vortexed and placed back on the block for 30 min. After all extraction was clear, double deionized water was slowly added to bring the final volume to 50 mL. The final diluted extract was used for determining ion element concentration using a plasma atomic emission spectrometer (ICP 9820; Shimadzu, Columbia, MD). About 30 mg of ground leaf samples were analyzed in a dry combustion analyzer (CHN 2000; Leco Corp., St. Joseph, MI) equipped with IR cell and thermal conductivity detectors for C and N concentrations, respectively (Hernandez-Ramirez et al., 2011).

Experimental design and data analysis. The experiment was arranged in a split-plot design, with salinity for the main plot and accession for the subplot. Each accession was randomly assigned within each treatment and each treatment was replicated three times (three pots). Analysis of variance was performed with SPSS (version 19.0; IBM Corp., Armonk, NY). The data from the salinity treatment were used for PCA. Based on the PCA results, accessions were divided into the tolerant, moderately tolerant, and sensitive group. Group mean for each trait was compared for illustrating the differences in various traits among the tolerant and sensitive materials.

Table 3. Mean values of plant height (HT), leaf fresh weight (LFW), leaf dry weight (LDW), leaf water content (LWC), concentration of N, C, P, K^+, Na^+, K^+/Na^+, Ca^{2+}, Mg^{2+}, Fe, B, Cu^{2+}, Mn^{2+}, and Mo^{2+} across eight accessions of perennial ryegrass under the nonsalinity control and 300 mM NaCl stress.

	Control		300 mM NaCl	
Traits	Range	Mean	Range	Mean
HT (cm)	10.4–17.7	13.6	4.1–6.1	4.9
LFW (g)	2.18–4.31	2.9	0.74–1.40	1.02
LDW (g)	0.32–0.56	0.41	0.14–0.35	0.26
LWC (%)	81.3–87.0	85.4	66.4–81.0	75.0
N ($mg \cdot g^{-1}$ DW)	43.5–51.5	4.9	30.0–38.9	33.2
C ($mg \cdot g^{-1}$ DW)	391.9–428.7	406.0	336.9–389.5	366.8
P ($mg \cdot g^{-1}$ DW)	4.67–7.04	5.7	3.9–6.7	5.1
K^+ ($mg \cdot g^{-1}$ DW)	42.3–71.2	59.3	29.5–38.5	34.3
Na^+ ($mg \cdot g^{-1}$ DW)	0.71–1.84	1.2	33.9–68.9	51.1
K^+/Na^+ (ratio)	37.7–88.0	54.0	0.50–1.09	0.74
Ca^{2+} ($mg \cdot g^{-1}$ DW)	4.4–8.0	5.7	3.6–5.9	4.6
Mg^{2+} ($mg \cdot g^{-1}$ DW)	2.4–3.5	2.8	1.8–2.7	2.1
Fe ($mg \cdot g^{-1}$ DW)	0.03–0.12	0.05	0.03–0.09	0.05
B ($mg \cdot g^{-1}$ DW)	0.006–0.04	0.02	0.006–0.07	0.04
Cu^{2+} ($mg \cdot g^{-1}$ DW)	0.009–0.04	0.03	0.009–0.04	0.02
Mn^{2+} ($mg \cdot g^{-1}$ DW)	0.06–0.15	0.08	0.05–0.14	0.08
Mo^{2+} ($mg \cdot g^{-1}$ DW)	0.003–0.02	0.009	0.02–0.04	0.03

Results and Discussion

Phenotypic variations and correlations among traits. Significant treatment effects were observed for all traits except P, Fe, and Mn^{2+} (Table 2). Significant accession effects were also shown on all traits except P, Mg^{2+}, Fe, and Cu^{2+} (Table 2). The interactions between accession and treatment were noted for LFW and concentration of N, K^+ B, and Cu^{2+} (Table 2).

A wide range in value of each trait was identified under salinity stress across eight accessions of perennial ryegrass (Table 3). Compared with the nonsalinity control, the mean values of HT, LFW, LDW, and LWC decreased ≈64%, 65.5%, 36.5%, and 12.2% under salinity stress, respectively (Table 3). The mean concentrations of N, C, P, K^+, Ca^{2+}, and Mg^{2+} ratio also decreased about 32.7%, 9.4%, 10.5%, 42.2%, 19.3%, and 25.0% at 300 mM NaCl, compared with the control, respectively. However, the mean concentration of Na^+, B, and Mo^{2+} increased under salinity stress, especially for Na^+ with a 42-fold increase in the mean concentration. The mean concentration of Fe and Mn^{2+} remained unchanged under the control and salinity treatment. Similar results were obtained from different soybean (*Glycine max*) accessions under salinity stress, whereas the HT, FW, DW, Ca^{2+}, K^+, and Mg^{2+} concentrations significantly

Table 4. Pearson correlation coefficients among plant height (HT), leaf fresh weight (LFW), dry weight (LDW), water content (LWC), concentrations of N, C, P, K^+, Na^+, K^+/Na^+ ratio, Ca^{2+}, Mg^{2+}, Fe, B, Cu^{2+}, Mn^{2+}, and Mo^{2+} across eight perennial ryegrass accessions under the nonsalinity control (upper diagonal) and 300 mM NaCl treatment (lower diagonal).

	HT	LFW	LDW	LWC	N	C	P	K^+	Na^+	K^+/Na^+	Ca^{2+}	Mg^{2+}	Fe	B	Cu^{2+}	Mn^{2+}	Mo^{2+}
HT		0.60**	0.18	0.78**	0.33	–0.56**	–0.17	0.63**	0.28	0.13	–0.16	–0.15	–0.27	–0.19	–0.23	–0.16	–0.23
LFW	0.27		0.84**	0.52*	0.25	–0.18	–0.27	0.48*	0.13	0.20	–0.42*	–0.47*	–0.23	–0.28	–0.34	–0.27	–0.28
LDW	0.25	0.62**		–0.02	0.05	0.32	–0.36	0.07	–0.15	0.27	–0.48*	–0.53**	0.03	–0.24	–0.28	–0.33	–0.37
LWC	0.02	0.2	–0.07		0.45*	–0.85	0.15	0.77**	0.49*	–0.1	0.01	0.04	–0.54**	–0.09	–0.21	0.01	0.07
N	0.15	0.36	0.19	–0.05		–0.41	–0.21	0.58**	0.18	0.16	0.11	0.14	–0.12	0.15	–0.07	0.17	0.16
C	0.41	0.35	0.07	0.41	0.37		–0.18	–0.77**	–0.51*	0.16	–0.37	–0.27	0.52*	–0.09	0.19	–0.16	–0.23
P	0.04	0.03	–0.08	0.09	0.52**	0.32		–0.16	0.25	–0.39	0.32	0.49*	–0.16	0.04	–0.08	–0.19	0.06
K^+	–0.08	–0.13	–0.13	–0.38	0.33	–0.40	0.12		0.47*	0.06	–0.01	–0.07	–0.42*	0.14	–0.21	0.22	0.27
Na^+	–0.34	–0.26	0.03	–0.35	–0.24	–0.87**	–0.08	0.37		–0.82**	–0.16	0.09	–0.4	0.42*	0.32	0.39*	0.33
K^+/Na^+	0.25	0.21	–0.06	0.06	0.53**	0.63**	0.23	0.15	–0.80**		0.15	–0.19	0.25	–0.36	–0.49*	–0.34	–0.26
Ca^{2+}	0.07	–0.13	–0.19	–0.46*	–0.08	–0.16	0.21	0.26	0.15	0.06		0.57**	–0.08	–0.08	–0.30	–0.18	0.01
Mg^{2+}	–0.04	–0.19	–0.15	–0.40	–0.20	–0.20	0.14	0.22	0.13	–0.06	0.51*		–0.31	–0.02	0.10	–0.23	–0.18
Fe	0.33	0.17	–0.08	0.04	0.46*	0.26	0.32	0.24	–0.17	0.31	0.10	0.11		–0.13	0.02	–0.08	–0.12
B	–0.44*	0.17	0.12	0.02	–0.14	–0.21	0.25	–0.02	0.29	–0.31	0.25	0.10	–0.25		0.34	0.64**	0.54**
Cu^{2+}	–0.14	0.48*	0.21	0.15	0.11	0.15	–0.14	–0.15	–0.13	0.02	–0.12	–0.16	0.30	0.28		0.45*	0.20
Mn^{2+}	–0.39	0.02	0.29	–0.25	0.04	–0.61**	–0.07	0.33	0.60**	–0.36	0.18	–0.11	–0.43*	0.44*	–0.10		0.83**
Mo^{2+}	–0.08	0.02	–0.05	–0.20	0.17	0.18	0.43*	0.29	–0.19	0.34	0.26	0.28	–0.22	0.38	–0.15	0.18	

*, ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively.

Table 5. Effects of salinity treatment on leaf fresh weight (LFW) and concentrations of N, K^+, B, and Cu^{2+} in eight perennial ryegrass accessions by split-plot analysis of variance.[z]

ID	LFW (g)		N (mg·g^{-1} DW)		K^+ (mg·g^{-1} DW)		B (mg·g^{-1} DW)		Cu^{2+} (mg·g^{-1} DW)	
no.[y]	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity
18	2.74 ± 0.4	1.40 ± 0.1*	49.3 ± 2.7	35.0 ± 2.4*	59.3 ± 4.1	35.1 ± 2.9*	0.020 ± 0.01	0.039 ± 0.01	0.027 ± 0.01	0.017 ± 0.002
29	3.07 ± 0.4	1.22 ± 0.1*	50.7 ± 2.1	38.9 ± 2.3*	62.1 ± 4.3	35.2 ± 1.1*	0.018 ± 0.003	0.006 ± 0.002*	0.020 ± 0.01	0.014 ± 0.001
89	2.39 ± 0.2	0.95 ± 0.2*	49.1 ± 1.3	36.0 ± 3.1*	58.5 ± 3.3	38.5 ± 0.6*	0.017 ± 0.01	0.027 ± 0.01	0.009 ± 0.001	0.015 ± 0.01
315	2.20 ± 0.4	0.74 ± 0.2*	48.6 ± 0.5	30.1 ± 3.0*	55.0 ± 5.4	30.9 ± 0.5*	0.024 ± 0.01	0.042 ± 0.001*	0.037 ± 0.001	0.015 ± 0.001*
451	3.10 ± 0.4	0.83 ± 0.1*	50.9 ± 0.4	32.1 ± 0.8*	71.2 ± 2.4	37.9 ± 6.2*	0.035 ± 0.01	0.068 ± 0.02	0.034 ± 0.01	0.011 ± 0.004*
466	2.18 ± 0.2	0.90 ± 0.2*	43.5 ± 2.3	30.0 ± 1.3*	42.3 ± 1.3	30.7 ± 1.2*	0.018 ± 0.001	0.034 ± 0.01	0.039 ± 0.01	0.009 ± 0.001*
556	4.31 ± 0.2	1.34 ± 0.2*	47.5 ± 0.6	31.5 ± 1.0*	67.0 ± 5.8	30.3 ± 1.0*	0.006 ± 0.0005	0.047 ± 0.01*	0.029 ± 0.01	0.042 ± 0.003
582	3.08 ± 0.3	0.77 ± 0.1*	51.5 ± 0.3	32.4 ± 0.5*	59.3 ± 4.8	37.0 ± 4.7*	0.019 ± 0.003	0.019 ± 0.01	0.035 ± 0.004	0.010 ± 0.002*

[z]Comparisons were made between the nonstress control and salinity treatment (300 mM NaCl) for a given accession and parameter. * indicates significance at $P < 0.05$.
[y]Experimental identification number.

Table 6. Principle component analysis of plant height (HT), leaf fresh weight (LFW), dry weight (LDW), water content (LWC), concentration of N, C, P, K^+, Na^+, K^+/Na^+ ratio, Ca^{2+}, Mg^{2+}, Fe, B, Cu^{2+}, Mn^{2+}, and Mo^{2+} across eight perennial ryegrass accessions under 300 mM NaCl stress.

Traits	PC1[z]	PC2	PC3
HT	–0.548	0.465	0.239
FW	0.429	0.753	–0.082
DW	–0.332	0.626	–0.354
LWC	0.785	0.505	0.119
N	0.636	0.131	0.452
C	0.735	0.183	–0.432
P	0.725	–0.229	0.359
K^+	0.295	–0.485	0.722
Na^+	–0.857	–0.178	0.391
K^+/Na^+ ratio	0.955	–0.059	–0.103
Ca^{2+}	–0.166	–0.913	–0.284
Mg^{2+}	0.454	–0.740	–0.263
Fe	0.670	–0.012	0.209
B	–0.675	–0.012	0.105
Cu^{2+}	–0.116	0.817	–0.133
Mn^{2+}	–0.282	0.130	0.739
Mo^{2+}	–0.273	–0.597	–0.319
PV[y]	40.2	24.9	13.4

[z]PC1, PC2, and PC3 are the first, second, and third principle component, respectively.
[y]Percentage variation.

decreased in the plants (Essa, 2002). In *Arabidopsis thaliana*, the concentrations of most of the nutrient elements decreased under salinity stress (Hill et al., 2013). Overall, our results supported these observations.

There were 20 positive correlations and 13 negative correlations among growth and nutrient elements under the nonstress control condition, whereas 10 positive correlations and 8 negative correlations were noted under 300 mM NaCl (Table 4). Specifically under salinity stress, HT was positively correlated with FW and LWC, whereas K^+/Na^+ ratio was positively correlated with C and N but negatively correlated with Na^+. In addition, Mn^{2+} was positively correlated with B and Na^+ but negatively correlated with C. P was positively correlated with N and Mo^{2+}. Positive correlations were also observed between Cu^{2+} and FW, between Fe and N, and between Ca^{2+} and Mg^{2+}. In addition, negative correlations were noted between B and HT, between Ca^{2+} and LWC, and between Na^+ and C. Less correlations under salinity stress may suggest that an element balance was partially disturbed because of high NaCl concentration. The positive and negative correlations of some elements suggested that C, P, N, Fe, and K^+/Na^+ could positively impact salinity tolerance, whereas Na^+, B, and Mn^+ might negatively influence salinity tolerance.

Interactive effects on traits. Although declines in LFW, N, and K^+ concentrations were found under 300 mM NaCl in all eight accessions, the magnitude of declines in these parameters differed in accessions (Table 5). Concentration of B decreased in accession 29 but increased in accession 315 and 556 under salinity stress, compared with the control, whereas B concentrations remained unchanged in other accessions (Table 5). Salinity decreased Cu^{2+} concentration in four accessions but not in other accessions. Nitrogen enables plants to improve growth and yield under salinity stress (Delgado et al., 1994). Salinity reportedly affected the accumulation of ammonium, nitrate, and free amino acids in plants (Amini and Ehsanpour, 2005). The lower reduction in N concentration could be associated with salinity tolerance.

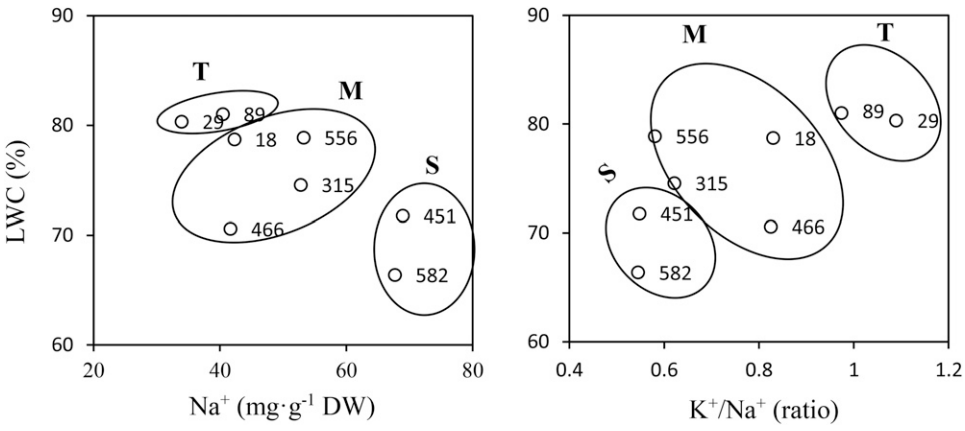

Fig. 1. Separation of perennial ryegrass accessions differing in sensitivities to salinity stress based on leaf water content (LWC) and Na^+ concentration and LWC and K^+/Na^+ ratio under 300 mM NaCl. Number represents each individual accession. T, M, and S represent the tolerant, moderately tolerant, and sensitive accessions, respectively.

PCA analysis. The PCA analysis revealed variations among the traits. The first (PC1), second (PC2), and third principal components (PC3) accounted for 40.2%, 24.9%, and 13.4%, respectively. PC1 showed the larger loading values for the K^+/Na^+ ratio (0.955), Na^+ (–0.857), and LWC (0.785) (Table 6). PC2 exhibited the larger loading values for LFW (0.753), Ca^{2+} (–0.913), and Cu^{2+} (0.817). PC3 had the larger loading values for Mn^{2+} (0.739) and K^+ (0.722) (Table 6). Based on the PC1 results, LWC, Na^+ concentration, and K^+/Na^+ ratio with larger loading values were chosen to evaluate salinity tolerance of accessions (Fig. 1). These parameters are often associated with salinity tolerance (Azadi et al., 2011; Tang et al., 2013b; Thalji and Shalaldeh, 2007). Specifically, accessions 29 and 89 were considered as the salinity tolerant materials (T). The four accessions of 18, 315, 466, and 556 were in the moderate-tolerant group (M), and accessions 451 and 582 were salinity sensitive accessions (S).

Growth responses to treatments. Compared with the control, the mean HT was reduced by 62.2%, 58.7%, and 67.5% for the T, M and S groups, respectively (Fig. 2). LWC decreased only 5.7% for T, 10.8% for M, and 20% for S. However, the reduction of LDW did not differ in the three groups (Fig. 2). Reduced growth was also observed in other plant species exposed to high salinity stress (Azadi et al., 2011; Brugnoli and Lauteri, 1991; Hu et al., 2012). Our results indicated that lower reductions on LWC could be associated with salinity tolerance in perennial ryegrass.

Responses of Na^+ and K^+/Na^+. Under the 300 mM NaCl treatment, Na^+ concentration was 37.2 $mg \cdot g^{-1}$ DW in the T group, which was significantly lower than the M (47.5 $mg \cdot g^{-1}$ DW) and S (68.3 $mg \cdot g^{-1}$ DW) groups (Fig. 3). The K^+/Na^+ ratio in the T group was significantly higher than that of the M and S groups under salinity stress (Fig. 3).

Cytosolic K^+ and Na^+ accumulations are mediated by ion transporters and channels (Zhu, 2003). The salt-tolerant *Populus euphratica* roots exhibited a higher capacity to extrude Na^+ than the salt-sensitive *Populus popularis* after exposure to salinity stress. As a result, Na^+ accumulation in *P. euphratica* was lower than *P. popularis* (Sun et al., 2009). Our results supported this observation. The salt-tolerant accessions could limit Na^+ accumulation and keep K^+ concentration stability under severe salinity stress. Similar results were also observed in barley and wheat (Azadi et al., 2011; Thalji and Shalaldeh, 2007). The results suggest that maintaining a relative higher K^+ concentration, lower Na^+ accumulation, and higher K^+/Na^+ ratio level is a crucial strategy for salinity tolerance in perennial ryegrass (Mäser et al., 2001).

Responses of C, P, Ca^{2+} and Mg^{2+} to treatments. Under 300 mM NaCl, the concentration of P increased 17.5% for the T group but decreased by 19.5% and 21% for the M and S groups, respectively; compared with the control (Fig. 4). The C concentration was significantly reduced by 5.5%, 9.4%, and 13.6% for the T, M, and S groups, respectively (Fig. 4). The reduction of the Ca^{2+} and Mg^{2+} concentrations was 27.9% and 17.5% for the T group, 15% and 23.4% for the M group, 17.8% and 30.3% for the S group under 300 mM NaCl, respectively, compared with the control (Fig. 4).

Plants often accumulate more carbohydrates under salinity stress (Nemati et al., 2011; Tang et al., 2013a). Salinity stress reduced the rate of photosynthesis, and the tolerant species could synthesize and maintain more carbohydrates than the sensitive species (Brugnoli and Lauteri, 1991). In this study, the

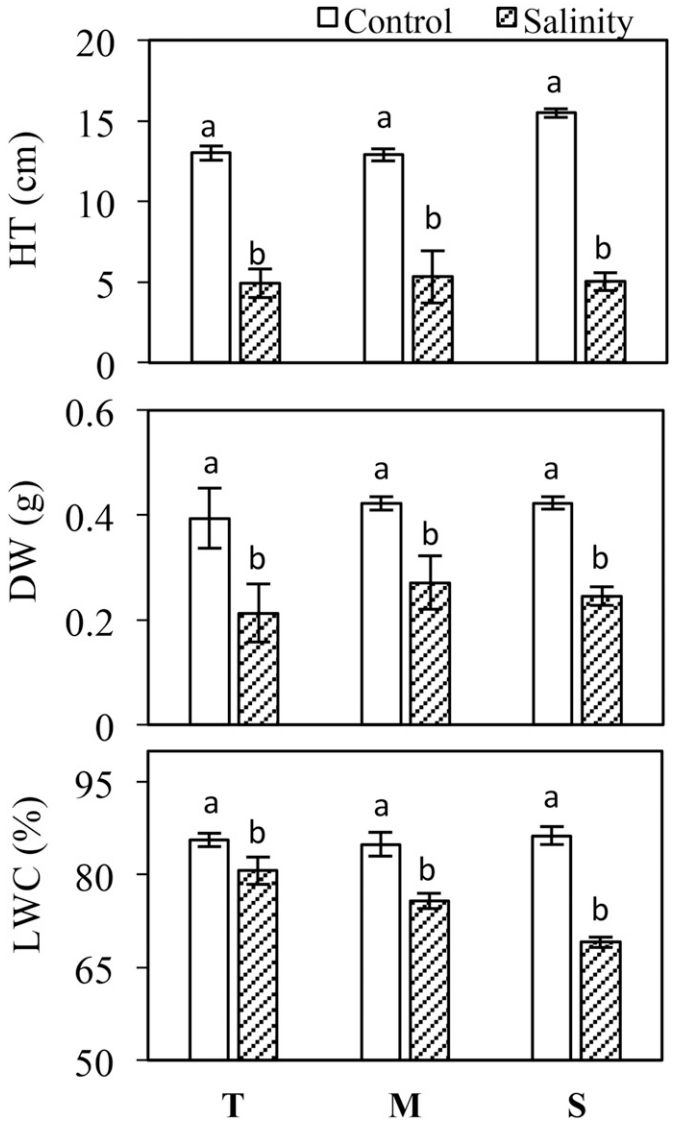

Fig. 2. Plant height (HT), leaf dry weight (LDW), leaf water content (LWC), and carbon concentration of perennial ryegrass as affected by 300 mM NaCl in the salinity tolerance group (T), moderately tolerant group (M), and the sensitive group (S) by split-plot analysis of variance. Comparisons are made between the control and salinity treatments. Means followed by the same letter within a group are not significantly different at $P < 0.05$. Bars indicate SD.

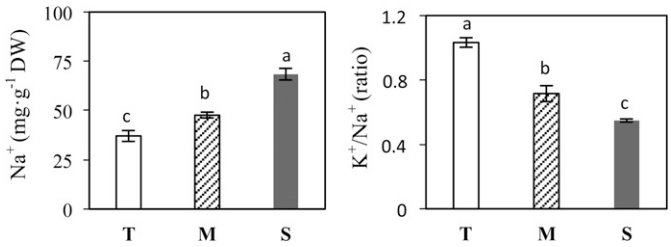

Fig. 3. Concentration of Na^+ and K^+/Na^+ ratio of perennial ryegrass as affected by 300 mM NaCl treatment in the salinity tolerance group (T), moderately tolerant group (M), and the sensitive group (S) by split-plot analysis of variance. Comparisons are made among the three groups. Means followed by the same letter are not significantly different at $P < 0.05$. Bars indicate SD.

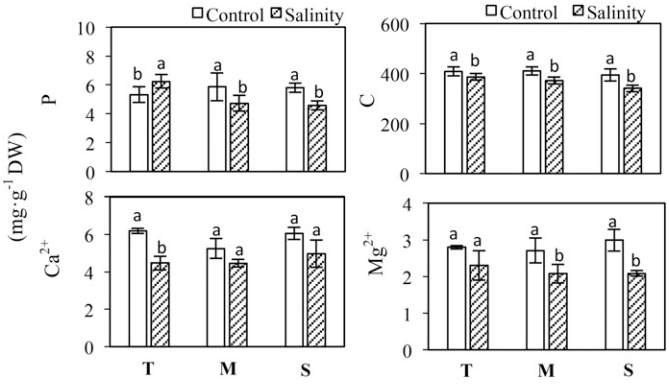

Fig. 4. Concentration of P, C, Ca^{2+}, and Mg^{2+} of perennial ryegrass as affected by 300 mM NaCl treatment in the salinity tolerance group (T), moderately tolerant group (M), and the sensitive group (S) by split-plot analysis of variance. Comparisons are made between the control and salinity treatments. Means followed by the same letter within a group are not significantly different at $P < 0.05$. Bars indicate SD.

unchanged C concentration in the tolerant accession demonstrated that salinity tolerance may be associated with higher capacity of maintaining carbon under salinity stress. Salinity decreased P uptake and the concentration of P in plant tissue (Grattan and Grieve, 1998; Sharpley et al., 1992) or caused changes in P concentration (Hawighorst, 2007). Our results were partially consistent with previous studies. The P accumulation in the M and S groups decreased under salinity, but the T group accumulated more P (Fig. 4). The results indicated that the P level in the plants is vital for salinity tolerance in perennial ryegrass.

Previous studies reported that high Ca^{2+} concentration could maintain a plant's capacity for nutrient uptake and transport under saline conditions (Fageria et al., 2011). In general, plant Mg^{2+} uptake is inhibited by high NaCl stress. However, Ca^{2+} is strongly competitive with Mg^{2+} at the binding sites of root plasma membranes, which can interfere with both Mg^{2+} and Ca^{2+} uptake under salinity stress. The relatively higher Mg^{2+} and lower Ca^{2+} accumulations in the T group and higher Ca^{2+} over Mg^{2+} accumulation in the M and S groups found in this study suggested that maintenance of a balance between Mg^{2+} and Ca^{2+} might be needed for acquiring salinity tolerance in perennial ryegrass accessions.

Responses of micronutrient elements to treatments. Mn^{2+} concentration did not differ in all three groups under salinity compared with the control (Fig. 5).

The concentration of Mo^{2+} increased 250.7% for the T group, 208.1% for the M group, and 145.7% for the S group exposed to 300 mM NaCl compared with the control. Salinity stress dramatically increased Fe concentration to 106.2% in the T group and decreased 28.7% in the M group and was unaffected in the S group compared with the control (Fig. 5).

Availability of micronutrients to plants may increase, decrease, or remain unchanged under salinity stress (Grattan and Grieve, 1998). In wheat, the leaf B concentration was unaffected by salinity (Bingham et al., 1987). In river red gum (*Eucalyptus camaldulensis*), salt solution reduced leaf B concentration (Grattan et al., 1997). The increased, decreased, or unchanged B concentration was not consistent with salinity tolerance of perennial ryegrass in this study (Table 5; Fig. 1), suggesting a complex role of B in salinity tolerance. Under 300 mM NaCl, increased Fe concentration in the T group and

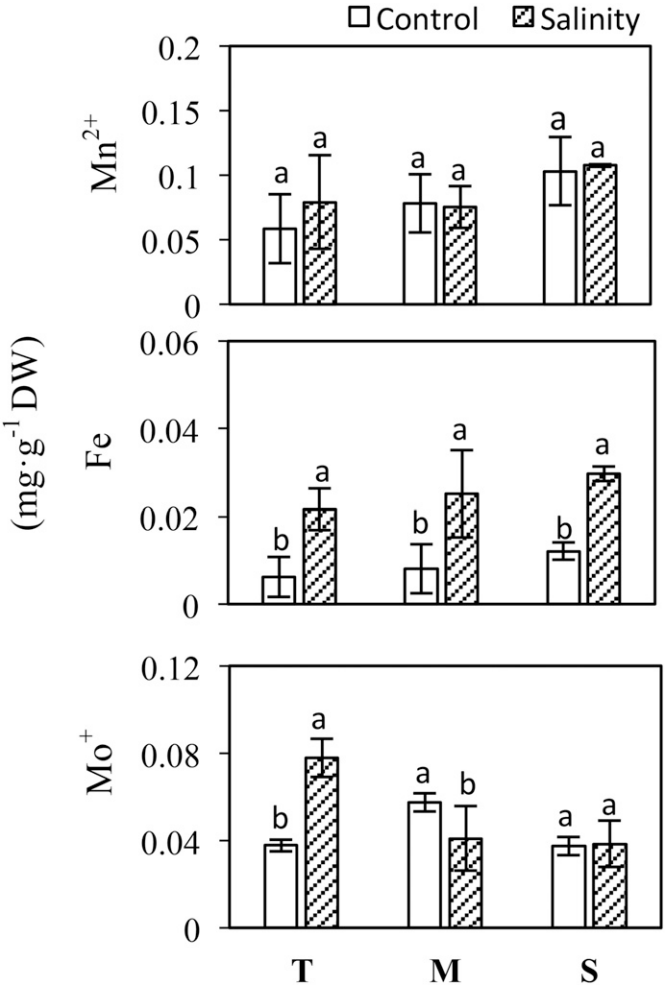

Fig. 5. Concentration of Fe, Mn^{2+}, and Mo^{2+} of perennial ryegrass as affected by 300 mM NaCl treatment in the salinity tolerance group (T), moderately tolerant group (M), and the sensitive group (S) by split-plot analysis of variance. Comparisons are made between the control and salinity treatments. Means followed by the same letter within a group are not significantly different at $P < 0.05$. Bars indicate SD.

decreased or unchanged Fe concentrations in the M and S groups suggested that Fe could play a role in resisting salinity stress in perennial ryegrass. Similar results were reported for tomato cultivars under salinity stress (Martinez et al., 1987).

Very little attention has been received toward salinity effects on Cu^{2+} and Mo^{2+} uptake in plants. In maize, Cu^{2+} concentration was variable under salinity; however, salinity increased Mo^{2+} concentration (Rahman et al., 1993). The reduced Cu^{2+} concentration found in the four accessions with less salinity tolerance demonstrated that higher concentration of Cu^{2+} could be associated with salinity tolerance. The role of Mn^{2+} in salinity tolerance is unclear. Salinity reduced Mn^{2+} uptake in dry bean [*Phaseolus vulgaris* (Doering et al., 1984)], and additions of Mn^{2+} to the culture solution increased barley salt tolerance (Cramer and Nowak, 1992). However, Mn^{2+} was unaffected by salinity stress in peanut [*Arachis hypogaea* (Chavan and Karadge, 1980)]. The unchanged Mn^{2+} concentration under salinity in all groups in our study suggested that Mn^{2+} might not be sensitive to a short period of salinity stress in perennial ryegrass accessions. The role of Mn^{2+} in salinity

tolerance needs to be further examined in perennial grass species exposed to a longer period of stress.

Conclusions

Salinity inhibited growth, reduced concentrations of N, C, Ca^{2+}, K^+, Mg^{2+}, Cu^{2+}, and K^+/Na^+ ratio increased Na^+ concentration in perennial ryegrass. The tolerant groups had relatively higher LWC and K^+/Na^+ ratio and concentrations of C, P, and Fe^{2+} and lower Na^+ concentrations than the other two groups, especially the sensitive groups. Genotypic variations of growth and nutritional responses of perennial ryegrass to salinity stress provide an important basis for further investigation of molecular mechanisms of salinity tolerance in perennial grass species.

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