Study on physiological and adaptational responses of turfgrasses to salinity stress

シバの塩ストレスに対する生理および適応応答に関する研究

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CHAPTER 1

INTRODUCTION

Salinity is one of the most serious problems limiting plant growth and productivity. Approximately 6% of the earth's land area (800 million hectares) is affected by either salinity or the associated condition of sodicity (Food and Agriculture Organization 2006). Salinity problem is increasing in turfgrass culture because of increased use of secondary, saline water sources, such as recycled water (Marcum 2008). Rapidly increasing populations and urbanization, especially in arid and semi-arid regions, are creating the freshwater shortage. The reuse of treated wastewater for irrigation of turfgrasses and landscapes is viewed as one way to use urban water resources more efficiently. In addition, seawater intrusion in coastal regions has increased problems associated with establishing and maintaining turfgrasses (Qian and Suplick 2001). The tsunami induced by the 2011 Off the Pacific Coast Tohoku Earthquake inundated a total agricultural land of approximately 23,600 ha from Iwate Prefecture to Chiba Prefecture, Japan (MAFF 2011). For these reasons, the needs for turfgrasses that are tolerant to stresses associated with salt-affected sites and even irrigation with recycled wastewater has increased in recent decades.

Turfgrass encompasses an extreme range in salinity tolerance, from salt-sensitive to extremely salt-tolerant (halophytic). Based on the maximum salinity (EC) at which shoot growth is reduced by approximately 50% (Marcum 2008), a salinity tolerance ranking of turfgrass species is provided below (Table 1.1). The unit in reporting salinity tolerance was modified to electrical conductivity (EC) of saturated soil paste (1 dS m^{-1} ≈ 11 mM NaCl ≈ 640 ppm NaCl).

Table 1. Salinity tolerance ranking of turfgrass species based on relative salinity tolerance according to Marcum, 2008.

Common Name	Salinity Tolerance Ranking[†]	Salinity Tolerance (EC_e in dS m^{-1})		Type[†††]
		Ave.[††]	Range	
Saltgrass	T	35	12-46	Warm
Seashore Dropseed	T	31	10-45	Warm
Seashore Paspalum	T	25	16-45	Warm
Alkaligrass	T	25	20-46	Cool
St. Augustinegrass	T	22	19-40	Warm
Bermudagrass	T	18	8-40	Warm
Zoysiagrass	T	14	6-40	Warm
Creeping bentgrass	MT	10	8-26	Cool
Tall fescue	MT	8	4-10	Cool
Buffalograss	MT	7	7-10	Warm
Perennial Ryegrass	MT	7	4-11	Cool
Creeping red fescue	MT	6	0-12	Cool
Gramagrass	MS	5	ND	Warm
Kentucky bluegrass	S	3-4	3-15	Cool
Carpetgrass	S	3	ND	Warm
Centipedegrass	S	2-3	ND	Warm
Bahigrass	S	1.25	3-6	Warm
Annual bluegrass	S	ND	0-2	Cool
Rough bluegrass	S	ND	0-3	Cool
Redtop	S	ND	0-2	Cool
Velvet bentgrass	S	ND	0-2	Cool

[†] S (sensitive) < 3 dS m^{-1}; MS (moderately sensitive) 3–6 dS m^{-1}; MT (moderately tolerant) 6–10 dS m^{-1}; T (tolerant) > 10 dS m^{-1}; ND (no data)

[††] Average salinity tolerance is an estimate of the maximum salinity at which the salinity at which shoot growth is reduced by approximately 50%.

[†††] Cool-season (C_3) and Warm-season (C_4) grasses. Cool-season grasses show optimum growth between 16 °C and 24 °C, and warm-season grasses show optimum growth between 27 °C and 35 °C.

Kentucky bluegrass (*Poa pratensis* L.), Tall fescue (*Festuca arundinacea* Schreb.) and four bermudagrass (*Cynodon* spp.) cultivars were used in this study. Kentucky bluegrass (KBG) is widely used for lawn, golf turf (except greens), athletic fields, and other general-purpose turfs in subarctic and temperate regions (Turgeon 2008). KBG has been generally ranked as one of salt-sensitive grass species with an average threshold EC of 3 dS m^{-1} (Carrow and Duncan 1998). A number of salinity studies have been done on KBG, reflecting its wide use among cool-season turfgrass. The 5000 ppm NaCl solution reduced germination of KBG seed by 50% and 10,000 ppm (15 dS m^{-1}) reduced germination nearly to zero (Liem *et al.* 1985). The majority of studies show there was not a very wide range of salinity tolerance among KBG cultivars compared with other turfgrasses (Marcum 2008). Robins *et al.* (2009) evaluated the salt tolerance of 67 Kentucky bluegrass and found that five accessions had tolerance as good as or better than the Tall fescue and Perennial ryegrass.

Tall fescue (TF) can adapt to a wide range of soil conditions. TF is becoming an increasingly important lawn species and is widely used as a utility turfgrass in both warm and cool subtropical climates (Turgeon 2008). TF is a moderately salt-tolerant species with an EC tolerance of 6-10 dS m^{-1} (Harivandi 1988). More salinity studies have been done on TF due to the widespread use for laws and roadsides in cooler climates. TF cv. Kentucky 31 was the most salt tolerant of ten species considered for use on salt-affected soil (Roberts and Zybura 1967). Alshammary *et al.* (2004) found that TF cv. Arid was more salt tolerant than KBG and 50% shoot growth reduction of TF was at 10.0 and 14.2 dS m^{-1} in the container experiments and hydroponic experiments, respectively. Kobayashi *et al.* (2004) reported that TF cv. Southern Cross was the most salt tolerant of six cool-season species and 50% dry matter reduction

occurred at 141.0 mmol L^{-1} NaCl in the hydroponic experiments,.

Bermudagrass species (*Cynodon* spp.), the most popular turfgrasses in warm regions worldwide for lawns, sports and along roadsides, are well-adapted to a wide range of soil conditions, being tolerant to drought and a broad salinity range (Carrow 1996; Marcum and Pessarakli 2006). Bermudagrass is ranked as having excellent salinity tolerance, tolerating ECe 8-40 dS m^{-1} (Marcum 2008). There have been a number of studies comparing salinity tolerance of several Bermudagrass cultivars (Dudeck and Peacock 1993; Dudeck *et al.* 1983; Francois 1988; Peacock *et al.* 2004). Differences in salinity tolerance among Bermudagrass cultivars have been noted. Salinity tolerance (according to the salinity level that causes 50% shoot growth reduction) ranged between 26 and 40 dS m^{-1} among 35 *Cynodon* spp. cultivars (Marcum and Pessarakli 2006).

Salinity can inhibit plant growth by low external water potentials, ion toxicity and ion imbalance (Munns 1993). Ionic and hyperosmotic stresses lead to secondary stress such as oxidative damage (Zhu 2001). Salt-tolerant plants accumulate inorganic ions and various organic osmolytes to balance external osmotic stresses through the process of osmoregulation, or osmotic adjustment. To avoid toxicity to the cytosol, Na^+ and Cl^- are compartmentalized mainly in the vacuoles of shoot and root cells (Flowers *et al.* 1977; Lerner *et al.* 1994). Under these conditions, the osmotic potential of the cytoplasm is maintained by the accumulation of compatible solutes such as proline, glycinebetaine, sugars, and cyclitols, which can be accumulated in sufficient concentrations to balance the osmotic potential of Na^+ and Cl^- accumulated in the vacuole (Flowers and Colmer 2008). To mitigate the oxidative damage induced by reactive oxygen species, plants employ a variety of enzymatic and non-enzymatic antioxidant defenses (Apel and

Hirt 2004). Numerous studies have compared relative salinity tolerance among turfgrass cultivars, however, only a few of them have attempted to elucidate tolerance mechanisms in turfgrasses. To date, relatively little progress has been made in breeding for improved salinity tolerance in turfgrass. Development of salt-tolerant cultivars is not simple because the trait is controlled by many physiological mechanisms and gene (Grover *et al.* 1999; Lee *et al.* 2005). A thorough knowledge of the physiology of salt tolerance of turfgrasses may aid in defining salt tolerance mechanisms and identifying criteria for turfgrass breeders in developing more salt-tolerant cultivars which can grow on sites with saline soil conditions, and normally not suitable for crop production. The objectives of this study were to compare the relative salinity tolerance and physiological responses of three turfgrass species to salinity and to elucidate the physiological adaptations of turfgrasses to salinity stress.

CHAPTER 2

Growth responses, ionic concentration, organic solute accumulation and osmotic adaptation in Kentucky bluegrass and Tall fescue under salinity stress

The detrimental effects of salinity on turfgrass growth include ion toxicity, osmotic stress, and nutritional disturbances. Salt tolerant plants have the ability to minimize these detrimental effects by producing a series of anatomical, morphological, and physiological adaptation (Alshammary *et al.* 2004). To avoid ion toxicity, Na^+, Cl^- and other inorganic ions are compartmentalized mainly into vacuoles of shoots and roots cells. Compatible solutes, such as organic solutes and K^+ are accumulated in cytoplasm for osmotic adjustment. The study in this chapter was conducted to compare growth responses, inorganic ion concentrations, compatible solutes accumulation and the contributions of specific inorganic ions and compatible solutes to osmotic potential in shoots and roots of KBG and TF in response to different levels of NaCl stress.

MATERIALS AND METHODS

Seeds of KBG (cv. Blue Star) and TF (cv. Little Hero) were sown in the growth chamber conditions which were maintained at 24 ± 2°C and 60% relative humidity, respectively. Three-week-old seedlings were transplanted to 4 L plastic pots filled with nutrient solution (Table 2) in a glasshouse. The solution was aerated constantly and replaced twice a week throughout the experiment.

Plants were cultured under non-saline conditions for 15 d to ensure full establishment before starting salinity treatments. The nutrient solution was salinized with NaCl to 50, 100, 150 and 200 mmol L^{-1}. Nutrient solution not containing NaCl (0 mmol L^{-1} NaCl) was prepared for the control treatment. Afterwards, plants in each treatment were grown for 40 days.

Table 2. Composition of nutrient solution.

Macro-nutrients (mol m^{-3})			**Micro-nutrients (g m^{-3})**		
N	2.0	NH_4NO_3	Fe	2.0	$FeSO_4 \cdot 7H_2O$
P	0.4	$NaH_2PO_4 \cdot 2H_2O$	Mn	0.5	$MnSO_4 \cdot 5H_2O$
K	2.0	KCl	B	0.2	H_3BO_3
Ca	1.0	$CaCl_2 \cdot 2H_2O$	Zn	0.1	$ZnSO_4 \cdot 7H_2O$
Mg	2.0	$MgSO_4 \cdot 7H_2O$	Cu	0.01	$CuSO_4 \cdot 5H_2O$
			Mo	0.005	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$

During the salinity treatment period, the plant growth parameters of shoot height, root length, dry weight (DW) of shoots and roots, turf quality and leaf firing were determined. Shoots and roots were washed with deionized water and dried at 70°C for 48 h to determine DW. Turf quality and leaf firing were visually estimated weekly as described by Alshammary *et al.* (2004). Turf quality was estimated based on a scale of 1-9, with 9 being green, dense and uniform, 1 being thin and completely brown, and 6 being the minimum acceptable level. Leaf firing was estimated as the total percentage of chlorotic leaf area, with 0% indicating no leaf firing and 100% indicating totally brown leaves.

Shoot and root samples were collected from three plants, thoroughly rinsed, cut into small pieces (5 mm length), placed in Eppendorf tubes perforated with four small holes, and immediately frozen in liquid nitrogen. The osmolality of the collected sample sap was analyzed with a vapor pressure osmometer (Osmometer 5520, Wescor, Logan, UT, US). The osmotic

potential (Ψ_s) was calculated by using the van't Hoff equation ($\Psi_s = -cRT$). The Ψ_s of each of Na^+, K^+, Cl^-, proline, and TSS was calculated as $\Psi_s = -nRT/V$, where n is the number of solute molecules and V is the volume (L). Osmotic coefficients of the solutes in tissue water were assumed to equal 1 (Song *et al.* 2006).

After harvest, shoots and roots were washed with deionized water and dried at 70°C for 48 h for determining inorganic ion concentrations and total soluble sugar (TSS). Dried samples were finely ground and digested in 1 mL sulfuric acid and hydrogen peroxide at 200°C on a dry block bath. The concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} were determined by atomic absorption spectrophotometry (Polarization Zeeman Z-6100; Hitachi, Ibaraki, Japan). Ground plant samples were heated in boiling deionized water in a beaker on a hot plate (250°C) for 1 min, then immediately filtered the extract through a Toyo No. 6 filter paper. Chloride and NO_3^- were determined using an ion chromatography (CDD-10A SP, HIC-10A Super; Shimadzu, Kyoto, Japan).

The proline concentration was determined by the method of Bates *et al.* (1972). Samples were ground in a mortar and homogenized in 120 mmol L^{-1} sulfosalicylic acid, then mixed with acid ninhydrin and glacial acetic acid and then boiled at 100°C for 1 h. The absorbance of the organic phase was determined at 520 nm.

TSS was extracted in 80% (v/v) ethanol in a boiling water bath until the extract became colorless and frozen (−20°C) until analysis (Angelov *et al.* (1993). TSS was estimated by the method of McCready *et al.* (1950) using anthrone in concentrated H_2SO_4. Spectrophotometric readings were taken at 630 nm. A standard curve was plotted with 0–200 ppm of glucose.

The experiment was set up as a completely randomized design with both species and five

salinity levels. The effects of species, salinity levels, and their interactions on variables were analyzed by two-way analysis of variance (ANOVA) with SPSS version 10.0J software (SPSS Japan, Inc.). When significant differences were detected at $P < 0.05$, mean values were compared by Duncan's multiple range tests.

RESULTS

Effects of salinity on plant growth

The height and DW of KBG and TF shoots decreased significantly with an increase in the concentration of NaCl (Table 3). Although the length and DW of KBG roots decreased significantly as salinity increased, the changes in TF were not significant. Compared to the control, the root length of TF increased at 100 mmol L^{-1} NaCl. However, the root DW of TF grown in the 100, 150 and 200 mmol L^{-1} NaCl treatments was reduced by 14.5, 21.7 and 26.1%, respectively. Similarly, compared to the control, the root DW of KBG at these salinity levels was reduced by 39.5, 61.8 and 80.3%, respectively.

Although turf quality declined as salinity increased in both species (Table 3), the turf quality of TF was higher during the experimental period. Turf quality of KBG decreased to 3.88 at 100 mmol L^{-1} NaCl, but TF maintained a minimal acceptable quality (6.69) at 100 mmol L^{-1}. Leaf firing in both species increased with an increase in salinity, reaching 56.7% in KBG and 29.5% in TF at 100 mmol L^{-1} NaCl. Leaf firing in KBG was markedly higher than that in TF at the same salinity levels. The results of growth parameters (Table 3) indicated that growth inhibition by NaCl was more severe in KBG than in TF.

Table 3. Effect of salinity on growth parameters in Kentucky bluegrass (KBG) and Tall fescue (TF).

NaCl (mmol L^{-1})	Shoot height (cm)	Root length (cm)	Shoot DW (g $plant^{-1}$)	Root DW (g $plant^{-1}$)	Turf quality	Leaf firing (%)
KBG						
0	32.20 ± 1.05a	31.59 ± 0.65a	2.49 ± 0.13a	0.76 ± 0.04a	8.62 ± 0.08a	13.52 ± 0.11e
50	27.42 ± 0.49b	24.90 ± 0.64b	1.65 ± 0.09b	0.65 ± 0.03b	6.25 ± 0.16b	32.87 ± 1.19d
100	22.40 ± 0.41c	22.61 ± 0.61c	1.25 ± 0.09c	0.46 ± 0.02c	3.88 ± 0.08c	56.71 ± 1.65c
150	20.70 ± 0.46c	19.57 ± 0.33d	0.96 ± 0.04d	0.29 ± 0.004d	2.70 ± 0.12d	74.32 ± 1.95b
200	18.03 ± 0.66d	14.86 ± 0.52e	0.57 ± 0.03e	0.15 ± 0.014e	2.27 ± 0.11e	84.24 ± 1.74a
TF						
0	40.84 ± 0.69a	34.37 ± 0.78a	2.83 ± 0.10a	0.69 ± 0.01a	8.80 ± 0.10a	9.33 ± 0.15e
50	36.26 ± 0.29b	33.90 ± 1.51a	2.20 ± 0.36b	0.67 ± 0.04a	8.13 ± 0.08b	15.51 ± 0.69d
100	30.39 ± 0.83c	39.48 ± 2.63b	1.32 ± 0.07c	0.59 ± 0.01b	6.69 ± 0.26c	29.54 ± 1.38c
150	25.75 ± 1.09d	29.99 ± 0.93a	1.36 ± 0.10c	0.54 ± 0.01b	5.68 ± 0.15d	56.13 ± 1.16b
200	22.88 ± 0.81e	29.47 ± 1.14a	1.14 ± 0.05c	0.51 ± 0.04b	4.32 ± 0.10e	70.28 ± 0.55a

Values in each column are the mean of five replicates ± S.E. Different letters indicate significant differences between means at $p < 0.05$ by analysis of variance.

Ion concentrations in shoots and roots

Na^+ in the shoots of KBG and TF increased as salinity increased (Fig. 1A); that in KBG increased significantly relative to the control. That in TF did not increase above 100 mmol L^{-1} NaCl. Na^+ was 30% to 122% higher in KBG than in TF at 50 to 200 mmol L^{-1} NaCl. K^+ in the shoots of KBG decreased as NaCl increased (Fig. 1B) However, That of TF decreased from 0 to 100 mmol L^{-1} NaCl and increased slightly at 150 and 200 mmol L^{-1} NaCl. K^+ was higher in TF than in KBG at the same salinity. Ca^{2+} and Mg^{2+} in the shoots of KBG and TF decreased as NaCl increased (Figs. 1C, D); Concentrations of these cations did not differ significantly between grasses except at 100 mmol L^{-1} NaCl.

Cl^- in the shoots of KBG increased as salinity increased (Fig. 2A). That of TF did not increase above 50 mmol L^{-1} NaCl (Fig. 2A). Cl^- was higher in KBG than in TF, except at 50 mmol L^{-1} NaCl. NO_3^- in the shoots of KBG decreased as salinity increased, but did not decrease

significantly above 100 mmol L^{-1} NaCl (Fig. 2B). That of TF decreased gradually as salinity increased (Fig. 2B). NO_3^- of shoots was 58% to 111% higher in TF than in KBG at 50 to 200 mmol L^{-1} NaCl.

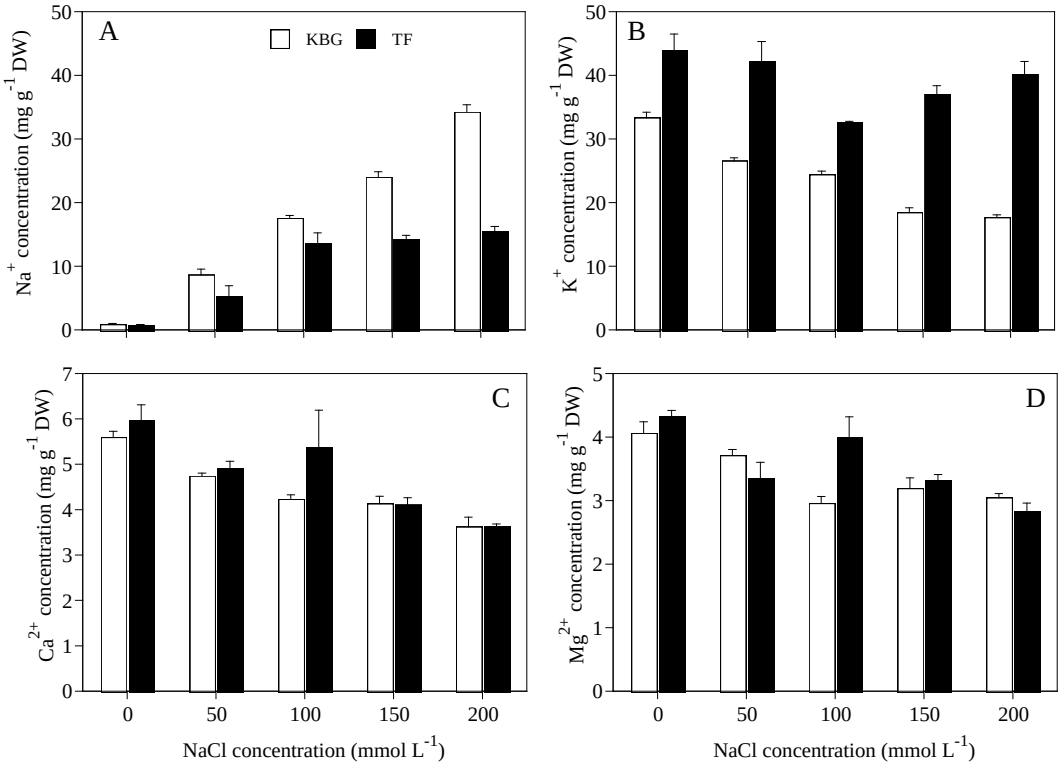

Figure 1 Cation concentrations in the shoots of KBG and TF at different NaCl concentrations. (A) Na^+, (B) K^+, (C) Ca^{2+}, and (D) Mg^{2+}. Bars indicate standard error (n = 3).

Na^+ in the roots of KBG and TF increased significantly as salinity increased, but that in KBG was significantly higher than in TF (Fig. 3A). Na^+ was 36% to 59% higher in KBG than in TF at 50 to 200 mmol L^{-1} NaCl. K^+ in the roots of KBG and TF decreased as salinity increased (Fig. 3B). K^+ was higher in TF than in KBG at all salinities. Ca^{2+} in the roots of KBG and TF did not change significantly under NaCl stress, except in KBG at 200 mmol L^{-1} (Fig. 3C). Mg^{2+} in the roots of KBG under salt stress was less than that in the control (Fig. 3D); however, that in TF increased slightly at NaCl concentrations above 50 mmol L^{-1} (Fig. 3D).

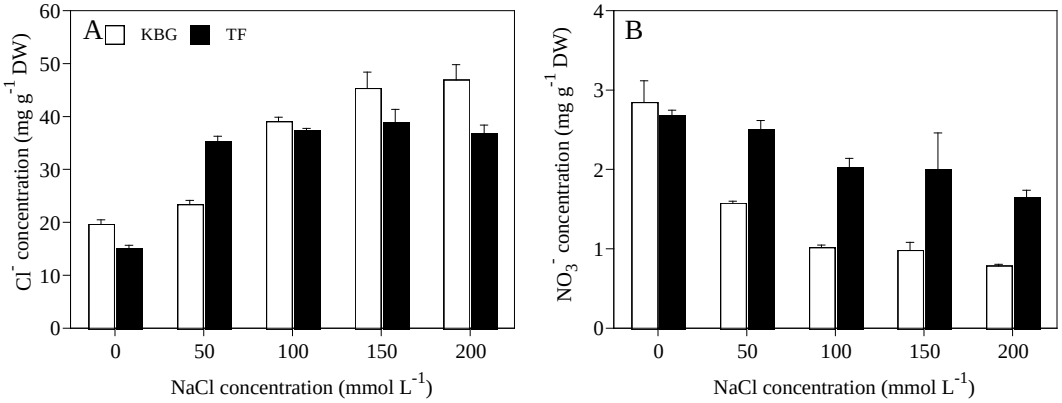

Figure 2 Anion concentrations in the shoots of KBG and TF at different NaCl concentrations. (A) Cl^- and (B) NO_3^-. Bars indicate standard error (n = 3).

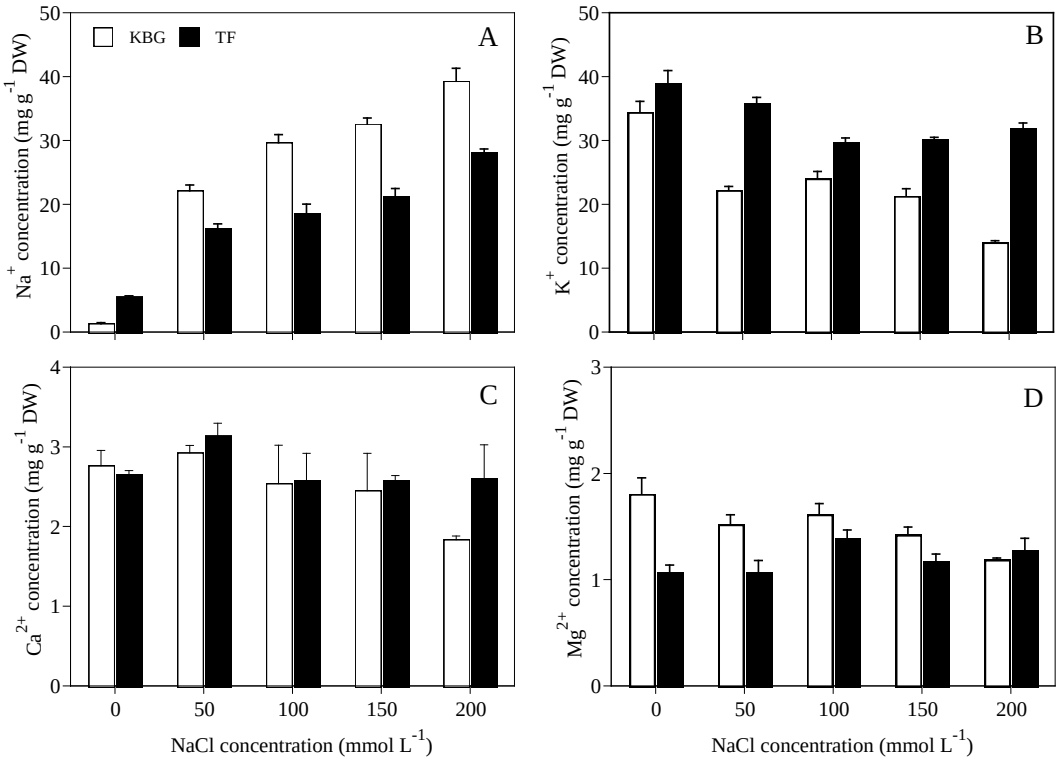

Figure 3 Cation concentrations in the roots of KBG and TF at different NaCl concentrations. (A) Na^+, (B) K^+, (C) Ca^{2+}, and (D) Mg^{2+}. Bars indicate standard error (n = 3).

Cl^- in the roots of KBG and TF increased significantly as salinity increased (Fig. 4A). Cl^- in KBG increased by 117% to 191% and that in TF increased by 39% to 99% at 50 to 200 mmol L^{-1} NaCl. Under salt stress Cl^- was higher in KBG than in TF. NO_3^- in the roots of KBG and TF

decreased significantly as salinity increased (Fig. 4B). The NO_3^- of root was 22% to 63% higher in TF than in KBG at 50 to 200 mmol L^{-1} NaCl.

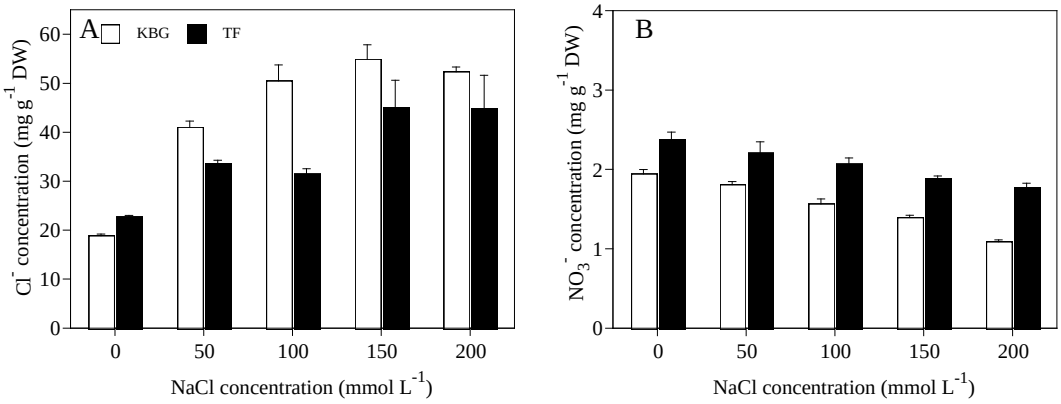

Figure 4 Anion concentrations in the roots of KBG and TF at different NaCl concentrations. (A) Cl^- and (B) NO_3^-. Bars indicate standard error (n = 3).

Na^+/K^+ ratio of shoots and roots and Na^+ and Cl^- ratios between roots and shoots

The Na^+/K^+ ratios in the shoots and roots of KBG increased significantly as salinity increased (Fig. 5). The shoot Na^+/K^+ ratio of TF did not change significantly above 100 mmol L^{-1} NaCl, but the root Na^+/K^+ ratio increased slightly (Fig. 5). Similar to Na^+, the Na^+/K^+ ratios in the shoots and roots of KBG were significantly higher than those of TF at the same salinity.

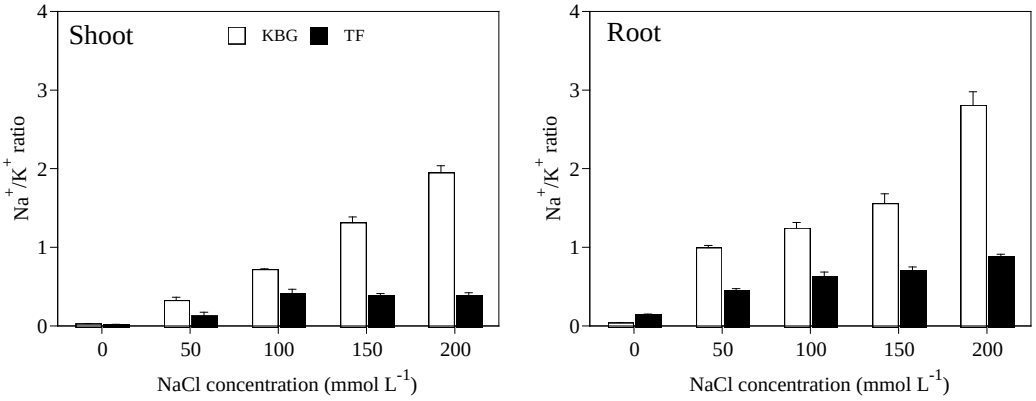

Figure 5 Na^+/K^+ ratio in the shoots and roots of KBG and TF at different NaCl concentrations. Bars indicate standard error (n = 3).

Root Na^+/shoot Na^+ ratio of both species decreased under salinity stress (Fig. 6A). It decreased gradually from 50 to 200 mmol L^{-1} NaCl in KBG. It increased significantly from control to 100 mmol L^{-1} NaCl, and then did not change significantly in TF. The root Na^+/shoot Na^+ ratio of TF was higher than that of KBG except at 100 mmol L^{-1} NaCl. Root Cl^-/shoot Cl^- ratios of KBG increased under salinity treatment, and exhibited the maximum ratio at 200 mmol L^{-1} NaCl. In comparison with control, Root Cl^-/shoot Cl^- ratios of TF decreased under salinity treatment (Fig. 6B). Root Cl^-/shoot Cl^- ratios of KBG were significant higher than those of TF at 50 and 100 mmol L^{-1} NaCl.

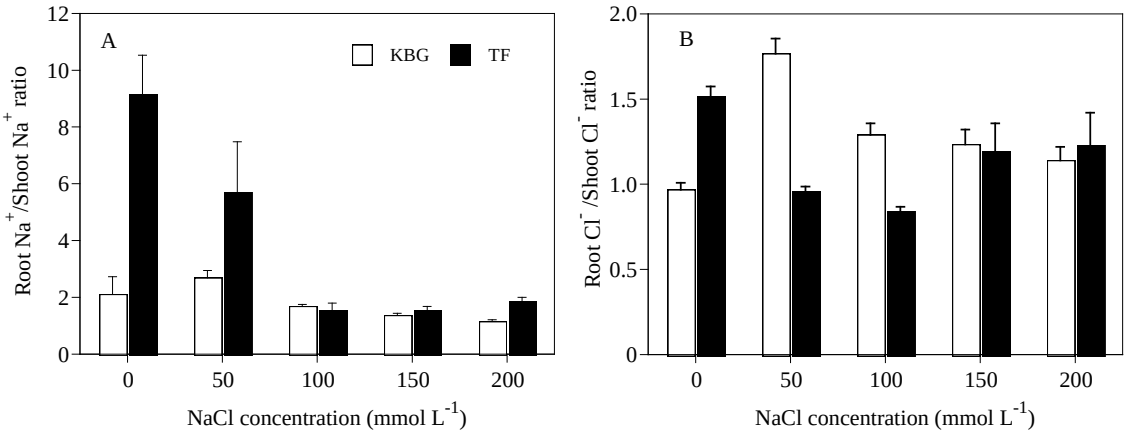

Figure 6 Root Na^+/shoot Na^+ (A) and root Cl^-/shoot Cl^- (B) ratios of KBG and TF at different NaCl concentrations. Bars indicate standard error (n = 3).

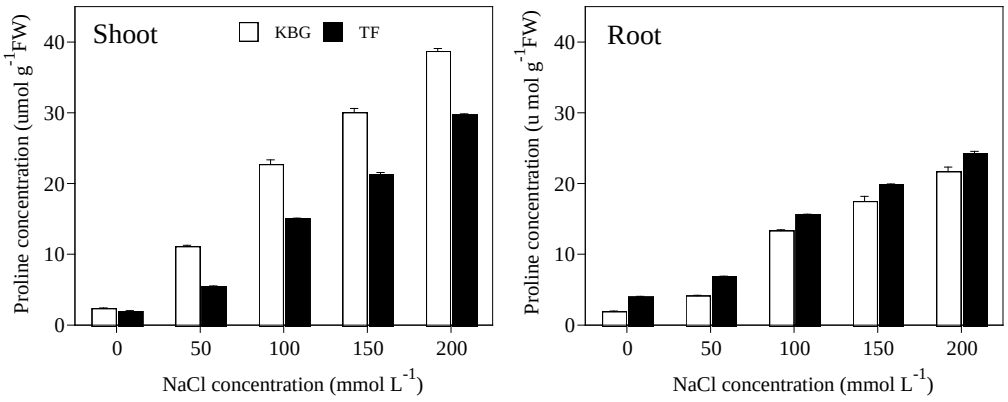

Figure 7 Proline concentrations in the shoots and roots of KBG and TF at different NaCl concentrations. Bars indicate standard error (n = 3).

Proline and TSS concentrations of shoots and roots

The proline concentrations in shoots and roots of both species increased significantly as salinity increased (Fig. 7). That in the shoots was significantly higher in KBG than in TF at the same salinity, but that in the roots was significantly higher in TF than in KBG.

TSS in the shoots and roots of KBG decreased gradually as salinity increased (Fig. 8). However, TSS in the shoots of TF increased as salinity increased to 100 mmol L^{-1} NaCl, but then decreased at higher salinities. TSS in the roots of TF increased significantly as salinity increased. TSS of shoots was 42% to 97% higher in TF than in KBG, and that of roots was 26% to 311% higher in TF than in KBG, at 50 to 200 mmol L^{-1} NaCl (Fig. 8).

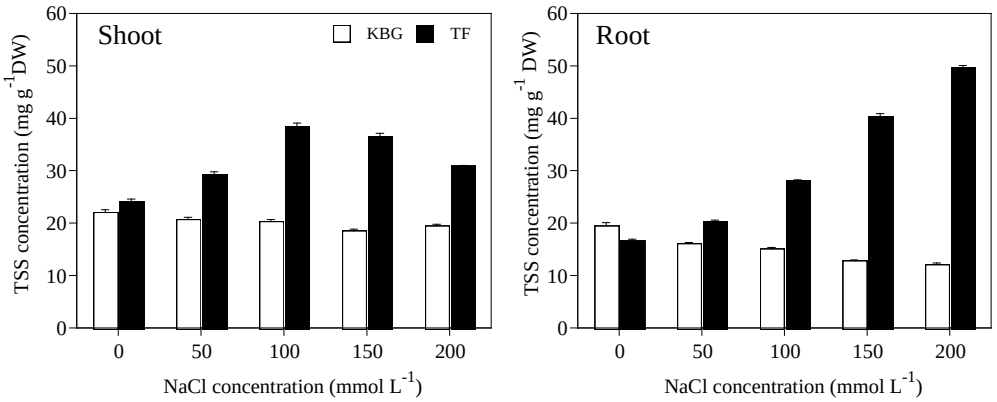

Figure 8 TSS concentrations in the shoots and roots of KBG and TF at different NaCl concentrations. Bars indicate standard error (n = 3).

Estimated contributions of inorganic ions and compatible solutes to osmotic potential

There was no difference in Ψ_s between the both species in control (Table 4 and Table 5). The 50, 100, 150 and 200 mmol L^{-1} NaCl treatments decreased the Ψ_s of the nutrient solution from −0.06 to −0.24, −0.43, −0.69 and −0.84 MPa, respectively. This resulted in a decline in root and shoot Ψ_s in both KBG and TF. Ψ_s became more negative in shoots of KBG and TF as salinity increased, and decreased more significantly in TF than in KBG (Table 4). The estimated contribution of shoot Na^+ to Ψ_s (C_{Na}) in KBG increased significantly as salinity increased; That

of TF remained unchanged from 100 to 200 mmol L^{-1} NaCl (Table 4). C_{Na} of KBG was significantly higher than that of TF. The estimated contributions of shoot Cl^- to Ψ_s (C_{Cl}) in KBG changed significantly from 100 to 200 mmol L^{-1} NaCl; That in TF under salt stress was significantly higher than in the controls and was not significant change 100 to 200 mmol L^{-1} NaCl (Table 4). The estimated contribution of shoot K^+ to Ψ_s (C_K) decreased significantly in KBG than TF as the concentration of NaCl increased (Table 4). The estimated contribution of shoot TSS (C_{TSS}) to Ψ_s in KBG increased at 150 and 200 mmol L^{-1} NaCl, but did not change significantly in TF (Table 4). However, C_{TSS} was significantly higher in TF than in KBG at the same salinity. The estimated contribution of shoot proline to Ψ_s (C_{Pro}) increased in both species as salinity increased (Table 4); it was lower than those of the other solutes.

Table 4. Effects of salinity on Ψ_s and the estimated contributions of Na^+ (C_{Na}), K^+ (C_K), Cl^- (C_{Cl}), TSS (C_{TSS}) and proline (C_{Pro}) to Ψ_s in shoots of Kentucky bluegrass (KBG) and Tall fescue (TF).

NaCl (mmol L^{-1})	Ψ_s (MPa)	C_{Na} (%)	C_{Cl} (%)	C_K (%)	C_{TSS} (%)	C_{Pro} (%)
KBG						
0	–1.14 ± 0.01a	1.90 ± 0.5d	27.94 ± 1.5bc	43.13 ± 1.1a	6.18 ± 0.1b	0.62 ± 0.02d
50	–1.19 ± 0.04a	15.57 ± 1.7c	26.30 ± 1.2bc	27.96 ± 1.6b	4.92 ± 0.1c	2.93 ± 0.06c
100	–1.57 ± 0.07b	23.29 ± 1.4b	33.41 ± 1.9a	19.05 ± 1.3c	5.62 ± 0.3bc	4.52 ± 0.24b
150	–1.75 ± 0.06c	23.84 ± 1.1b	29.92 ± 1.3ab	10.70 ± 0.7d	7.85 ± 0.4a	4.84 ± 0.31b
200	–1.82 ± 0.09c	27.82 ± 1.4a	24.54 ± 1.7c	8.73 ± 0.5d	7.87 ± 0.3a	6.27 ± 0.19a
TF						
0	–1.07 ± 0.04a	1.08 ± 0.3c	14.72 ± 1.0c	40.55 ± 1.3a	23.73 ± 0.7a	0.53 ± 0.01d
50	–1.62 ± 0.08b	5.28 ± 2.1b	28.34 ± 1.6a	32.47 ± 3.0b	18.20 ± 1.2b	0.97 ± 0.06d
100	–1.80 ± 0.08b	13.08 ± 1.9a	22.48 ± 0.9b	17.92 ± 0.9c	16.70 ± 0.9b	2.43 ± 0.11c
150	–2.11 ± 0.08c	12.56 ± 0.6a	22.81 ± 1.5b	19.85 ± 0.8c	17.42 ± 1.0b	3.11 ± 0.21b
200	–2.26 ± 0.06c	13.27 ± 0.7a	21.34 ± 0.9b	20.50 ± 1.6c	18.76 ± 0.9b	3.88 ± 0.20a

Values in each column are means of five replicates ± SE. Different letters indicate significant differences between means at $P = 0.05$ according to Duncan's multiple range tests.

Similar to Ψ_s in shoots, Ψ_s in roots of KBG and TF became more negative as salinity increased, and the difference between KBG and TF was large at 200 mmol L^{-1} NaCl (Table 5). The estimated contribution of root Na^+ to Ψ_s (C_{Na}) in KBG and TF increased significantly as salinity increased (Table 5). C_{Na} was higher in KBG than in TF. The estimated contribution of root Cl^- to Ψ_s (C_{Cl}) in KBG increased with salinity treatment compared with the control. That in TF also increased at 50, 150 and 200 mmol L^{-1} NaCl (Table 5). However, there was no significant change in estimated contribution of root Cl^- to Ψ_s (C_{Cl}) between both species under

Table 5. Effects of salinity on Ψ_s and the estimated contributions of Na^+ (C_{Na}), K^+ (C_K), Cl^- (C_{Cl}), TSS (C_{TSS}) , and proline (C_{Pro}) to Ψ_s in roots of Kentucky bluegrass (KBG) and Tall fescue (TF).

NaCl (mmol L^{-1})	Ψ_s (MPa)	C_{Na} (%)	C_{Cl} (%)	C_K (%)	C_{TSS} (%)	C_{Pro} (%)
KBG						
0	–0.52 ± 0.01a	2.75 ± 0.2c	23.99 ± 0.8c	38.67 ± 2.8a	4.67 ± 0.1d	0.95 ± 0.05c
50	–0.75 ± 0.01b	25.31 ± 0.8b	29.99 ± 0.6a	14.76 ± 0.4b	8.95 ± 0.3c	1.46 ± 0.03c
100	–0.83 ± 0.01c	23.48 ± 1.2b	25.47 ± 1.7bc	11.35 ± 0.7bc	9.13 ± 0.2c	4.50 ± 0.07b
150	–0.92 ± 0.02d	25.05 ± 1.3b	27.98 ± 1.1ab	9.89 ± 0.7cd	10.11 ± 0.4b	5.11 ± 0.40b
200	–0.88 ± 0.02d	29.03 ± 1.8a	25.56 ± 0.9bc	6.24 ± 0.2d	11.22 ± 0.4a	6.53 ± 0.37a
TF						
0	–0.46 ± 0.03a	8.21 ± 0.3d	21.89 ± 1.2a	34.18 ± 3.0a	14.90 ± 0.8a	2.11 ± 0.15c
50	–0.73 ± 0.03b	17.25 ± 1.0c	23.20 ± 1.0a	22.73 ± 1.4b	15.21 ± 0.9a	2.51 ± 0.17c
100	–0.78 ± 0.04b	18.82 ± 1.2bc	20.38 ± 0.8a	17.35 ± 0.9c	16.69 ± 1.3a	5.39 ± 0.42ab
150	–0.94 ± 0.02c	21.22 ± 1.4ab	27.62 ± 3.9a	17.38 ± 0.3c	15.76 ± 0.4a	5.68 ± 0.12a
200	–1.40 ± 0.02d	21.99 ± 0.7ab	24.75 ± 3.7a	14.70 ± 0.6c	12.13 ± 0.4b	4.71 ± 0.17b

Values in each column are means of five replicates ± SE. Different letters indicate significant differences between means at P = 0.05 according to Duncan's multiple range tests.

NaCl treatments. The estimated contribution of K^+ to Ψ_s (C_K) in roots was similar to that in shoots: C_K decreased in KBG and TF as salinity increased, and more significant reduction was observed in KBG (Table 5). The estimated contribution of root TSS to Ψ_s (C_{TSS}) in KBG increased as salinity increased (Table 5); that in TF did not change significantly, except at 200

mmol L^{-1} NaCl (Table 5). The estimated contribution of root TSS to Ψ_s (C_{TSS}) of TF was significantly higher than that of KBG at the same salinity. The estimated contribution of root proline to Ψ_s (C_{Pro}) increased in KBG and TF as salinity increased (Table 5).

DISCUSSION

Plant species differ greatly in their growth response to salinity. It is important to study the physiological characterization of plants to saline conditions to define relative salinity tolerance. Parameters, such as shoot growth, root mass, root length, and turf quality are well suited for examining salinity tolerance in turfgrass species (Alshammary *et al.*, 2004). In this study, the growth parameters of root length, root DW, leaf firing and turf quality revealed that TF was more salt tolerant than KBG, corroborating the findings of Alshammary *et al.* (2004) and Marcum (2008). Shoot growth in both species decreased significantly with increasing salinity (Table 3). Marcum (2008) reported that the shoot growth of salt-sensitive to moderately-tolerant turfgrass species declined linearly with increased salinity stress. However, TF exhibited high root length and root DW, which results in increased root/shoot ratios under salt stress. The increase in root/shoot ratio of TF can maintain an optimal water balance between the root water absorption and shoot transpirational area. An inability to adjust the root/shoot ratio in KBG may explain the relatively poor salinity tolerance in this species.

The suppression of turfgrass growth due to salinity stress was manifested as a decrease in turf quality and an increase in leaf firing. Both turf quality and leaf firing were significantly affected with increasing salinity. Compared to their respective controls, the decrease in turf quality and

increase in leaf firing under salt stress was larger for KBG than for TF, indicating that TF was more tolerant to salinity than KBG. Our results show that KBG and TF retained a minimal acceptable quality in the 50 and 100 mmol L^{-1} NaCl, respectively.

Na^+ and Cl^- are the dominant toxic ions in saline soil. Higher Na^+, often in conjunction with Cl^- results in specific ion toxicity and growth inhibition (Ashraf and Harris 2004; Zhu *et al.* 2008). High Na^+ can replace Ca^{2+} and decline cell membrane integrity of root for water and nutrient uptake. Accumulation of Na^+ in leaf leads to dehydration, reduced turgor and death of cells; Accumulation of Cl^- in leaf can lead to leaf burn and desiccation in sensitive plants (Carrow *et al.* 2001). In the present study, KBG accumulated higher Na^+ and Cl^- than TF in both shoots and roots. This result indicates the injurious effect of ion toxicity for KBG was greater than that for TF.

High salinity can affect essential cation uptake and nutrient balance. At the level of individual cells, one of the most damaging consequences of salt stress is an influx of Na^+ and a decrease in K^+ in plant tissues (Li *et al.* 2010). K^+ is often considered as second in importance behind N as a nutrient in turfgrass. K^+ strongly influences turfgrass tolerance to drought, low temperature, high temperature, wear and salinity stresses (Carrow *et al.* 2001). In comparison to the control, the concentrations of K^+ in the shoots and roots of both species decreased with salt stress. Those in TF were significantly higher than in KBG at the same salinity (Figs. 1B, 3B). K^+ was the most important cation related to the shoot and root growth of many plants subjected to salt stress (Marschner 1995; Grattan and Grieve 1999; Lee *et al.* 2007). The higher K^+ concentration in TF might be one of the reasons for the better growth under salt stress.

The Ca^{2+} and Mg^{2+} concentrations in the shoots of both species and Mg^{2+} in the roots of

KBG decreased as salinity increased (Figs. 1C, 1D, 3C), and the decreased extent of Ca^{2+} and Mg^{2+} concentrations in the roots were higher in KBG than that in TF. The decreases were probably due to interactive substitution with Na^{+} (El-Hendawy *et al.* 2005). Saline induced Ca^{2+} deficiency may reduce certain salinity tolerance such as ion exclusion and selective transport. Ca^{2+} improves the tolerance of higher plants to salt stress, and its availability plays a major role in counteracting salt stress (Breckle 2002, Carrow *et al.* 2001). However, we found no significant difference in Ca^{2+} concentration in shoots between KBG and TF under salinity stress. Mg^{2+} deficiency is relatively common in turfgrass grown in soils naturally when high Na^{+} is present. A high external Na^{+} concentration will easily displace Mg^{2+} and decrease Mg^{2+} uptake (Carrow *et al.* 2001). However, Mg^{2+} in TF roots did not decrease under salt stress (Fig. 3D). It should be further investigated whether Mg^{2+} accumulation is a special adaptation of TF under salt stress.

Under saline condition, Cl^{-} competes with NO_3^{-} and depresses its uptake, which may cause the ion imbalance (Hu *et al.* 2005). N is usually the most growth-limiting nutrient for plants. Thus, the great decrease in the NO_3^{-} concentration of KBG may reduce its growth significantly. NO_3^{-} in the shoots and roots of TF was higher than that in KBG, indicating that effects of salinity on nitrate uptake vary considerably with species. TF maintains more healthy growth and stress tolerance by active NO_3^{-} accumulation under salt stress.

High salinity can affect essential ion uptake and nutrient balance. Compared to TF, KBG accumulated higher Na^{+} and Cl^{-} concentrations in shoots and roots, which reduced K^{+}, Ca^{2+}, Mg^{2+}, and NO_3^{-} concentrations greatly. The Na^{+} and Cl^{-} toxicity and nutrient imbalance was more significant in KBG than in TF under salt stress. The difference in concentrations of K^{+} and

NO_3^- between KBG and TF appeared to be related to salinity tolerance. These results would be important information for managing this species in salt-affected environments. When turfgrasses grown on salt-affected soils or irrigated with recycled wastewater, the increase in K and N fertilizer properly may improve plant growth.

The significant differences in K^+ concentration in both species were observed with apparent relationship to salinity tolerance (Figs. 1B, 3B). Comparisons between KBG and TF exhibited some difference in Na^+/K^+ ratios in the shoots and roots with increasing salinity. The Na^+/K^+ ratios in the shoots and roots of KBG increased significantly as salinity increased. However, those of TF were significantly lower than those of KBG under salt stress (Fig. 5). The Na^+/K^+ ratio of TF was higher in the roots than in the shoots, and did not reach 1 in each organ. The capacity of plants to maintain a low cytosolic Na^+/K^+ ratio is likely to be one of the key determinants of salt tolerance (Maathuis and Amtmann 1999; Yeo 1998). This result indicates that TF had a better selectivity for K^+ over Na^+ than KBG via roots and transporting K^+ from roots to shoots under salt stress.

Salinity tolerance is associated with the capacity to limit uptake and transport of saline ions from the root zone to aerial parts (Greenway and Munns 1980). In both species, Na^+ concentrations were lower in shoots than in roots (Figs. 1A, 3A). Thus, the roots play an important role in limiting the transport of Na^+ to the shoots of both species. However, TF had a lower Na^+ concentration in the roots and a higher root Na^+/shoot Na^+ ratio compared with KBG (Fig. 3A, 6). This result indicates a strong ability of TF to limit Na^+ uptake by roots and to prevent Na^+ transport from roots to shoots.

Osmotic adjustment is one of the main strategies by which plants ensure water uptake during

salinity or drought stress conditions (Greenway and Munns 1980). In TF, shoot and root osmotic potential changed significantly as salinity increased (Tables 4, 5). TF was able to maintain stable gradient of Ψ_s between shoots, roots and saline nutrient solution, suggesting that osmotic adjustment is an important mechanism in relation to TF's better salinity tolerance. Na^+, Cl^-, and K^+ were the principal inorganic ions for osmotic adjustment in both species under salt stress (Tables 4, 5). However, their contributions to the total measured osmotic potential differed with salinity treatment and grass species. Under salt stress, Na^+ and Cl^- made a larger contribution to osmotic potential in KBG than in TF. The availability of Na^+ and Cl^- as cheap osmoregulator is generally beneficial. However, excessive accumulation of Na^+ and Cl^- results in ion toxicity and growth inhibition. The estimated contribution of K^+ to Ψ_s (C_K) in KBG decreased significantly as salinity increased. However, TF had a higher and stable C_K from 100 to 200 mmol L^{-1} NaCl. K^+ has been reported to be involved in activation of several enzymes, membrane transport, maintenance of cystolic osmotic potential, and maintenance of osmotic potential in vacuoles (Marschner 1995). These results suggest that TF has a strong mechanism for active uptake of K^+ as an osmoregulator for osmotic adjustment.

Osmotic potential of the cytoplasm is maintained by the accumulation of organic solutes to osmotically balance Na^+ and Cl^- in the vacuole. In this study, TSS plays an important role in balancing osmotic potential under salt stress. C_{TSS} in KBG decreased as salinity increased, but that in TF was significantly higher at the same salinity (Tables 4, 5). These results suggest that TF, but not KBG, can efficiently mitigate NaCl stress by accumulating TSS for osmotic adjustment. Sugar accumulation is strongly correlated with salt tolerance (Streeter *et al.* 2001; Taji *et al.* 2002). More TSS was accumulated in TF than in KBG at the same salinity (Fig. 8),

indicating the importance of TSS in salinity tolerance of TF.

The accumulation of proline in response to abiotic stresses contributes to osmotic adjustment in the cytoplasm (Ashraf and Foolad 2007). The proline concentrations in shoots and roots of both species increased significantly as salinity increased (Fig. 7). However, it contributed less than 7% of the total osmotic potential (Tables 4, 5). Thus, neither species could accumulate enough proline to mitigate NaCl stress by osmotic adjustment. The accumulation of proline by many plant species under salt stress has been correlated with salinity tolerance, and the concentration of proline is generally higher in stress-tolerant than in stress-sensitive plants (Ashraf and Foolad 2007). However, this correlation is controversial: for example, under salt stress, proline levels of the salt sensitive KBG were similar to those of salt tolerant alkalinegrass (Torello and Rice 1986); The accumulation of proline in rice and KBG coincided with a sharp increase in leaf burn (Lutts *et al.* 1999; Qian *et al.* 2001), indicating a symptom of salt injury rather than salt tolerance. In this study, the accumulated proline was very low and accumulation pattern was not related to salinity tolerance suggested that proline accumulation was a reaction to salt stress and not a salinity tolerance mechanisms in KBG and TF.

The percentages of individual estimated contributions to osmotic potential under salt stress show that KBG achieved osmotic adjustment more by inorganic ions than through organic osmolytes. The high reliance on inorganic ions for osmotic adjustment is vital for water uptake under saline environment. However, osmotic adjustment of TF was achieved by both inorganic ion and organic osmolytes based on percentage contribution to Ψ_s, respectively. TF maintained higher concentrations of K^+ and TSS in the cytoplasm to achieve cytoplasmic osmotic adjustment, which may explain its better salinity tolerance than KBG.

SUMMARY

In this study, we investigated changes of growth, ionic concentrations and compatible solutes in KBG (a salt-sensitive species) and TF (a moderately salt-tolerant species) in response to elevated NaCl concentration. TF exhibited better root growth and turf quality than KBG under salinity stress. Na^+ and Cl^- concentrations in shoots and roots increased with increasing salinity in both turfgrasses. KBG accumulated more Na^+ and Cl^- under NaCl stress than did TF. NaCl stress induced more significantly mineral nutrient imbalances in KBG than in TF. The concentrations of K^+, Ca^{2+}, Mg^{2+} and NO_3^- in TF were much less affected than those in KBG. TSS concentration of TF was significantly higher than that of KBG under elevated NaCl concentration. These results indicate that the different physiological responses of KBG and TF to salt stress were highly related to ionic distribution and the accumulation of compatible osmolytes. TF had a high salt tolerance due to maintenance of higher root growth, restricted uptake of Na^+ and Cl^- in conjunction with maintenance of higher K^+ in shoots and roots and the accumulation of enough total soluble sugars to make osmotic adjustment.

CHAPTER 3

Comparative lipid peroxidation and antioxidative enzymes of Kentucky bluegrass and Tall fescue under salinity stress

High salt concentrations in soil cause ionic stress, hyperosmotic stress and secondary stresses such as oxidative stress by increasing reactive oxygen species (ROS) including superoxide radicals ($O^{\cdot}_2^-$), hydrogen peroxides (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$) (Botella *et al.* 2005). To mitigate the oxidative damage induced by ROS, plants employ a variety of enzymatic and non-enzymatic antioxidant defenses. Enzymatic antioxidant systems typically consist of several antioxidant enzymes that participate in the detoxification of ROS. In this chapter, the change in lipid peroxidation in terms of malondialdehyde (MDA) concentration, and the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxide (APX) and glutathione reductase (GR) in the shoots and roots of Kentucky bluegrass (KBG) and Tall fescue (TF) under salinity stress were investigated.

MATERIALS AND METHODS

The plant materials and salt stress treatments were the same as those described in the Chapter 2. After harvested, fresh shoot and root samples were collected from both species for enzyme analysis. Samples were frozen in liquid nitrogen immediately after harvesting and stored at −70°C until enzyme assays were performed. For protein and enzymes extractions, samples (0.15 g of shoot and 0.2 g of root) were ground to a powder using a mortar and pestle pre-cooled with

liquid nitrogen and homogenized in 50 mmol L^{-1} potassium phosphate buffer (pH 7.8) containing 1 mmol L^{-1} EDTA, 1 mmol L^{-1} ascorbate and 2% (w/v) polyvinylpolypyrrolidone. Homogenates were then centrifuged at 20,000 *g* for 30 min at 4°C and supernatants were divided into two parts. One part was used directly for CAT, APX and GR assays, while the other was transferred to a dialysis membrane (Wako, Osaka, Japan) and dialyzed at 4°C in 10 mmol L^{-1} potassium phosphate buffer (pH 7.8) for 12 h (buffer was changed at 3 h intervals). After centrifuging the dialyzed solution at 13,000 *g* for 20 min, the supernatant was used for the SOD assay and total protein determination.

Lipid peroxidation was determined in terms of MDA concentration using the thiobarbituric acid (TBA) method described by Heath and Packer (1968). The absorbance of the supernatant was read at 532 nm. The measurements were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. MDA concentration was calculated using an extinction coefficient of 155 mmol L^{-1} cm^{-1}.

SOD was assayed according to Tanaka and Sugahara (1980). The reaction was initiated by the addition of xanthine oxidase and the absorbance was measured at 550 nm. One unit of SOD was defined as the amount of enzyme that inhibits the rate of cytochrome c reduction by 50%.

CAT activity was measured using the method of Aebi (1984) with slight modifications. CAT activity was calculated using an extinction coefficient of 0.04 mmol L^{-1} cm^{-1} and one unit of CAT activity was defined as the amount of enzyme required to decompose 1 μmol of H_2O_2 per minute.

APX activity was determined by measuring the decrease in ascorbate oxidation at 290 nm (Nakano and Asada 1981). The concentration of oxidized ascorbate was calculated using an

extinction coefficient of 2.8 mmol L^{-1} cm^{-1} and one unit of enzyme was defined as 1 μmol ascorbate oxidized per minute.

GR activity was assayed by measuring the decrease in absorbance at 340 nm (Tanaka *et al.* 1988). GR activity was calculated using an extinction coefficient of 6.2 mmol L^{-1} cm^{-1} for NADPH and one unit of enzyme was defined as the amount of enzyme required to oxidize 1 μmol of NADPH per minute.

The activities of the enzymes were expressed as U mg^{-1} protein. Total soluble protein content was determined using bovine serum albumin as a standard (Bradford 1976).

All data were evaluated by one-way analysis of variance (ANOVA) using SPSS version 10.0J software for Windows (SPSS, SPSS Japan Inc., Japan). Comparison between means was performed using Duncan's multiple range test at a 5% level.

RESULTS

Lipid peroxidation

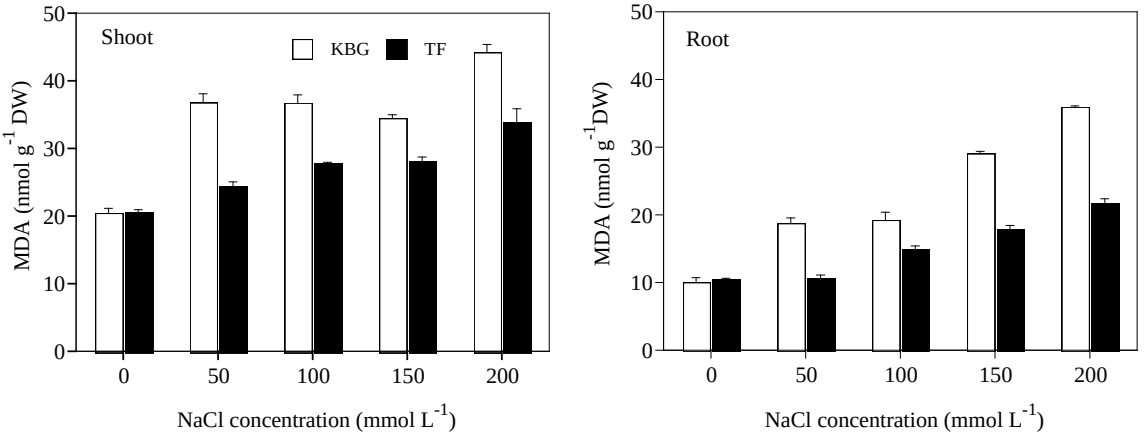

Figure 9 MDA cocentration in the shoots and roots of Kentucky bluegrass (KBG) and Tall fescue (TF) under different NaCl treatments. Bars indicate the standard error (n = 3).

Lipid peroxidation of both turfgrass species was determined in terms of MDA concentration (Fig.9). The MDA concentration in shoots and roots of both species increased significantly with

increasing salinity. The accumulation of MDA in the shoots and roots of KBG was higher than that observed in TF, indicating that the increase in lipid peroxidation induced by NaCl stress was higher in KBG than in TF.

Activities of antioxidative enzymes

The SOD activities in the shoots of both turfgrass species and the activities in the roots of TF increased under NaCl stress, whereas SOD activities in the roots of KBG showed no significant change with increasing salinity (Fig. 10). The SOD activities in the shoots and roots of TF were higher than those in KBG, especially at higher salinities. The increase in SOD activities in TF in response to NaCl treatment was larger than that observed in KBG. Compared to the control, the SOD activities at 200 mmol L^{-1} NaCl increased by 114.6% for shoots and 87.6% for roots of TF, while SOD activities in the shoots and roots of KBG increased by 21.4% and 11.0%, respectively.

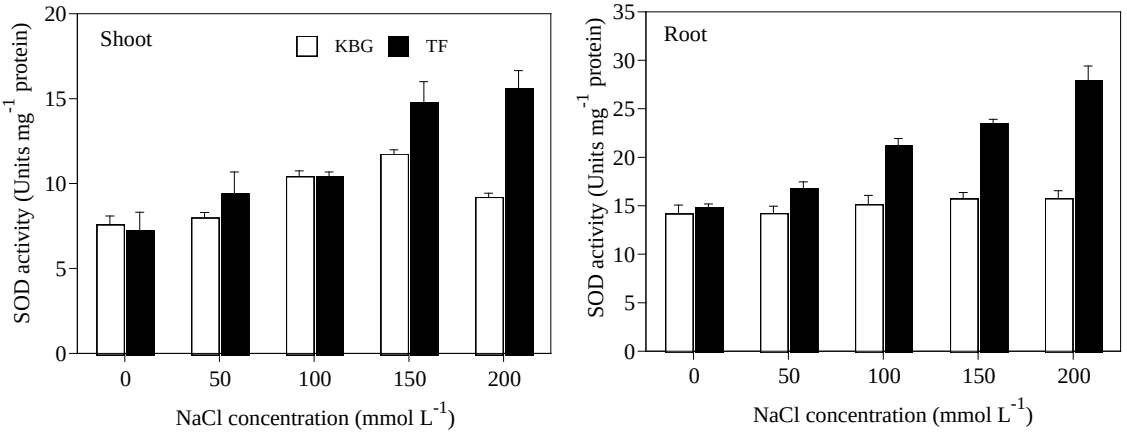

Figure 10 SOD activities in the shoots and roots of Kentucky bluegrass (KBG) and Tall fescue (TF) under different NaCl treatments. Bars indicate the standard error (n = 3).

CAT activities in the shoots and roots of KBG were reduced with an increase in NaCl

concentrations (Fig. 11). The CAT activities in the shoots of TF were higher in control than in salinity conditions; however, CAT activities did not change between 50 and 200 mmol L^{-1} NaCl (Fig. 11). The CAT activities in the roots of TF were higher in salt-stressed plants than in control plants. The CAT activities in the shoots and roots of KBG were significantly lower than those in TF for all salinity treatments.

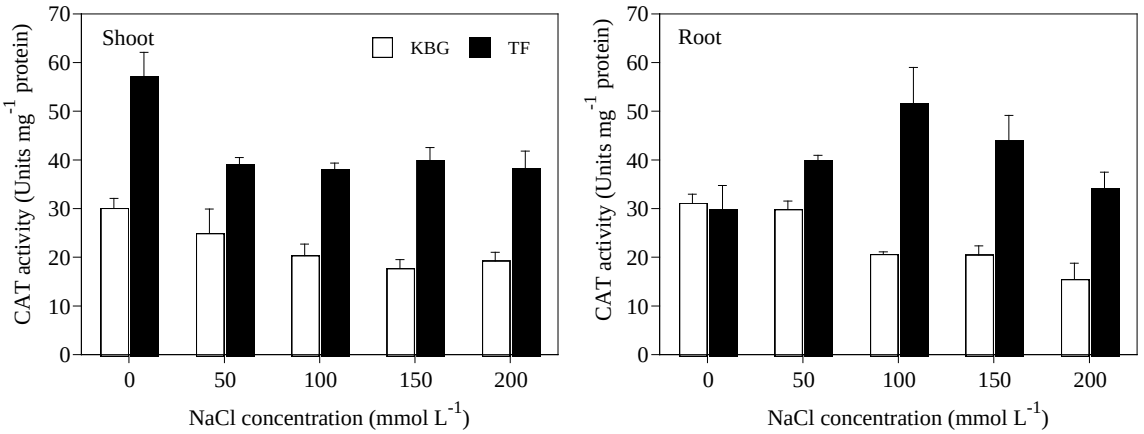

Figure 11 CAT activities in the shoots and roots of Kentucky bluegrass (KBG) and Tall fescue (TF) under different NaCl treatments. Bars indicate the standard error (n = 3).

APX activities in the shoots of KBG did not change significantly, and decreased with increasing salinity in roots of KBG (Fig. 12). At 200 mmol L^{-1} NaCl, root CAT activities in KBG decreased to 56% of the control. Activities of APX in the shoots of TF did not show a significant change under salt stress. However, the activities of APX in the roots of TF were higher under conditions of salt treatment compared to those of the control. The APX activities of both shoots and roots were significantly lower for KBG than for TF under salt stress (Fig. 12). NaCl affected APX activities in the roots, but not in the shoots.

The activities of GR in the shoots of KBG and TF were decreased by 50 mmol L^{-1} NaCl (Fig. 13). However, there was no significant difference in the GR activities of plants subjected to 100

mmol L^{-1} to 200 mmol L^{-1} NaCl in both turfgrass species. GR activity in TF roots was highest at

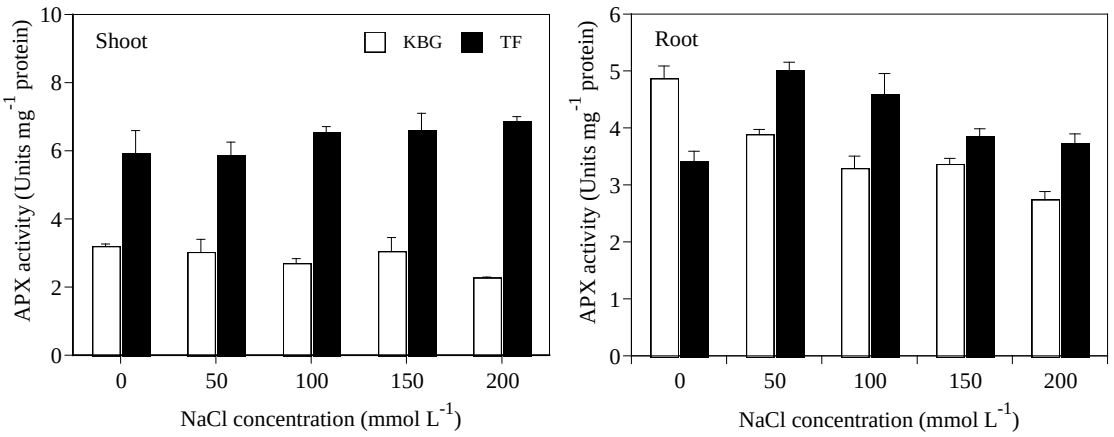

Figure 12 APX activities in the shoots and roots of Kentucky bluegrass (KBG) and Tall fescue (TF) under different NaCl treatments. Bars indicate the standard error (n = 3).

50 mmol L^{-1} NaCl and there was no significant difference in the 100 to 200 mmol L^{-1} NaCl treatments. Root GR activities in KBG increased gradually with an increase in salinity and were higher than the GR activities of TF at 100, 150 and 200 mmol L^{-1} NaCl (Fig. 13); However, there was no effect of turfgrass species on GR activities in the roots.

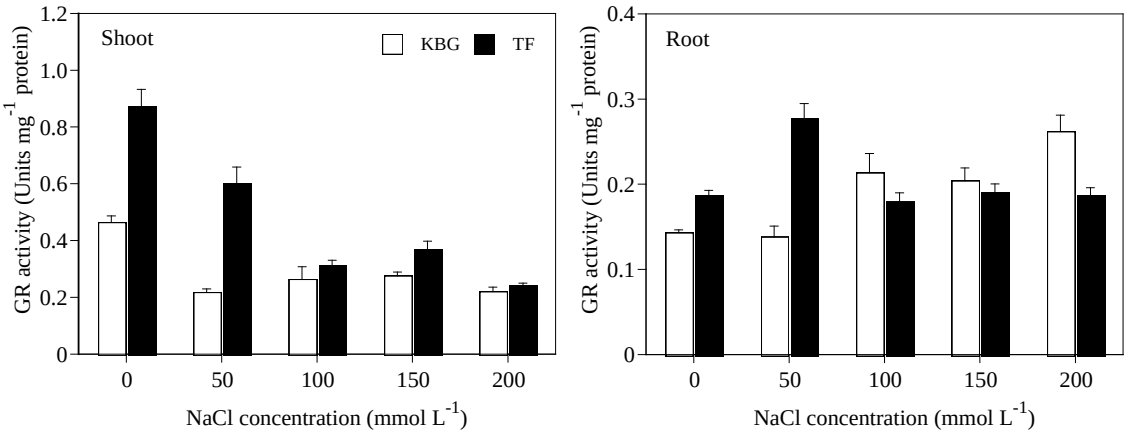

Figure 13 GR activities in the shoots and roots of Kentucky bluegrass (KBG) and Tall fescue (TF) under different NaCl treatments. Bars indicate the standard error (n = 3).

DISCUSSION

To keep ROS under control, plants have evolved an efficient antioxidant defense system. In

the present study, the changes of MDA concentration, SOD, CAT, APX and GR activities suggest that oxidative stress is an important component of salt stress in KBG and TF.

The MDA concentration increased with increasing salt stress in the shoots and roots of both turfgrass species (Fig. 9), indicating that cell membranes were damaged in both turfgrass species. Growth inhibition, decreased turf quality, and increased leaf firing are associated with cell membrane damage due to salinity-induced lipid peroxidation (Huang *et al.* 2001). The MDA concentration in TF was significantly lower than that in KBG under salt stress, indicating that less lipid peroxidation occurred in response to the higher activities of SOD, CAT, and APX observed in TF. In KBG, a significant increase in MDA levels appeared to be correlated with a decrease in the activities of CAT and APX under salt stress, with SOD activity in the root remaining unchanged. The higher MDA concentration in KBG implies that the oxidative damage was severer in KBG and that the antioxidant defense mechanisms of KBG were less effective than those of TF. Similar evidence of lipid peroxidation and antioxidant activity has been reported by other researchers (Shalata and Tal 1998; Amor *et al.* 2006; Pérez-López *et al.* 2009; Seckin *et al.* 2010).

In the antioxidative systems of plants, SOD plays a central role in the enzymatic defense system by catalyzing the dismutation of $O^{\cdot}{}_2^-$ to H_2O_2 and oxygen (Bowler *et al.* 1992). In our study, SOD activities increased significantly under conditions of salt stress in the shoots of both species and in the roots of TF. The increase in SOD activity in the shoots and roots of TF was larger than that observed in KBG, implying that TF has better $O^{\cdot}{}_2^-$ radical scavenging ability.

In addition to being an important agent of cellular toxicity, the product of SOD activity, H_2O_2, is also an important signal molecule between environmental stress and adaptive response (Foyer

and Noctor 2003; Seckin *et al.* 2010). In plants, CAT and APX are considered to be the most important enzymes regulating intercellular levels of H_2O_2. CAT is primarily localized in peroxisomes and glyoxysomes where it breaks down H_2O_2 into water and oxygen. Amor *et al.* (2007) reported that H_2O_2 accumulation under salinity stress was related to a decrease in CAT activity. Our data showed that CAT activity decreased in both the shoots and roots of KBG under salt stress. However, CAT activity remained unchanged in the shoots and roots of TF, indicating that the scavenging of H_2O_2 by TF is more effective than by KBG.

Like CAT, APX plays a key role in protecting the plant against oxidative stress by scavenging H_2O_2 in the chloroplasts, cytosol, mitochondria and peroxisome of cells (Asada 2006). In the present study, TF exhibited higher APX activities than KBG under salt stress. We also found that the increase in APX activity was accompanied by higher CAT activities in TF, implying that TF has a more effective H_2O_2 scavenging mechanism than KBG.

The activity of GR, which catalyzes the NADPH-dependent reduction of GSSG, is important in combating oxidative stress arising from abiotic stress (Foyer *et al.* 1991). Several researchers have suggested that a salt-induced increase in GR activity is more prevalent in salt-tolerant cultivars than in salt-sensitive cultivars (Hernández *et al.* 2000; Meloni *et al.* 2003; Demiral and Türkan 2005). However, GR activity decreased in the shoots of both turfgrass species in this study. Our findings are in agreement with those of Shalata and Tal (1998), who reported a decrease in GR activity in the leaves of both salt-tolerant and salt-sensitive tomato plants under salt stress. Interestingly, GR activity increased with NaCl concentration in the roots of KBG (Fig. 13), being greater in KBG than in TF at 100, 150 and 200 mmol L^{-1} NaCl. The reduced level of GR activities in shoots of KBG and TF could decrease the GSH/GSSG ratios. Gechev *et al.*

(2005) reported that cell growth arrest and blocked cell division is associated with low GSH/GSSG ratio and GSH depletion. The transgenic tobacco plants overexpressing GR had both high levels of GSH and increased tolerance to oxidative stress (Broadbent *et al.* 1995). Therefore, GR may be a key enzyme for development of salt-tolerant turfgrass cultivars.

Our results demonstrated that membrane lipid peroxidation occurred in both turfgrass species. Compared to KBG, the lower level of lipid peroxidation observed in TF suggests that TF has the more effective antioxidant defense system. The increased SOD activity in TF, which corresponded to increased CAT and APX activities, plays a central role in scavenging O_2^- and H_2O_2. The high SOD activity in the shoots of KBG indicated that H_2O_2 was produced from the conversion of $O^{\cdot}{}_2^-$. However, compared to the control plants, the relatively lower activities of CAT, APX and GR in salt-stressed KBG shoots indicated that H_2O_2 scavenging was less effective in the salt-stressed plants. In fact, this surplus H_2O_2 may be the main reason for the extensive lipid peroxidation and growth inhibition observed in KBG.

To the best of our knowledge, this is the first study to demonstrate changes in the antioxidant systems of the cool-season turfgrasses, KBG and TF, under salinity stress. Our results demonstrated that salinity stress had a significant impact on the antioxidative enzymes in both turfgrass species. KBG, a salt sensitive cool-season turfgrass, lost ROS scavenging ability under salt stress. Although TF, a moderately salinity-tolerant turfgrass species, could eliminate ROS by increasing antioxidative enzyme activity, the observed decrease in the DW and turf quality of TF, as well as increased leaf firing and MDA concentration under salinity stress, indicate that the antioxidant defense system in TF could not prevent cell injury completely.

SUMMARY

This study has shown the effects of salinity stress on MDA concentration and antioxidant activities in KBG and TF. The MDA concentration under salinity stress was lower in TF than in KBG in both shoots and roots. Activities of SOD in the shoots of both species increased with salinity stress, but the activities of CAT and APX decreased in KBG. While no significant difference in the activities of CAT and APX was observed in TF, the activities of these salt-induced, antioxidative enzymes in the shoots of TF were higher than in KBG. In roots of KBG, SOD and GR activities increased and CAT and APX activities decreased in comparison with the control. In roots of TF, salinity increased the activities of SOD, CAT, and APX. The differences in antioxidative enzyme activities of shoots and roots may explain the salt tolerance of KBG and TF. TF exhibited a better antioxidant defense system against oxidative stress and lipid peroxidation by maintaining higher SOD, CAT and APX activities than KBG.

CHAPTER 4

Growth responses and ionic concentrations of four bermudagrass cultivars under salinity stress

Salinity tolerance (according to the salinity level that causes 50% shoot growth reduction) ranged between 26 and 40 dS m^{-1} among 35 *Cynodon* spp. cultivars (Marcum and Pessarakli 2006). Cultivars within a given species that exhibit superior salinity tolerance must possess different physiological mechanisms from the least tolerant types. In this chapter, the relative salinity tolerance, ion concentrations and osmotic potential in leaves and roots of four bermudagrass cultivars were investigated.

MATERIALS AND METHODS

Four bermudagrass cultivars (Riviera, Sundevil 2, Savannah, and Blackjack) were used for this study. Seeds were sown into plastic containers filled with vermiculite in a glasshouse under natural light. One-month-old seedlings (at the two-leaf stage) were transferred into 4 L plastic pots. The pots contained constantly aerated half-strength Hoagland No. 2 solution (Hoagland and Arnon 1950), modified by the addition of Fe-EDTA to supply 3 mg L^{-1} of Fe. Nutrient solutions were renewed weekly throughout the experiment. To ensure establishment and adaptation, all grasses were maintained in nutrient solution for 4 weeks before the initiation of salinity treatments. Shoots were clipped weekly at a mowing height of 5 cm recommended by Turgeon (2008) throughout the experiments.

Five salinity levels of 0 (control), 100, 200, 300, and 400 mmol L^{-1} were prepared by dissolving NaCl in nutrient solution. The hydroponic system was comprised of 20 (five salinity levels and four replications) pots for each cultivar. To avoid osmotic shock, salinity was gradually increased by 50 mmol L^{-1} per day until a final salinity level of 400 mmol L^{-1} NaCl was reached. Afterwards, plants in each treatment were grown for 35 days. Shoots and roots were clipped 1 day before the initiation of salinity treatments. Roots were clipped at a length of 15 cm to provide a common baseline for subsequent measurements. Data collection began at this point.

Shoot clippings were collected weekly for a total of three harvests during the experiment. The clippings were oven-dried at 70°C for 72 h, and weighed for dry weight (DW). Three of these clippings were combined to determine clipping shoot FW and DW for each cultivars. Root FW and DW, and root length were measured at the end of 35 d of salinity treatment. The relative water content (RWC) of shoots and roots is described by RWC (%) =

$$[(FW - DW)/FW] \times 100\%$$

After removal of all external salts with deionized water, the leaf and root samples were collected from three individual plants of each NaCl treatment and cut into small pieces (5 mm in length) for determination of the osmotic potential (Ψ_s). The osmolality of the collected sample sap was analyzed using a vapor pressure osmometer (Osmometer 5520; Wescor, Logan, UT, US). The osmotic potential was calculated using the van't Hoff equation ($\Psi_s = -cRT$).

At the end of experiment, leaves were thoroughly rinsed with deionized water to remove external salts, and then allowed to air-dry before being clipped. Roots were separated from the shoots and also rinsed with deionized water. Clipped leaves and roots were dried at 70°C for 72

h, and the samples were ground in a blender for the ionic analyses. Dried samples were digested in 1 mL sulfuric acid and hydrogen peroxide at 200°C on a dry block bath. The concentrations of Na^+, K^+ were determined by atomic absorption spectrophotometry (Polarization Zeeman Z-6100; Hitachi, Ibaraki, Japan). Chloride was determined by using an ion chromatography (CDD-10A SP, HIC-10A Super; Shimadzu, Kyoto, Japan).

All data were subjected to ANOVA using SPSS 16.0J software (SPSS Japan Inc.) and mean separations were performed using Duncan's multiple-range test at the 5% level. The relationship between salinity level and relative shoot growth (RSG) was described by quadratic regressions.

RESULTS

Plant growth

Shoot DW of all cultivars decreased significantly with increasing salinity (Table 6). However, a difference between the cultivars in response to salinity was apparent. In comparison to the control, the shoot DW at 400 mmol L^{-1} NaCl was reduced by 48.9% for Riviera, 60.1% for Blackjack, 71.4% for Savannah, and 79.0% for Sundevil 2. All four cultivars showed an increase in root length and root DW at moderate salinity levels, followed by a decrease to the highest salinity level (Table 6). Compared with the control, the root lengths of Riviera and Blackjack increased significantly at 100 and 200 mmol L^{-1} NaCl. Those of Savannah and Sundevil 2 increased significantly at 100 mmol L^{-1} NaCl. The root DW of Riviera and Blackjack was significantly larger than that of control at 100, 200, and 300 mmol L^{-1} NaCl and did not change significantly at 400 mmol L^{-1} NaCl (Table 6). The root DW of Savannah increased significantly compared with that of control at 100 and 200 mmol L^{-1} NaCl, and then declined to the same

level of control thereafter. Sundevil 2 showed the maximum root DW at 100 mmol L^{-1} NaCl. At 400 mmol L^{-1} NaCl, the root DW of Savannah and Sundevil 2 decreased by 14.1% and 28.8% compared with the control, respectively.

Table 6. Effects of NaCl stress on the growth of the shoots and roots of four bermudagrass cultivars.

Cultivar	NaCl (mmol L^{-1})	Shoot DW (g)	Root DW (g)	Root length (cm)	Shoot RWC (%)	Root RWC (%)
Riviera	0	1.47±0.03 a	0.24±0.01 b	37.8±1.0 b	89.7±0.6 a	82.6±0.3 a
	100	1.41±0.04 ab	0.32±0.01 a	47.3±1.5 a	86.5±0.2 b	80.1±0.2 b
	200	1.30±0.06 b	0.35±0.01 a	46.5±1.2 a	79.8±0.1 c	78.0±0.3 c
	300	0.87±0.03 c	0.33±0.02 a	38.3±1.5 b	78.1±0.4 d	74.8±0.4 d
	400	0.75±0.07c	0.23±0.02 b	32.9±0.3c	76.4±0.4 e	76.0±0.3 e
Blackjack	0	1.45±0.05 a	0.26±0.004 c	37.1±1.1 b	88.6±0.6 a	83.0±1.0 a
	100	1.30±0.06 b	0.35±0.01 a	44.7±1.2 a	86.7±0.1 b	80.2±0.7 b
	200	1.21±0.05 b	0.33±0.04 ab	43.7±1.5 a	81.3±0.5 c	78.1±1.4 b
	300	0.80±0.03 c	0.29±0.01 bc	34.2±1.0 b	79.0±0.4 d	73.7±0.4 c
	400	0.58±0.01 d	0.26±0.02 c	29.6±2.1 c	75.1±0.4 e	71.9±0.6 c
Savannah	0	1.43±0.05 a	0.24±0.01 b	39.1±0.6 b	88.4±0.4 a	83.0±1.0 a
	100	1.25±0.01 b	0.34±0.03 a	45.7±2.0 a	81.8±0.2 b	80.2±0.8 b
	200	1.12±0.05 c	0.32±0.01 a	39.0±0.7 b	75.5±0.4 c	78.1±1.4 b
	300	0.73±0.01 d	0.22±0.01 b	32.5±1.1 c	75.3±0.6 c	73.8±0.4 c
	400	0.41±0.01 e	0.23±0.01 b	28.6±2.0 c	73.9±0.7 d	71.9±0.5c
Sundevil 2	0	1.49±0.02 a	0.26±0.02 ab	36.7±1.2 ab	88.1±0.2 a	81.4±0.4 a
	100	1.29±0.07 b	0.29±0.01 a	39.9±0.3 a	81.5±0.6 b	71.6±2.9 b
	200	1.00±0.02 c	0.26±0.01 ab	34.4±1.4 b	75.5±0.4 c	64.0±1.0 c
	300	0.58±0.02 d	0.22±0.02 bc	27.7±1.0 c	72.7±0.5 d	61.5±0.8 c
	400	0.31±0.01 e	0.18±0.01 c	22.4±1.1 d	71.2±0.3 e	59.6±1.8 c

Values of a parameter for each cultivar followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple-range test. Data are mean ± SE (n=4).

The RWC of the shoots and roots decreased significantly with increasing salinity in all four cultivars (Table 6). Riviera and Blackjack had significantly higher water contents in the shoots

and roots. Savannah had intermediate RWC, and Sundevil 2 had the lowest water contents among the cultivars at all salinity levels. In all cultivars, water contents in the shoots were higher than those in roots, except in Savannah at 200 mmol L^{-1} NaCl.

Quadratic decreases in relative shoot growth (as a percent of control) were observed with increasing salinity levels for all cultivars (Table 7). The predicted salinity level causing 50% reduction of relative shoot growth was 400.4 mmol L^{-1} NaCl in Riviera, 369.6 mmol L^{-1} NaCl in Blackjack, 305.3 mmol L^{-1} NaCl in Savannah, and 279.6 mmol L^{-1} NaCl in Sundevil 2. According to the salinity level causing 50% shoot DW reduction, the ranking for salinity tolerance of four bermudagrass cultivars was Riviera > Blackjack > Savannah > Sundevil 2.

Quadratic regressions of relative shoot growth (RSG, %) and predicted salinity levels causing 50% reduction (S50R) in RSG of four bermudagrass cultivars under salinity stress.

Cultivar	Regression	R^2	S50R (mmol L^{-1})
Riviera	$Y=101.69-0.049X-0.0002\ X^2$	0.88	400.4
Blackjack	$Y=100.05-0.062X-0.0002\ X^2$	0.92	369.6
Savannah	$Y=99.5-0.071X-0.0003\ X^2$	0.97	305.3
Sundevil 2	$Y=101.25-0.155X-0.0001\ X^2$	0.98	279.6

Ionic concentrations

Leaf and root Na^+ concentrations increased with increasing salinity for all cultivars (Fig. 14). Riviera, the most salt-tolerant cultivar, had the lowest leaf Na^+ concentration, and Sundevil 2, the most salt-sensitive cultivar, , had the highest leaf Na^+ concentration at the same salinity levels. The most dramatic differences in Na^+ concentration among the cultivars were at 300 and 400 mmol L^{-1} NaCl. The leaf Na^+ concentrations in Blackjack, Savannah, and Sundevil 2 were 24%, 60%, and 122% higher than in Riviera at 300 mmol L^{-1} NaCl, and were 24%, 40%, and 107% higher than in Riviera at 400 mmol L^{-1} NaCl, respectively. There was no significant difference in

root Na^+ concentrations among Riviera, Blackjack, and Savannah except at 300 mmol L^{-1} NaCl. Sundevil 2 had the highest Na^+ concentration from 200 to 400 mmol L^{-1} NaCl. The root Na^+ concentrations of Sundevil 2 were 45%, 60%, and 43% higher than those of Riviera at 200, 300, and 400 mmol L^{-1} NaCl, respectively. Root Na^+ concentrations were higher than leaf Na^+ concentrations for all cultivars at each salinity treatment, except for Sundevil 2 at control.

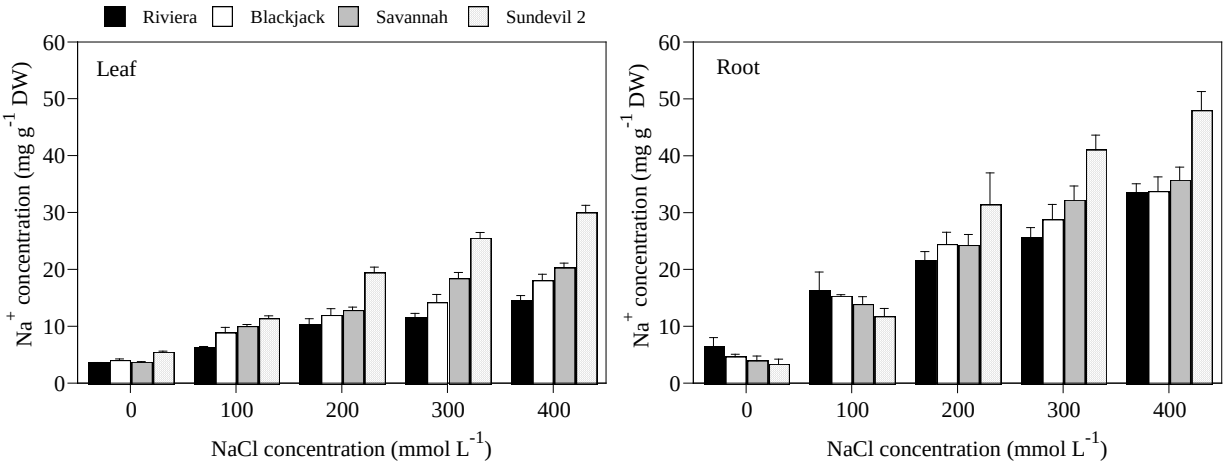

Figure 14 Na^+ concentrations in the leaves and roots of the four bermudagrass cultivars at 0, 100, 200, 300, and 400 mmol L^{-1} NaCl. Data are the means ± standard error (n = 4).

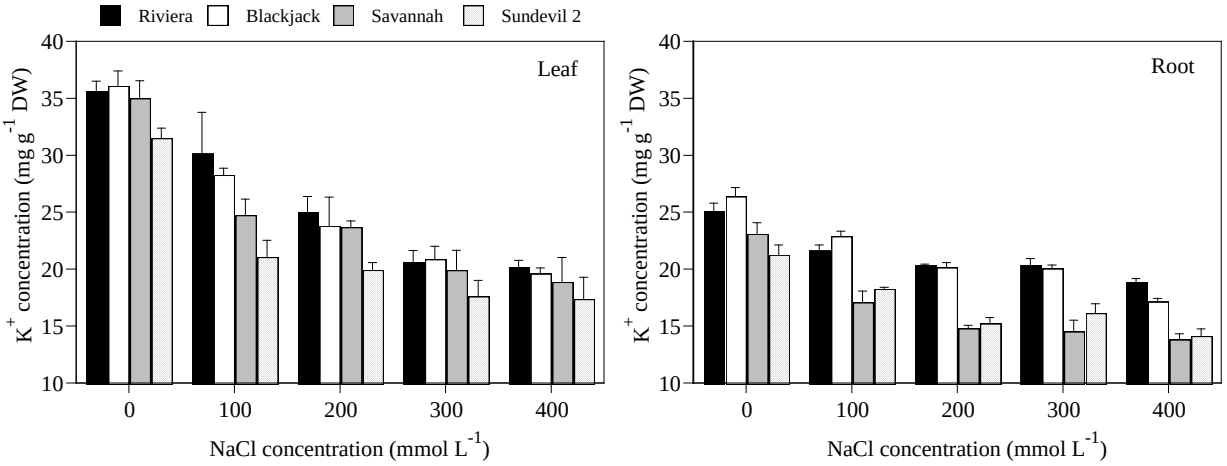

Figure 15 K^+ concentrations in the leaves and roots of the four bermudagrass cultivars at 0, 100, 200, 300, and 400 mmol L^{-1} NaCl. Data are the means ± standard error (n = 4).

Leaf and root K^+ concentrations of all cultivars decreased significantly with increasing salinity (Fig. 15). The leaf K^+ concentration of Riviera was higher than that of Sundevil 2 at the

same salinity levels. However, there was no significant difference in leaf K^+ concentrations among Riviera, Blackjack, and Savannah, except at 100 mmol L^{-1} NaCl. The root K^+ concentrations of Riviera and Blackjack were higher than those of Savannah and Sundevil 2, and those of Savannah were the lowest from 100 to 400 mmol L^{-1} NaCl. For all cultivars, leaf K^+ concentrations were higher than root K^+ concentrations at a same salinity level.

The Cl^- concentration in the leaf and root of four cultivars increased with increasing salinity (Fig. 16). In general, those in the leaf and root of Riviera were significantly lower than those in the other three cultivars, except at 0 and 100 mmol L^{-1} NaCl. Those in the leaf and root of Blackjack, Savannah, and Sundevil 2 did not change significantly at 200 and 300 mmol L^{-1} NaCl. However, those in the leaf and root of Blackjack were lower than those in Savannah and Sundevil 2 at 400 mmol L^{-1} NaCl.

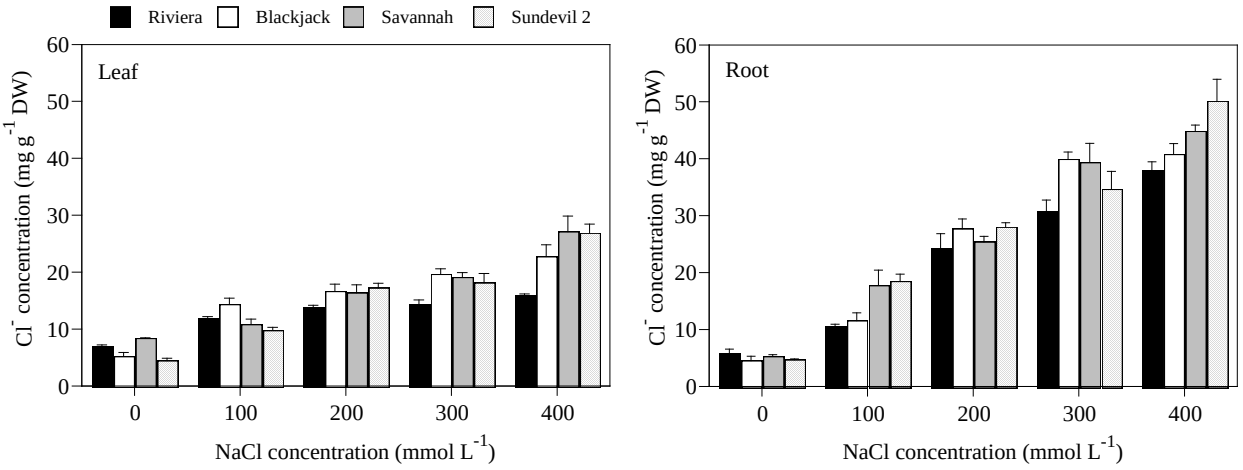

Figure 16 Cl^- concentrations in the leaves and roots of the four bermudagrass cultivars at 0, 100, 200, 300, and 400 mmol L^{-1} NaCl. Data are the means ± standard error (n = 4).

K^+/Na^+ ratios, Na^+ and Cl^- ratios between root and leaf

Leaf and root K^+/Na^+ ratios decreased significantly with increasing salinity for all cultivars (Fig. 17). The overall leaf K^+/Na^+ ratios was highest in Riviera, followed by Blackjack and

lowest in Sundevil 2 at each salinity treatment. However, there were no significant difference in root K^+/Na^+ ratios among four cultivars under salinity stress. The leaf K^+/Na^+ ratios were higher than root K^+/Na^+ ratios for all cultivars at a same salinity level.

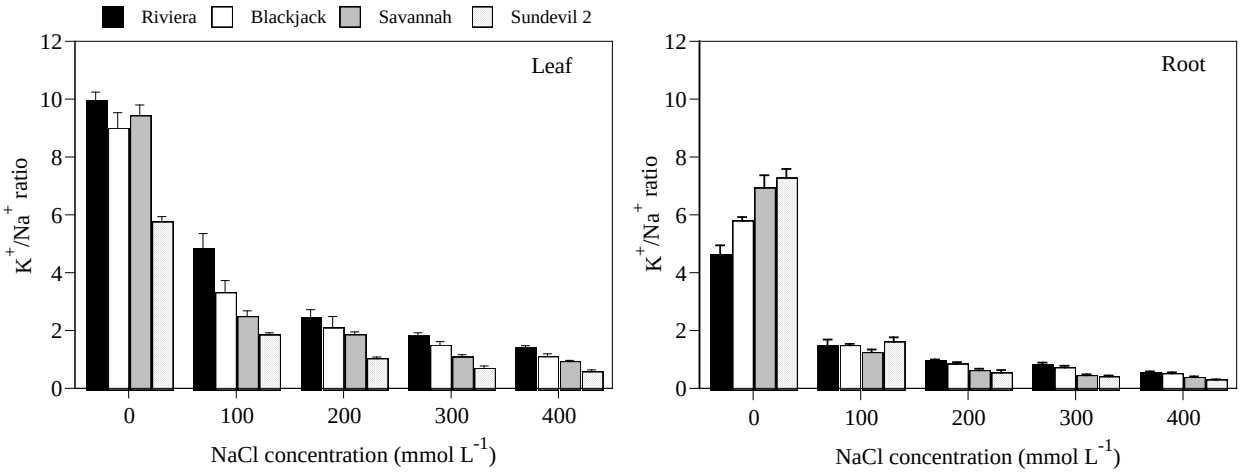

Figure 17 K^+/Na^+ ratios in the leaves and roots of the four bermudagrass cultivars at 0, 100, 200, 300, and 400 mmol L^{-1} NaCl. Data are the means ± standard error (n = 4).

All four cultivars increased in Root Na^+/leaf Na^+ ratios under salinity stress, relative to the control (Fig. 18A). However, the root Na^+/leaf Na^+ ratios did not change significantly between 200 and 400 mmol L^{-1} NaCl for each cultivars. At each salinity level, the root Na^+/leaf Na^+ ratios was Riviera > Blackjack > Savannah > Sundevil 2. The root Cl^-/leaf Cl^- ratios of Riviera increased with increasing salinity, and were higher than those in other cultivars from 200 to 400 mmol L^{-1} NaCl (Fig. 18B). The root Cl^-/leaf Cl^- ratios of Blackjack, Savannah and Sundevil 2 were higher than that of control, except at 100 mmol L^{-1} NaCl for Blackjack.

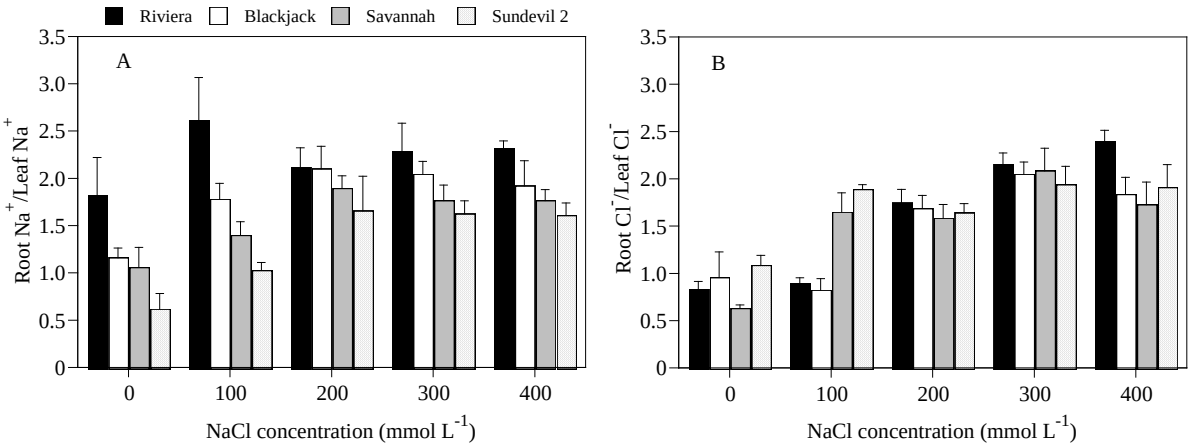

Figure 18 Root Na^+/leaf Na^+ (A) and root Cl^-/leaf Cl^- (B) ratios in the four bermudagrass cultivars at 0, 100, 200, 300, and 400 mmol L^{-1} NaCl. Data are the means ± standard error (n = 4).

Relationships between osmotic potential and ionic concentration

Table 8. Effects of NaCl stress on the osmotic potential (–MPa) of the leaves and roots of four bermudagrass cultivars.

NaCl (mmol L^{-1})	Riviera	Blackjack	Savannah	Sundevil 2
Leaf				
0	−1.14 ± 0.01	−1.04 ± 0.02	−1.04 ± 0.01	−0.98 ± 0.02
100	−1.39 ± 0.05	−1.41 ± 0.02	−1.51 ± 0.03	−1.56 ± 0.06
200	−1.67 ± 0.02	−1.84 ± 0.02	−1.85 ± 0.04	−2.11 ± 0.05
300	−1.86 ± 0.02	−1.99 ± 0.07	−2.17 ± 0.04	−2.47 ± 0.07
400	−2.08± 0.04	−2.47 ± 0.06	−2.69 ± 0.08	−3.08 ± 0.10
Column Mean	−1.63 ± 0.08	−1.75 ± 0.11	−1.85 ± 0.13	−2.04 ± 0.17
Root				
0	−0.63 ± 0.04	−0.36 ± 0.04	−0.34 ± 0.02	−0.31 ± 0.04
100	−1.00 ± 0.08	−0.69 ± 0.02	−0.89 ± 0.04	−0.73 ± 0.03
200	−1.40 ± 0.03	−1.16 ± 0.04	−1.28 ± 0.02	−1.19 ± 0.07
300	−1.77 ± 0.01	−1.72 ± 0.04	−1.69 ± 0.03	−1.77 ± 0.05
400	−1.99 ± 0.02	−2.22 ± 0.03	−2.31 ± 0.08	−2.31 ± 0.07
Column Mean	−1.36 ± 0.11	−1.23 ± 0.16	−1.30 ± 0.16	−1.26 ± 0.17

Values in each column are the mean of four replicates ± S.E.

The osmotic potentials (Ψ_s) became more negative in both leaves and roots of all cultivars with increasing salinity (Table 3). Leaf Ψ_s exhibited significant differences among cultivars at each salinity level. The most tolerant Riviera tended to maintain higher leaf Ψ_s than others. Root

Ψ_s were significantly different among cultivars except at 300 mmol L^{-1} NaCl (Table 3). The leaf and root Ψ_s were lower than those of the growing media (an average of –0.93 MPa) for all cultivars. Leaf and root Na^+ and Cl^- concentrations were strongly ($R^2 \geq 0.77$) correlated with the Ψ_s (Fig. 19), indicating that Na^+ and Cl^- are important factors involved in osmotic adjustment in all four cultivars.

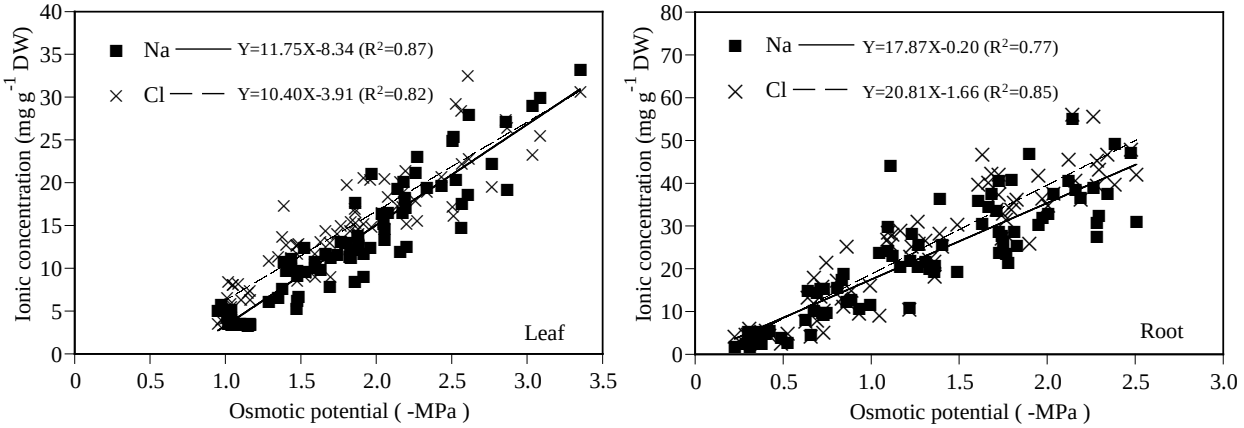

Figure 19 Relationships between the measured osmotic potential and ionic concentrations in the leaves and roots of the four bermudagrass cultivars at 0, 100, 200, 300, and 400 mmol L^{-1} NaCl (n = 80).

DISCUSSION

Salt stress results in a considerable decrease in the dry weights of plants due to an inhibition of cell division and loss of cell turgor (Chartzoulakis and Klapaki 2000; Neumann *et al.* 1988). Our results show that shoot DW of all four bermudagrass cultivars were significantly decreased by salinity stress (Table 6). There was a large difference in salt tolerance among four bermudagrass cultivars, which was Riviera > Blackjack > Savannah > Sundevil 2. The root DW and root length increased at moderate salinity levels (Table 6). This root growth stimulation in all four cultivars may be an adaptive response to salinity stress, because a concurrent increase in

root growth and decrease in shoot growth would increase the root/shoot ratio, and would therefore increase water absorption to alleviate osmotic stress. Mechanisms of adaptation to salinity stress can be very specific for different cultivars. For example, Riviera had a higher shoot weight than the other cultivars at the same level of salinity stress, and its root weight and root length increased markedly to compensate for the greater evaporative surface of the shoots. This root growth stimulation under moderate salinity has been reported previously in relatively salt-tolerant grasses (Alshammary *et al.* 2004; Dudeck *et al.* 1983; Dudeck and Peacock 1993).

The results of relative water content of all four cultivars (Table 6) indicated that maintaining higher relative water content may be responsible for higher growth rates under salt stress. Osmotic stress due to a lack of osmotic adjustment inhibits water uptake and can lead to physiological drought (Marcum 1999). However, the osmotic potentials in the shoots and roots were more negative than the corresponding level in the growing media in all cultivars, which indicates that all cultivars achieved complete osmotic adjustment (Table 8). Water contents in the shoots and roots of all four cultivars decreased with increasing salinity (Table 6), indicating that the cultivars may have achieved complete osmotic adjustment by solute accumulation and tissue dehydration. Marcum and Murdoch (1990) reported that osmotic adjustment of bermudagrass was not achieved exclusively by solute accumulation but also by tissue dehydration. In our study, the Na^+ and Cl^- concentrations were strongly and significantly negatively correlated with osmotic potential (Fig. 19). Riviera, the most salt-tolerant cultivar, maintained a less negative osmotic potential, which may have resulted from the low ionic concentrations and high relative water contents in this cultivar. In contrast, Sundevil 2, the most salt-sensitive cultivar, exhibited the lowest osmotic potential. This was due to relatively high ionic concentrations concurrent with

very low relative water contents. Our results suggested that Riviera might have achieved its superior osmotic adjustment and salt tolerance by greater solute accumulation and less tissue dehydration than in the more salt-sensitive cultivars.

Na^+ and Cl^- were important factors involved in the limitation of plant growth under salt stress. Riviera accumulated less Na^+ and Cl^- than did Sundevil 2, especially at higher NaCl concentrations (Fig. 14). Salinity tolerance in grasses has been associated with the exclusion of Na^+ and Cl^- from the shoots (Marcum 2006; Marcum and Murdoch 1994). In this study, the most salt-tolerant cultivars had less Na^+ and Cl^- accumulation than the least salt-tolerant cultivars (Fig. 14, 15) Riviera maintained lower leaf and root ion concentrations, resulting in superior salinity tolerance. Bermudagrass cultivars have bicellular salt glands on the leaf surface. The salt gland excretion efficiency is an important factor in the process of salt ion regulation and the resultant salinity tolerance, which has been measured in Chapter 5.

Riviera also accumulated the lowest amounts of Na^+ and Cl^- in its roots. In all cultivars, the concentrations of Na^+ and Cl^- in the roots were higher than those in the leaves (Figs. 14, 16). Riviera had the lowest Na^+ concentration in its leaves and the highest root Na^+/leaf Na^+ ratio among the four cultivars. In contrast, Sundevil 2 maintained the highest Na^+ concentration and the lowest root Na^+/leaf Na^+ ratio among the cultivars (Figs. 14, 18A). Similar to the root Na^+/leaf Na^+ ratio, Riviera had the highest root Cl^-/leaf Cl^- ratios at 200, 300, and 400 mmol L^{-1} NaCl (Fig. 18B). These results suggest that the high salinity tolerance of Riviera may result from its ability to reduce uptake of Na^+ and Cl^- better than the other cultivars. The ability to prevent the entry of Na^+ and Cl^- into the leaves was strong in Riviera. Therefore, preventing Na^+ and Cl^- transport from the roots to the leaves appears to be an important mechanism that results in the

different salt tolerance levels among the bermudagrass cultivars.

Potassium concentrations in leaves and roots decreased significantly with increasing salinity, and those in the leaves were higher than those in the roots in all four cultivars (Fig. 15). Potassium played an essential role in the growth of all four cultivars under salinity stress. These results indicate that a decrease in the K^+ concentration results in growth inhibition in bermudagrass, and might prevent water absorption under salt stress. Potassium is involved in the activation of several enzymes, in membrane transport and in the maintenance of osmotic potential in vacuoles and guard cells (Lee *et al.* 2007; Marschner 1995). The K^+ concentration differed significantly among cultivars, and appeared to be related to salt tolerance. Riviera had higher leaf and root K^+ than the other cultivars. This suggests that the roots of salt-tolerant cultivars selectively accumulate K^+ and transport it to the leaves.

Comparison of the most and least tolerant cultivars revealed some difference in the K^+/Na^+ ratio with increasing salinity (Fig. 17). Riviera maintained the highest leaf K^+/Na^+ ratio among the cultivars at all salinity levels. The maintenance of higher shoot K^+/Na^+ ratios has been linked to salinity tolerance in turfgrasses (Marcum 2008; Marcum and Murdoch 1990; Qian *et al.* 2001). Leaf K^+/Na^+ ratios were much higher than 1 in Riviera and Blackjack at all salinity levels (Fig. 17), indicating a strong capacity for the selective transport of K^+ from roots to leaves under salinity stress.

SUMMARY

Bermudagrass cultivars exhibited marked differences in their responses to salinity. The reduction in relative shoot growth with increasing salinity indicated a salinity tolerance decreasing in the order of Riviera > Blackjack > Savannah > Sundevil 2. Shoot growth, root growth, and relative water content of shoots and roots of all cultivars were significantly affected by NaCl stress. However, root length and root dry weight of the salt-tolerant cultivars Riviera and Blackjack increased significantly compared with the control. Tissue Na^+ and Cl^- concentrations in all cultivars increased with increasing salinity. The most salt-tolerant cultivar, Riviera, accumulated less Na^+ and Cl^- in leaves and roots and more K^+ in leaves than the least tolerant cultivar, Sundevil 2. All grasses exhibited complete osmotic adjustment, and osmotic adjustment has significant correlative with the Na^+ and Cl^- in leaves and roots. Our results indicate that the salinity tolerance of bermudagrass is associated with root growth stimulation in conjunction with leaf and root Na^+ and Cl^- regulation and the maintenance of high leaf K^+ levels.

CHAPTER 5

Salt glands and salt gland excretion efficiency of bermudagrass cultivars to salinity stress

Salt glands are found in a number of warm-season (C_4) turfgrass, including gramagrass, bermudagrass, zoysiagrass, buffalograss, saltgrass and dropseeds (Marcum 2008). Salt gland is a major means of saline ions excretion in leaf of salt-tolerant turfgrass. Salt gland excretion rates have been associated with salt tolerance ability and is a major physiological adaption associated with salt tolerance. The characteristic of salt glands and salt gland excretion efficiency of bermudagrass cultivars was determined in this chapter. To compare the structure of salt glands, salt glands of Japanese lawngrass were also observed in this study.

MATERIALS AND METHODS

The plant materials and salt stress treatments were the same as those described in the Chapter 4. The presence of salt glands in the leaves was determined according to the method of Alshammary *et al.* (2004), with a slight modification. Leaf segments of 0.5 to 1 cm in length were fixed in 2.5% glutaraldehyde in 0.1 mol L^{-1} sodium cacodylate buffer at pH 7.2 for 24 h, and then washed three times in the sodium cacodylate buffer for 20 min. The segments were post-fixed in 2% osmium tetroxide in sodium cacodylate buffer for 1.5 h, washed three times in a buffer for 10 min, and then washed twice in distilled water for 10 min. Fixed leaf segments were dehydrated in a graded ethanol series, followed by critical-point drying with liquid CO_2. Mounted specimens were sputter-coated with 12 nm gold–palladium particles, and observed with

a scanning electron microscope (S-2380N, Hitachi Ltd., Tokyo, Japan) at 15 kV and Miniscope® (TM3000 Hitachi Ltd., Tokyo, Japan).

Rates of ion (Na^+, Cl^- and K^+) excretion by salt glands

Rates of ion excretion by salt glands were determined according to the method of Marcum (1999). Briefly, the shoots were rinsed to remove external salts, and the plants were then grown for 1 week. We then carefully excised four to eight full mature leaves and placed them in 10 mL of distilled water within a scintillation vial, sealed the vial, and then shook the vial for 10 s, which was sufficient to dissolve all of the excreted salt crystals. Leaves were removed and dried at 70°C for 48 h, then weighed. Vials were resealed and subsequently analyzed to determine the ion concentration as described in Chapter 2. Ion excretion rates are expressed as mg/g leaf dry weight per week.

RESULTS

Structure of salt glands

Salt crystals were clearly discernible on leaf surfaces of all bermudagrass cultivars growing in saline solution (Fig. 20A). Salt glands were present on both abaxial and adaxial leaf surfaces of bermudagrass and Japanese lawngrass. Salt glands appeared between the costal zone of leaf epidermis in longitudinal rows, and parallel to rows of stomates (Fig. 20B, C).

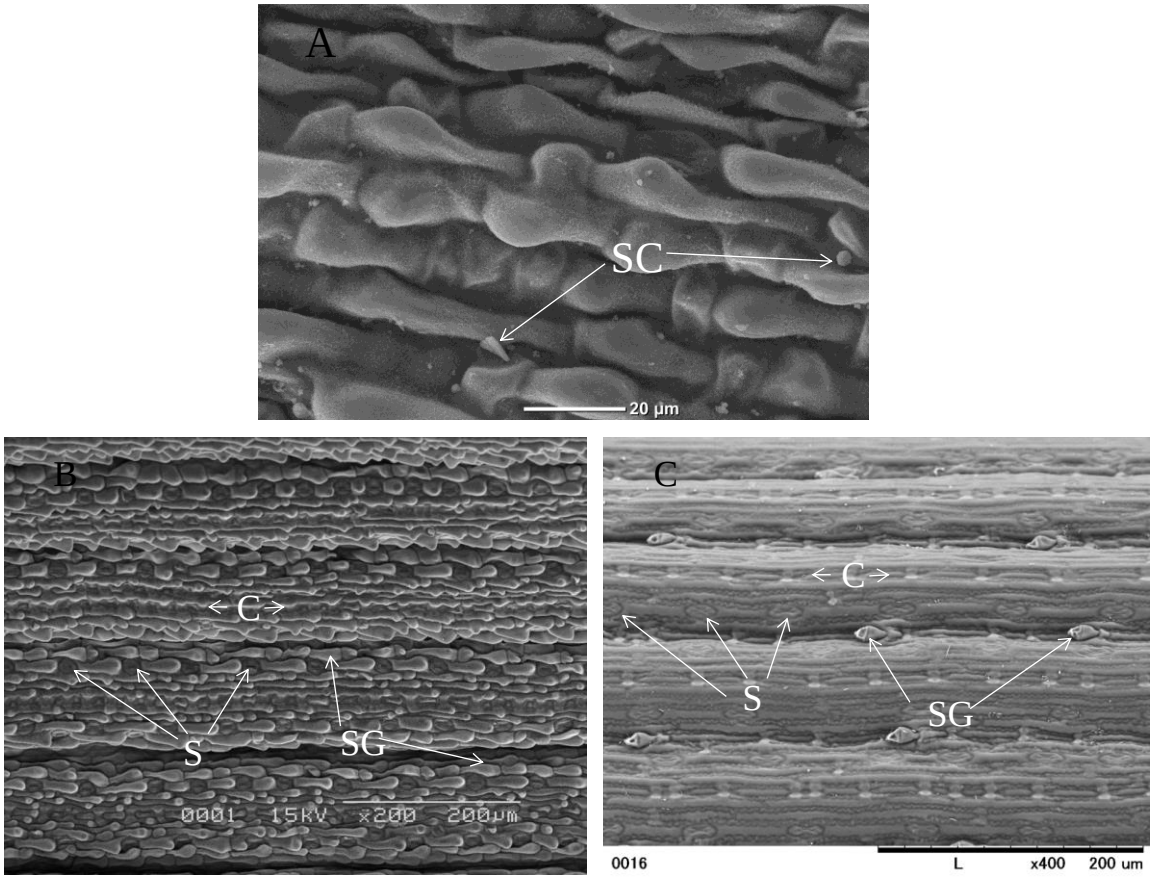

Figure 20 Scanning electron micrograph of adaxial leaf surfaces, (A: bermudagrass) and overview of location of salt gland relative to other structure (B: bermudagrass; C: Japanese lawngrass). SC = salt crytal; C = costal zone of leaf epidermis; S = stomata; SG = salt gland.

The glands were situated atop the leaf epidermis, in a recumbent position (Fig. 21A, C). In both species, glands were bicellular, with a basal and cap cell, and basal cell, and the basal cell semi-imbedded into the leaf epidermis (Fig. 21A, C). Salt glands of bermudagrass were surrounded by numerous papillae and appeared move vertical in orientation due to imbedded basal cell (Fig. 21A, B).

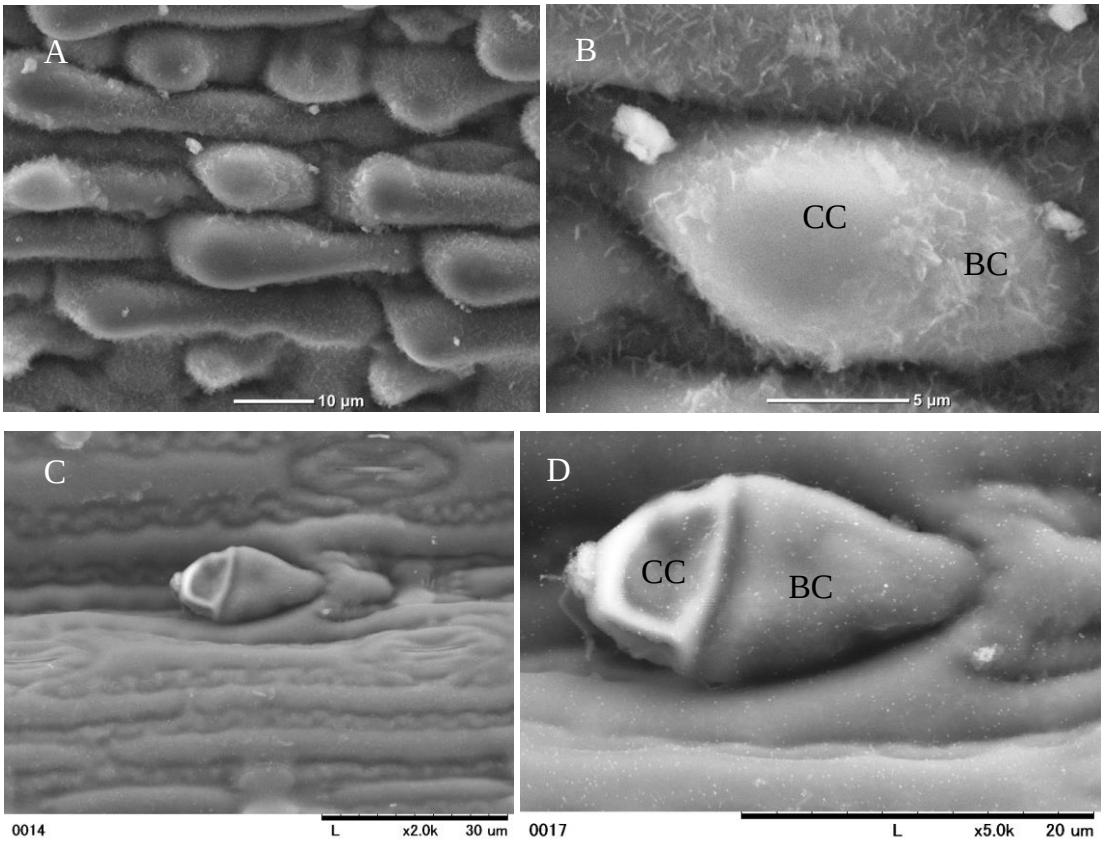

Figure 21 Scanning electron micrographs of salt glands in adaxial leaf surfaces of bermudagrass (A and B) and Japanese lawngrass (C and D). CC = cap cell; BC = basal cell.

Ion excretion rate by leaf salt glands

The leaf salt glands of all four cultivars excreted ions under salinity stress (Fig. 22). Sodium excretion in Riviera, Blackjack, and Sundevil 2 did not change significantly as salinity increased (Fig. 22A). However, Na^+ excretion in Savannah increased with increasing salinity to 300 mmol L^{-1} NaCl, and then decreased at 400 mmol L^{-1} NaCl. The amount of Na^+ excreted was significantly higher in Riviera and Blackjack than in Savannah and Sundevil 2. Potassium excretion in Riviera increased under salinity stress and was significantly higher than other cultivars (Fig.22B). In comparison to control, K^+ excretion of Blackjack, Savannah and Sundevil 2 did not change significantly except Sundevil 2 at 400 mmol L^{-1} NaCl. Chloride excretion in

Riviera and Blackjack increased with increasing salinity (Fig. 22C). That in Savannah increased to a salinity of 200 mmol L^{-1} NaCl and then decreased. The excretion in Sundevil 2 did not change significantly with increasing salinity. In addition, more Na^+ and Cl^- were secreted than K^+ in each cultivar under salinity stress.

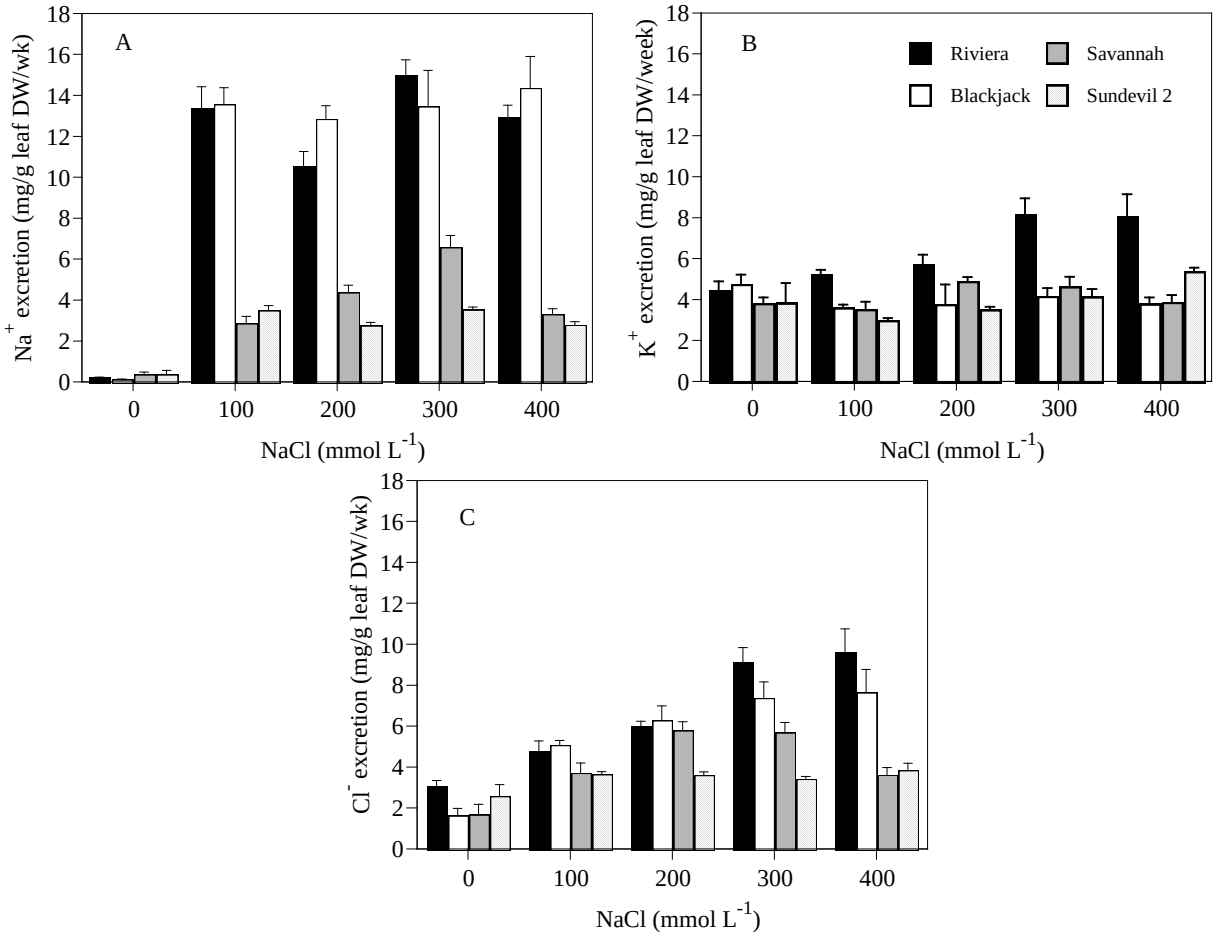

Figure 22 Leaf salt gland Na^+ (A), K^+ (B) and Cl^- (C) excretion rates of the four bermudagrass cultivars at 0, 100, 200, 300, and 400 mmol L^{-1} NaCl. Data are the means ± standard error (n = 4).

The salt glands excretion rates were influenced by NaCl concentration in the growing solution. Increasing NaCl concentrations generally stimulate excretion up to an optimal level. The Na^+ and Cl^- excretion rates of control plants were higher than those of plants under salinity stress (Fig. 22A, C). Riviera and Savannah exhibited the maximum Na^+ excretion rates at 300 mmol L^{-1} NaCl. Blackjack and Sundevil 2 maintained the stable Na^+ excretion rates from 100 to

400 mmol L^{-1} NaCl (Fig. 22A); The Cl^- excretion rates of Riviera and Blackjack were gradually increased with increasing salinity and no significant change at 300 and 400 mmol L^{-1} NaCl. Savannah exhibited the maximum Cl^- excretion rates at 200 mmol L^{-1} NaCl. Sundevil 2 maintained the stable Cl^- excretion rates under salinity stress (Fig. 22C).

DISCUSSION

Bicellular leaf salt glands, which eliminate excess saline ions from shoots by active excretion, have been reported in a number of salt-adapted grass species (Marcum 2008). Salt glands can be easily observed on grass leaves, which is a potential tool for salt-tolerant bermudagrass cultivars breeding. Results of this study showes that salt tolerance among bermudagrass cultivars have been correlated with salt glands ionic excretion rate. Riviera and Blackjack maintained higher Na^+ and Cl^- excretion rates than Savannah and Sundevil 2 at the same level of salt stress. High rates of Na^+ and Cl^- excretion by leaf salt glands were associated with lower leaf Na^+ and Cl^- concentrations, indicating that salt gland excretion efficiency is an important factor in the regulation of ions and the resultant salinity tolerance of bermudagrass. Salt gland excretion rates and salinity tolerance were highly correlated to leaf salt gland density or the number of glands per unit of leaf area in *Zoysia* spp. (Marcum 1998; Marcum and Murdoch 1990). Therefore, the density and size of salt glands in the leaf surface among bermudagrass cultivars need further investigation.

Evidence that salt gland ion excretion in grass is an active, metabolically driven process is varied, including effects of temperature, light, oxygen pressure and metabolic inhibitors on

excretion rate (Marcum 2008). Comparison of salt gland excretion rates among studies is difficult due to the varying factors such as growing environment, duration to salt stress, and plant leaf age. The salt glands excretion rates were influenced by NaCl concentration in the growing solution and different among cultivars. However, there was no difference in salt gland density between a plant grown in saline conditions and one grown in salt-free conditions. The salt gland activity was found to be heritable, not induced by salt stress, among *Zoysia* genotypes (Marcum and Pessarakli 2006). Riviera may bear with certain salt glands density due to its genetics. The salinity tolerance of Bermudagrass cultivars were correlated with salt glands density and salt gland saline ion excretion rates.

SUMMARY

Bicellular leaf salt glands were observed in both abaxial and adaxial leaf surfaces of all four bermudagrass cultivars. Salt glands ion excretion was highly selective for Na^+ and Cl^- over K^+. Ion excretion rates were different depending on cultivars and correlated with salinity tolerance. Riviera excreted more Na^+ and Cl^- than the other cultivars through leaf salt glands. Salinity tolerance in bermudagrass is associated with leaf saline ion excretion, which might prove an effective selection tool in breeding salt-tolerant cultivars.

GENERAL DISCUSSION

Salinity had a significant impact on the growth of turfgrasses in this study. The shoot growth of KBG, TF and bermudagrass cultivars decreased significantly with increasing salinity (Table 3, 6). However, the root growth of TF and bermudagrass (dry weight or root length) was stimulated at moderate salinity (Table 3,6). Growth parameter, such as shoot DW, root mass and turf quality have been reported to be excellent criteria to determine salinity tolerance of turfgrass (Marcum 2008). Based on the turf quality, leaf firing percentage and 50% shoot growth reduction in this study, the ranking of salinity tolerance decreased in this order: bermudagrass, TF and KBG. The tolerance of bermudagrass cultivars was Riviera > Blackjack > Savannah > Sundevil 2. Differences in salinity tolerance of turfgrasses are associared with various adaptation mechanisms. Root growth stimulation in bermudagrass maintained higher root/shoot ratios than that of TF under moderate salinity. The salinity tolerant cultivar Riviera also exhibited more significant root growth than other bermudagrass cultivars. A higher root/shoot ratio, which can increase water absorption/transpiration area, seemed to be ralated to salinity tolerance in this study.

Increasing salinity reduces the water potential of the soil water, which results in inhibition of water uptake by plants. However, KBG, TF and bermudagrass cultivars maintained complete osmotic adjustment under salinity stress. However, the process of osmotic adjusment differed with grass species. Salt-sensitive KBG and moderately salt-tolerant TF accumulated Na^+ and Cl^- for osmotic adjustment under salt stress (Tables 4, 5). Na^+ and Cl^- made a larger contribution to osmotic potential in KBG than in TF. The availability of Na^+ and Cl^- as cheap osmoregulator is generally beneficial. However, excessive accumulation of Na^+ and Cl^- results in ion toxicity and

growth inhibition. TF accumulated more compatible solute (TSS) than KBG (Fig.8). These results indicated that KBG achieved osmotic adjustment more by inorganic ions than through organic osmolytes. The high reliance on inorganic ions for osmotic adjustment is vital for water uptake under saline environment. However, osmotic adjustment of TF was achieved by both inorganic ion and organic osmolytes. Na^+ and Cl^- concentrations were also mainly instrumental for osmotic adjustment in bermudagrass cultivars (Fig. 19). Na^+ and Cl^- concentrations were highly associated with osmotic potential in all turfgrasses in this study.

Na^+ concentrations in shoots were lower than in roots in KBG and TF (Figs. 1A, 3A). Similar with KBG, Na^+ concentrations of all bermudagrass cultivars were lower in leaves than in roots under salinity treatment (Fig. 14). The specific ion toxicity was minimal by excluding saline ions from shoots. TF exhibited higher root Na^+/shoot Na^+ ratio compared with KBG (Fig. 6). This result indicates a strong ability of TF to prevent Na^+ transport from roots to shoots. The most salt-tolerant Riviera had the highest root Na^+/leaf Na^+ ratio and Cl^-/leaf Cl^- ratio among the four cultivars (Fig. 18). Salt-tolerant bermudagrass cultivars were able to limit saline ions accumulation in the aerial parts. These results indicated that the relative salinity tolerance was related to regulate saline ions distribution in roots and shoots.

Salinity stress can affect essential ion uptake and nutrient balance. High levels of Na^+ and Cl^- decreased the K^+, Ca^{2+}, Mg^{2+} and NO_3^- concentrations in KBG and TF (Fig.1, 2, 3, 4). The K^+ concentrations of bermudagrass cultivars also decreased with increasing salinity (Fig. 15). The degree of ion imbalance caused by salinity stress varies among plant species and within a species. The nutrient imbalance was more significant in KBG than in TF under salt stress. There was a significant difference in leaf and root K^+ concentration between the most (Riviera) and least

(Sundevil 2) salinity tolerant bermudagrass cultivars. Maintenance of high shoot K^+/Na^+ ratios, necessary for proper cellular enzymes function, has been linked to salinity tolerance in turfgrass (Marcum 2008). The Na^+/K^+ ratios in the shoots and roots of KBG increased significantly as salinity increased. However, under salt stress those of TF were significantly lower than those of KBG (Fig. 5). Riviera maintained the highest leaf K^+/Na^+ ratio among the cultivars at all salinity levels (Fig. 17). Uptake and accumulation of K^+ and selectivity for K^+ over Na^+ may occur via root and shoot mechanism in turfgrass. In terms of the understanding of salt tolerance and its evolution, saline ion regulation or selectivity might be important for the physiological and biochemical bases of salinity tolerance.

The results suggested that salinity tolerance of KBG and TF differed in inorganic and compatible solute accumulation for osmotic adjustment. KBG achieved osmotic adjustment more by inorganic solutes than compatible solute based on percentage of their contribution. However, TF accumulated both inorganic solutes and compatible solute (TSS) for osmotic adjustment (Table 4, 5). Na^+ and Cl^- are compartmentalized mainly into the vacuole to avoid toxicity. Compatible solutes are accumulated in cytoplasm for osmotic adjustment and osmoprotection. Higher K^+ and TSS concentrations of TF may explain its better salinity tolerance than KBG. However, the ability to accumulate organic solutes on a whole-cell basis is metabolically expensive and therefore limited. Marcum (2008) reported that turfgrass typically accumulate saline ions for osmotic adjustment, and only glycinebetaine can accumulate enough for cytoplasm osmotic adjustment in salt-tolerant turfgrass. The glycinebetaine concentration of bermudagrass cultivars used in this study needs further investigation.

Salinity induces oxidative stress by increasing ROS. The Na^+ concentration of KBG and TF

was significantly higher in the salt-stressed plants than in the control (Fig. 1, 3). Therefore, the salinity stress induced the accumulation of Na^+, and simultaneously increased the production of ROS due to the excessive ion uptake. The MDA concentration of KBG and TF increased under salinity stress. KBG has higher MDA concentration than TF (Fig. 9). MDA is considered to be an indicator of lipid peroxidation induced by ROS under stress conditions. The increasing MDA concentration of KBG and TF indicated that cell membrane lipid peroxidation has occurred. KBG received more severe lipid peroxidation than TF. Plants normally develop antioxidant defense systems that scavenge ROS and protect cells from oxidative stress injury (Bowler *et al.* 1992). In this study, TF exhibited higher SOD, CAT and APX activities than KBG (Fig. 10, 11, 12), which indicated that TF maintained a higher ability to scavenge ROS and was able to curtail lipid peroxidation and cell membrane damage effectively. An understanding of the role of antioxidants and their signal induction during salinity stress can provide basic knowledge for advancement of turfgrass research. The role of antioxidants of bermudagrass cultivars should be further investigation.

The appearance of salt crystals on leaf surface is indicative of active salt excretion (Fig. 20). Bicellular salt glands were observed in leaf surface of all bermudagrass cultivars (Fig. 21), and salinity tolerance was correlated with salt gland excretion efficiency. The most salt-tolerant Riviera has higher salt gland excretion rate to exclude salt by salt glands (Fig. 22).

In conclusion, KBG, TF and bermudagrass cultivars exhibited differential growth and physiology under salinity stress. These physiological and adaptational responses may aid in understanding salinity tolerance mechanisms and for developing salt-tolerant cultivars.

GENERAL SUMMARY

Increased need for salt-tolerant turfgrasses continues due to increased use of recycled saline water for turfgrass irrigation in the arid and semiarid regions. Turfgrasses growing on saline environments suffer from salinity stress. This study was conducted to determine the salinity tolerance, growth and physiological responses of Kentucky bluegrass (KBG), Tall fescue (TF) and four bermudagrass cultivars (Riviera, Blackjack, Savannah and Sundevil 2). KBG and TF were subjected to 0, 50, 100, 150 and 200 mmol L^{-1} NaCl and bermudagrass cultivars were treated with 0, 100, 200, 300, and 400 mmol L^{-1} NaCl. Salinity tolerance was assessed according to the turf quality, leaf firing and relative shoot growth. The ranking of salinity tolerance was bermudagrass > Tall fescue > Kentucky bluegrass. Salinity tolerance among Bermudagrass cultivars was Riviera > Blackjack > Savannah > Sundevil 2. KBG, TF and bermudagrass cultivars exhibited differential physiological and adaptational response to salinity stress.

Maintenance of relatively stable root growth of TF and root growth stimulation of bermudagrass was a potential adaptive mechanism for salinity tolerance. The most salt-tolerant Riviera has the most significant root growth under moderately salinity among bermudagrass cultivars. Root growth stimulation can increase in root/shoot ratios, and therefore increase in water absorption/transpiration area to resist saline osmotic stress.

Complete osmotic adjustment was observed in all turfgrasses studied in this study. Saline ions (Na^+ and Cl^-) were predominant solute for osmotic adjustment. These saline ions regulation may be one of key mechanisms of salinity tolerance in turfgrasses. The Na^+ and Cl^- concentrations in root were higher than those in shoot or leaf in all turfgrasses. The root

Na^+/shoot Na^+ ratio of TF was higher than that of KBG. The root Na^+/leaf Na^+ ratio of Riviera was the highest among bermudagrass cultivars. Root plays an important role in limiting the transport of Na^+ to the shoot in turfgrass. The exclusion of saline ions from shoot reduces the toxicity to plant growth, which associate with salinity tolerance of turfgrasses studied in this study.

Salt-sensitive KBG had higher shoot and root Na^+ and Cl^- concentrations than moderately salt-tolerant TF. The least salt-tolerant Sundevil 2 maintained the highest leaf and root Na^+ and Cl^- concentrations among four bermudagrass cultivars. KBG typically accumulated saline ions for osmotic adjustment. TF had better salt tolerance than KBG due to maintain a lower level of Na^+ and Cl^- in conjunction with the accumulation of enough total soluble sugars to make osmotic adjustment. Bermudagrass could regulate leaf and root Na^+ and Cl^- at low level by saline ion excretion via leaf salt glands. Salinity tolerance of bermudagrass cultivars was correlated to salt gland excretion efficiency. The different ionic concentrations in KBG, TF and bermudagrass had an apparent relationship to salinity tolerance in turfgrasses.

Uptake and accumulation of K^+ and selectivity for K^+ over Na^+ might play an important role in salinity tolerance. The K^+/Na^+ ratios in the shoots and roots of KBG decreased significantly as salinity increased. However, under salt stress those of TF were significantly higher than those of KBG. This result indicates that TF had a better selectivity for K^+ over Na^+ than KBG via roots and transporting K^+ from roots to shoots under salt stress. In bermudagrass cultivars, Riviera maintained the highest leaf K^+/Na^+ ratio at all salinity levels, indicating a strong capacity for the selectivity for K^+ over Na^+ under salinity stress.

In addition, TF accumulated higher level of total soluble sugar for osmotic potential in the

cytoplasm. TF exhibited a better antioxidant defense system against oxidative stress and lipid peroxidation by maintaining higher antioxidant activities.

In conclusion, developing salt-tolerant turfgrasses has become imperative as increase in recycled water irrigation. Turfgrasses have developed various survival mechanisms in response to salinity stress and showed a wide range in salinity tolerance. Salinity tolerance was related to inhibit the accumulation of Na^+ and Cl^-, which helps to maintain ion balance; and accumulate sugars, which decrease the osmotic potential of the cytoplasm; keep lower MDA and higher antioxidative enzyme activities, which improve the oxidative stress and lipid peroxidation. A thorough knowledge of the salinity tolerance mechanisms might be useful for developing salt-tolerant turfgrasses.

摘 要

乾燥地および半乾燥地では、淡水資源の不足と土壌塩類化のために耐塩性の強いシバの需要が増している。塩ストレスはシバの成長の抑制を招く重要な非生物学的要因の一つである。本研究ではケンタッキーブルーグラス(KBG)、トールフェスク(TF)と 4 品種のバミューダグラス (BG) (Riviera、Blackjack、Savannah、Sundevil 2)を供試植物として、塩ストレス下での生育及び生理特性に関する研究を行った。KBG と TF は基本培養液に NaCl を 0、50、100、150、200 mmol L^{-1} 含むように調製した処理液を用いて栽培した。 BG は基本培養液に NaCl を 0、100、200、300、400 mmol L^{-1} 含むように調製した処理液を用いて栽培した。シバの品質，葉の枯死割合および茎葉の相対成長量を植物種間で比較し，耐塩性を判定した。耐塩性の総合評価では BG ＞ TF ＞ KBG となった。4 品種の BG の耐塩性の総合評価は Riviera ＞ Blackjack ＞ Savannah ＞ Sundevil2 となった。

TFとBGの根の乾物重および根長が塩ストレスによって受ける影響は相対的に小さかった。100、200 mmol L^{-1} NaClでRivieraの根の成長が有意に上昇した。耐塩性の強いシバの全重は減少するが、対照区に比べ茎葉/根比が大きくなることは、根機能が高まることを示している。これらはシバの塩分適応性の特徴と考えられた。

全てのシバは完全に浸透圧調節を行うことができた。それはNa^+とCl^-を大量に蓄積することによって行われた。Na^+とCl^-による調節がシバの耐塩性の特徴であることが明らかになった。全てのシバで根のNa^+とCl^-の濃度は茎葉より高かった。根のNa^+/茎葉のNa^+の比率はTFがKBGより高かった。また、Rivieraの根と葉のNa^+の比率は4品種のBGの中で最も高かった。全てのシバはNa^+とCl^-を大量に蓄積したが、耐塩性の強い品種は根から

茎葉への輸送を抑制した。また、茎葉でのNa^+とCl^-の蓄積やそれに伴う無機元素の減少が成長に与える影響は耐塩性の高い品種の方小さいと考えられた。

KBGとTFの茎葉と根におけるNa^+とCl^-の濃度はNaCl濃度の上昇にともなって上昇したが、KBGはTFよりも多く蓄積した。感受性が高いSundevil 2のNa^+とCl^-の濃度は4品種のBGの中で最も高かった。TFがNa^+やCl^-の蓄積を抑制することによってイオンバランスを維持すること、および糖を蓄積することによって細胞質の浸透ポテンシャルを低下させ、塩に対してより強い抵抗性を獲得することが示された。BGは葉表面の塩類線から塩を効率的に排出することにより、茎葉での塩濃度を低く維持していると考えられる。

耐塩性が高いシバは体内の Na^+と Cl^-濃度が過剰になると茎葉から排出して、Na/K 比の上昇を維持することが明らかにされた。TF は KBG より低い MDA 含有率と高い抗酸化酵素活性を示した。これらのことから、細胞の過酸化防止と H_2O_2 など活性酸素の酵素的な消去機能と耐塩性との間に強い関係があると考えられた。

本研究によって得られたシバの耐塩性機構の概要は次のとおりである。耐塩性の強いシバは Na^+や Cl^-の蓄積を抑制することによってイオンバランスを維持すること、糖を蓄積することによって細胞質の浸透ポテンシャルを低下させること、および低い MDA 含有率と高い抗酸化酵素活性を維持することによって、塩に対してより強い抵抗性を獲得することが示された。これらの成果は今後沿岸地や塩類集積地の緑化再生に用いる耐塩性の強い品種の選抜と開発に利用することができる。

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LIST OF PUBLICATIONS

1. R. XU, M. YAMADA and H. FUJIYAMA. 2013. Lipid Peroxidation and Antioxidative Enzymes of Two Turfgrass Species Under Salinity Stress. Pedosphere. 23(2), 213–222 (Chapter 3).

2. RAN XU and Hideyasu FUJIYAMA. 2013. Comparison of ionic concentration, organic solute accumulation and osmotic adaptation in Kentucky bluegrass and Tall fescue under NaCl stress. Soil Science and Plant Nutrition. 59, 168–179 (Chapter 2).