DISSERTATION

SALINITY TOLERANCE AND ASSOCIATED SALINITY TOLERANCE MECHANISMS OF FOUR TURFGRASSES

Submitted by
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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SAAD FARHAN ALSHAMMARY ENTITLED SALINITY TOLERANCE AND ASSOCIATED SALINITY TOLERANCE MECHANISMS OF FOUR TURFGRASSES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

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ABSTRACT OF DISSERTATION

SALINITY TOLERANCE AND ASSOCIATED SALINITY TOLERANCE MECHANISMS OF FOUR TURFGRASSES

The need for salinity tolerant turfgrasses is increasing because of the increased use of effluent or other low quality water for turfgrass irrigation. Greenhouse container and hydroponic experiments were conducted to determine the relative salinity tolerance, growth characteristics, and physiological responses (especially water and ion relations) of 'Challenger' Kentucky bluegrass (*Poa pratensis* L.) (KBG), 'Arid' tall fescue (*Festuca arundinacea* Schreb) (TF), 'Fults' alkaligrass (*Puccinellia distans* (L.) Parl.) (AG), and saltgrass (*Distichlis spicata* (Torr.) Beetle) (SG). Salinity treatments were applied for 8 weeks using $1NaCl{:}1CaCl_2$ solution at 2.0, 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m. Based on data on shoot dry mass, KBG, TF, AG, and SG experienced a 50% shoot growth reduction at 5.5, 14.2, 23.0, and 34.5 dS/m, respectively, suggesting the ranking of salinity tolerance was SG > AG > TF > KBG. Leaf firing of KBG, TF, and AG increased as salinity increased, but no injury was noticeable in SG. Salinity caused root cortex cells to collapse in KBG at 14.1 dS/m and in TF at 23.5 dS/m. Alkaligrass and SG only had a few cell collapses even at 23.5 dS/m. Osmotic adjustment (OA) occurred in all species under salinity stress. However, in KBG and TF, the contribution of Na^+ and Cl^- to OA increased and became the major contributors at high levels of salinity, whereas Na^+ and Cl^- contributions to OA in SG were maintained at stable levels as salinity increased from 4.7 to 23.5 dS/m. As salinity increased, the contribution of unidentified osmolytes to OA increased in SG and decreased in KBG and TF. The proportion of K^+ to OA in AG and TF was lower than SG but higher than KBG. The ability to maintain a K^+/Na^+ ratio close to or above 1 appeared to be important for these grasses to tolerate high salinity. Saltgrass, AG, TF, and KBG could maintain a shoot K^+/Na^+ ratio of 1 when salinity levels were less than 22.3, 13.6, 7.4, and 3.7 dS/m, respectively. Salt glands present in SG, root growth stimulation of SG and AG, maintenance of high root to shoot ratio in TF, synthesis of compatible solutes, regulation of ion concentrations, and maintenance of high K^+/Na^+ ratio in shoots are important salinity tolerance mechanisms among these grasses.

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Chapter One

Growth Responses of Four Turfgrasses to Salinity

ABSTRACT

The need for salinity tolerant turfgrasses is increasing because of the increased use of effluent or other low quality waters for turfgrass irrigation. Greenhouse container and hydroponic experiments were conducted to determine the relative salinity tolerance, growth responses, and salinity tolerance mechanisms of 'Challenger' Kentucky bluegrass (*Poa pratensis* L.) (KBG), 'Arid' tall fescue (*Festuca arundinacea* Schreb) (TF), 'Fults' alkaligrass (*Puccinellia distans* (L.) Parl.) (AG), and a saltgrass (*Distichlis spicata* (Torr.) Beetle) collocation (SG). In the container experiments, irrigation waters at different salinity levels were applied to experimental plants grown in plastic pots filled with a mix of sand and Isolite. In the hydroponic experiment, grasses were grown in saline solution at 2.0, 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m. Kentucky bluegrass, TF, AG, and SG experienced a 50% shoot growth reduction at 5.5, 14.2, 23.0, and 34.5 dS/m, respectively, and a 50% root growth reduction at 7.9, 21.5, 30.4 and 40.8 dS/m, respectively. Root to shoot ratio of KBG remained constant, whereas root to shoot ratios of TF, AG, and SG increased at all salinity levels. Leaf firing of KBG, TF, and AG increased as salinity increased, but no injury was noticeable in SG. Salinity caused root cortex cells to collapse, in KBG at 14.1 dS/m and in TF at 23.5 dS/m. Alkaligrass and SG only had a few cell collapses even at 23.5 dS/m. Bi-cellular salt glands were observed only on leaves of SG. Based on these results, we ranked the salinity tolerance of selected grasses as: SG > AG > TF > KBG. Salt glands present in SG, root growth stimulation of SG and AG, and maintenance of relatively high root to shoot ratio in TF are apparent adaptive mechanisms exhibited by these grasses for salinity tolerance.

INTRODUCTION

Soil salinity, defined as an accumulation of excess soluble salts in soils, is considered to be one of the major factors that reduce plant growth in many regions in the world. Throughout the world turfgrass has been used in landscapes and household lawns. In a number of western states, fresh water shortage has resulted in restrictions on the use of potable water for landscape irrigation. Consequently, secondary water sources are increasingly being used to irrigate large turf facilities (Arizona Department of Water Resources,1995; California State Water Resources,1993). Seawater intrusion in the coastal states (McCarty and Dudeck, 1993; Murdoch, 1987) and the use of salt for deicing roadways of the northern USA (Hanes et al., 1970) have added to the salinity problems in turfgrass culture. Therefore, the need for salt tolerant turfgrasses has increased (Harivandi et al., 1992).

The detrimental effects of salinity on turfgrass health include osmotic stress, ion toxicity, and nutritional disturbances (Greenway and Munns,1980; Lauchli, 1986; Cheeseman, 1988). Salt tolerant plants have the ability to minimize these detrimental effects by producing a series of anatomical, morphological, and physiological adaptations (Poljakoff-Mayber, 1975 and 1988), such as an extensive root system and salt secreting glands on the leaf surface (Liphschitz and Waisel, 1974; Oross and Thomson, 1982; Gould and Shaw, 1983; Oross et al., 1985; Gorham et al., 1985, Sinha et al., 1986; Marcum and Murdoch, 1990a ; Marcum et al., 1998).

Variations in salt tolerance among turfgrasses has been demonstrated in many studies based on the effects of salinity on turf growth and quality (Dudeck et al.,1983;

Horst and Taylor, 1983; Horst and Beadle, 1984; Torello and Symington, 1984; Chetelat and Wu, 1986; Dean et al., 1996; Marcum and Kopec, 1997; Marcum et al., 1998; Qian et al., 2000 and 2001). Limited information is available concerning the mechanisms of salinity tolerance in different turfgrass species (Torello and Rice, 1986; Marcum and Murdoch ,1994; Marcum et al., 1998; Marcum, 1999).

Kentucky bluegrass (*Poa pratensis* L.), native to Europe, is the most widely used cool-season turfgrass in the temperate and subarctic regions of North America. Tall fescue (*Festuca arundinacea* Schreb) is a cool-season turfgrass best adapted to the transition zone in the U.S. Alkaligrass (*Puccinellia distans* (L.) Parl.) is typically found inhabiting saline and alkaline sites throughout cooler portions of North America. Kentucky bluegrass, tall fescue, and alkaligrass are members of *Festucoideae* subfamily. Saltgrass (*Distichlis spicata* (Torr.) Beetle), a member of *Chlorideae* subfamily, is a warm season grass showing great salt tolerance. A breeding project is in progress at Colorado State University to develop turf-type saltgrass, for which more information is needed concerning the growth responses to different salinity levels.

The major objectives of this study were to: (1) determine the relative salt tolerance and growth responses of four turfgrass species to salinity, (2) determine the salt tolerance mechanisms for the selected grasses.

MATERIALS AND METHODS

Container Experiment

Plant Materials and Growth Conditions

The experiments were carried out from 27 April 1998 to 11 August 1998 and repeated from 15 May 1999 to 28 September 1999, using plastic pots (20 cm in diam. and 20 cm in depth) in a greenhouse at Colorado State University. The plastic pots were filled with 7 kg of a mix of 50 sand: 50 Isolite (w/w). The bulk density of the potting mix was 1.06 g/cm^3. Isolite is a soil amendment derived from diatomaceous earth that reportedly has a high water holding capacity, low cation exchange capacity, and high porosity. The greenhouse temperature was maintained between 25°C day/18 °C night. Before planting, a 6N- 2P- 0K slow release organic fertilizer (Milorganite) was mixed into the top 10 cm of soil in each pot at the rate of 61 kg N ha^{-1}. 'Challenger' Kentucky bluegrass (*Poa pratensis* L.) (KBG), 'Arid' tall fescue (*Festuca arundinacea* Schreb) (TF), 'Fults' alkaligrass (*Puccinellia distans* (L.) Parl.) (AG), and a saltgrass (*Distichlis spicata* (Torr.) Beetle) collocation (SG) were transplanted from sod pieces to the plastic pots and grown for 8 weeks with non-saline irrigation water. Grasses were clipped weekly throughout the experiment to 5 cm for KBG, TF, and AG, and to 3 cm for saltgrass.

Salinity Treatment and Data Collection

Irrigation waters at different salinity levels were prepared by the addition of 1NaCl: 1$CaCl_2$ (w/w) to tap water to obtain desired EC values. Saline waters at 1.7, 4.7, and 9.4 dS/m along with tap water as the control were applied to KBG and TF (Study I), whereas saline waters at 14.1, 18.8, 23.5 dS/m along with the control were applied to AG and SG (Study II). To avoid salinity shock, salinity levels were gradually increased by daily increments of~1.57 dS/m for KBG and TF, and 4.7 dS/m for AG and SG. The irrigation waters were applied for a period of 6 weeks.

During the salinity treatment period, data were collected on leaf firing, turf quality, shoot growth, root growth, and the presence of salt glands. Leaf firing and turf quality were visually estimated weekly. Leaf firing was estimated as the total percentage of chlorotic leaf area, with 0% corresponding to no leaf firing, and 100% as totally brown leaves. Turf quality was estimated based on a scale of 1 to 9, with 9 as green, dense, and uniform turf, and 1 as thin and completely brown turf. Shoots were clipped weekly, washed with deionized water, and dried at 70°C for 24 hours to determine dry weight. At the termination of the experiment shoots were harvested, and roots were clipped. Both shoots and roots were washed with deionized water, and dried at 70°C for 24 hr to determine root dry weight. Root to shoot ratio was calculated based on root dry weight and cumulative shoot dry weight.

The presence of salt glands in the leaves was determined by scanning electron microscopy. Leaf segments 0.5 to 1 cm long were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 for 24 hr, and then washed twice in a 0.1 M sodium cacodylate buffer for 15 minutes each. Leaf segments were post fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer at pH 7.2 for 1.5 hr, then washed three times in a buffer for 10 min each, and washed twice in distilled water for 10 min each. 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, and observed with a Philips SEM-505 scanning electron microscope (FEI Company, Hillsboro, OR) at 20 kV.

Hydroponic Experiment

Plant Materials and Growth Conditions

The experiment was conducted from 14 July 1999 to 13 November 1999, and repeated from 14 April 2000 to 13 August 2000, in a greenhouse at Colorado State University with a solution culture system. Four grasses as described previously were planted into plastic cups (9 cm in diam and 4 cm deep). The cups were filled with a 1cm layer of coarse, sterilized silica sand. The cup bottom was removed and covered with nylon screen to hold sand and allow roots to grow through. Twelve cups were placed into the holes of a plywood lid and suspended over a 50 liter tank, each tank containing 48 liters of full strength Hoagland solution (Hoagland and Arnon, 1950), which was replaced weekly. Air-stones connected to an electric pump provided aeration in each tank. The greenhouse mean temperatures were maintained between 25°C day and 18°C night. Grasses were transplanted and grown for 8 weeks in non-saline water in order to achieve full establishment. One day before starting salinity treatments, roots were clipped at the base of the cups, giving a common baseline for subsequent root measurements, and shoots were clipped to a 3 cm height.

Salinity Treatment and Data Collection

Salinity treatments were applied by the addition of 1NaCl: 1$CaCl_2$ (w/w) to Hoagland solution to obtain EC values of 2.0 (control), 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m. To avoid salinity shock, salinity levels were gradually increased by daily increments of~2.35 dS/m. After the targeted salinity levels were achieved, salinity treatments were continued for a period of 8 weeks. Grasses were clipped weekly to 5 cm for KBG, TF, and AG, and 3 cm for SG throughout the experiments.

Data on leaf firing, turf quality, shoot and root dry weight, and root to shoot ratio were collected as described in the container experiments. Root length was measured as root extension (in cm) from the base of the cup to the farthest extending root. Root cross structure was determined by scanning electron microscope observation. Root segments (5 mm long) were cut at 5 cm from the tip of roots. Sample fixation, preparation, and SEM examination were the same as described previously for leaf samples.

Data Analysis

In the container experiments, a completely randomized design was used with three replications for each treatment. In the hydroponic experiments, a split plot design was used with salt treatment (tank) being the main plot, and grass species within each tank being the subplot. Each treatment had 3 replications. Since experiment (time) X treatment interactions were not significant, data from both experiments were combined; therefore the number of replications was doubled. Combined data were analyzed by analysis of variance (SAS Institute 1989). Treatment means were separated by Fisher's protected LSD. Regression analysis was used to determine the relationships between each variable and the salinity level. The generated regression equations were used to calculate the salinity levels that caused 50% growth reduction of shoots and roots.

RESULTS

Container Experiments

Study I (Kentucky Bluegrass and Tall Fescue)

Relative shoot growth (as a percent of control) decreased with increasing salinity in both species (Fig. 1.1A). Likewise, root growth of KBG decreased as salinity levels increased (Fig. 1.1B). However, root growth of TF did not change significantly. Regression analysis indicated that KBG experienced a 50% shoot and root growth reduction at 4.9 and 5.8 dS/m, respectively, whereas 50% growth reduction of TF was caused by 10.0 dS/m for shoots and 19.6 dS/m for roots.

Growth of TF roots was less adversely affected by salinity than that of shoots, leading to a significant shift in the root to shoot ratio in favor of the root. Tall fescue had a higher root to shoot ratio than KBG at 4.7 and 9.4 dS/m salinity levels (Fig. 1.1C).

Leaf firing percentage for both species increased as salinity increased, reaching ~50% for KBG and ~20% for TF at 9.4 dS/m (Fig. 1.2A). Although, turf quality decreased for both species at 4.7 and 9.4 dS/m, TF exhibited better turf quality than KBG when irrigated with saline water at 4.7 and 9.8 dS/m (Fig. 1.2B). During the study period, TF maintained a minimal acceptable quality even at 9.8 dS/m, whereas turf quality of KBG dropped to an unacceptable level when salinity reached 4.7 dS/m.

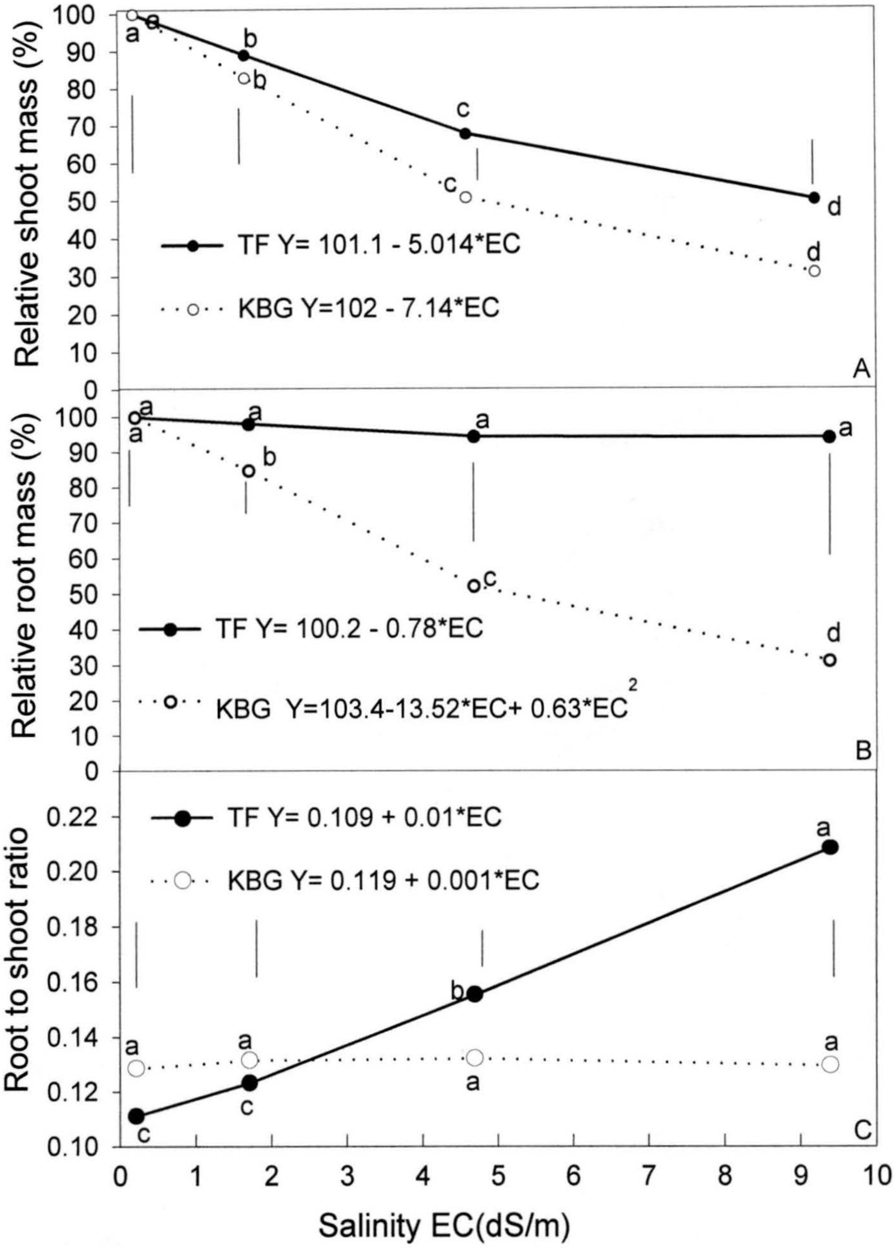

Fig. 1.1. Relative shoot and root growth and root to shoot ratio of tall fescue (TF) and Kentucky bluegrass (KBG) irrigated with saline solution at 0.2, 1.7, 4.7, and 9.4 dS/m. Vertical bars indicate least significant difference (*P* =0.05) between species within a given salinity level. Different letters indicate significant difference (*P* =0.05) among salinity treatments for each species.

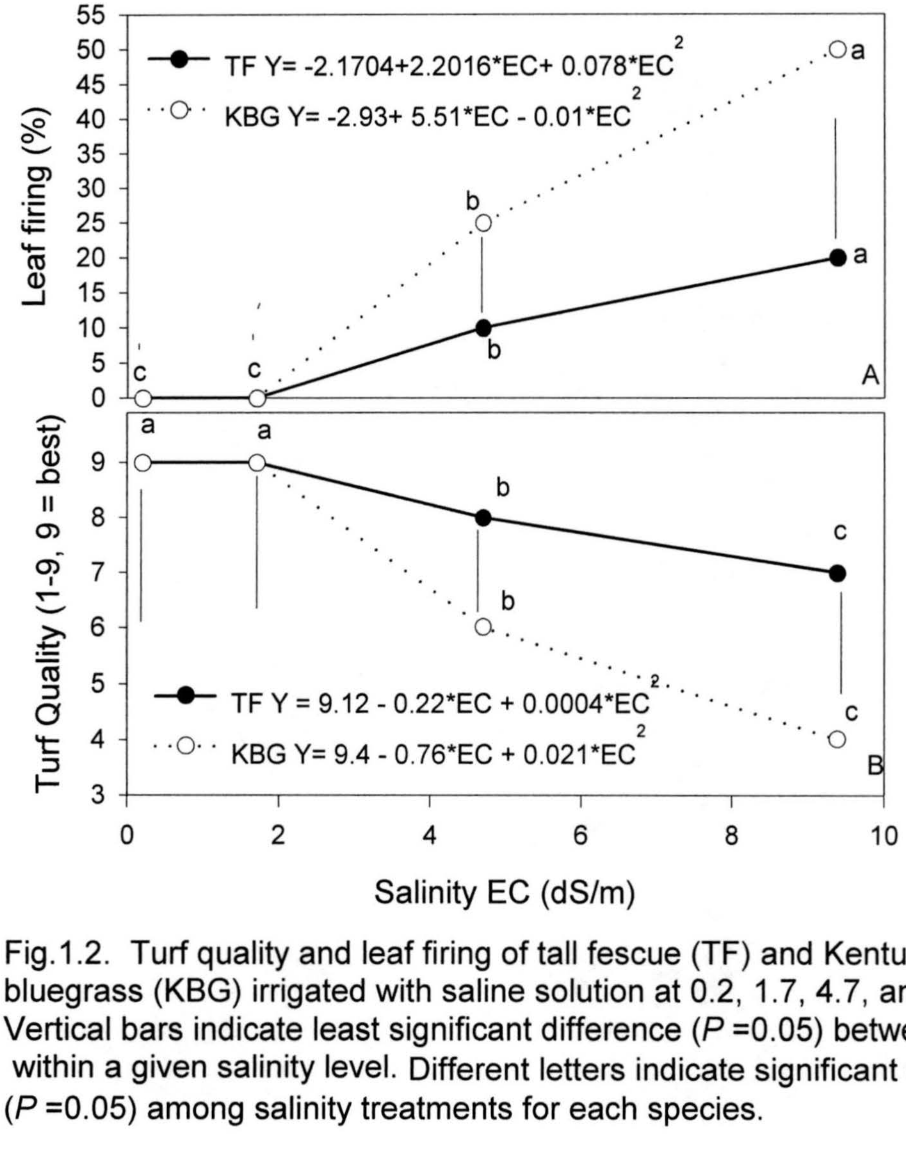

Fig.1.2. Turf quality and leaf firing of tall fescue (TF) and Kentucky bluegrass (KBG) irrigated with saline solution at 0.2, 1.7, 4.7, and 9.4 dS/m. Vertical bars indicate least significant difference (*P* =0.05) between species within a given salinity level. Different letters indicate significant difference (*P* =0.05) among salinity treatments for each species.

Study II (Alkaligrass and Saltgrass)

Differences in relative shoot growth were found between AG and SG (Fig. 1.3A). While shoot growth of AG decreased as salinity increased, the shoot growth of SG did not change with increasing levels of salinity. Root growth of SG was about 90% higher in salinity treatments than in the control. Although not statistically significant, AG exhibited a slight increase in root mass at 14.1 dS/m, then decreased as salinity increased further. Alkaligrass experienced a 50% shoot growth reduction at 20.0 dS/m and 50% root growth reduction at 24.9 dS/m. Regression analysis predicted that 50% growth reduction of SG would occur at 34.9 dS/m for shoots and at 41.0 dS/m for roots. As salinity increased from control to 23.5 dS/m, root to shoot ratio of AG and SG exhibited a 3-fold and 2-fold increase, respectively. Alkaligrass consistently produced much higher root to shoot ratios than SG at all salinity levels (Fig. 1.3C).

Leaf firing of AG increased linearly with increasing salinity, but leaf firing of SG did not occur until the level of salinity reached 18.8 dS/m. Leaf firing of AG and SG (Fig. 1.4A) was 70% and 15%, respectively, at 23.5 dS/m. Under non-saline condition, AG produced a turf quality rating 4 units higher than SG. Turf quality was significantly decreased for AG with increasing salinity, but for SG turf quality at 18.8, and 23.5 dS/m was reduced only slightly (Fig. 1.4B).

Scanning electron microscopy revealed salt secreting glands only on the leaf surfaces of saltgrass (Fig. 1.5). These salt glands were located at leaf surfaces in longitudinal rows parallel to rows of stomata. Salt glands were bi-cellular with a basal and a cap cell. Ion secretion activity of salt glands in SG will be further discussed in Chapter 3.

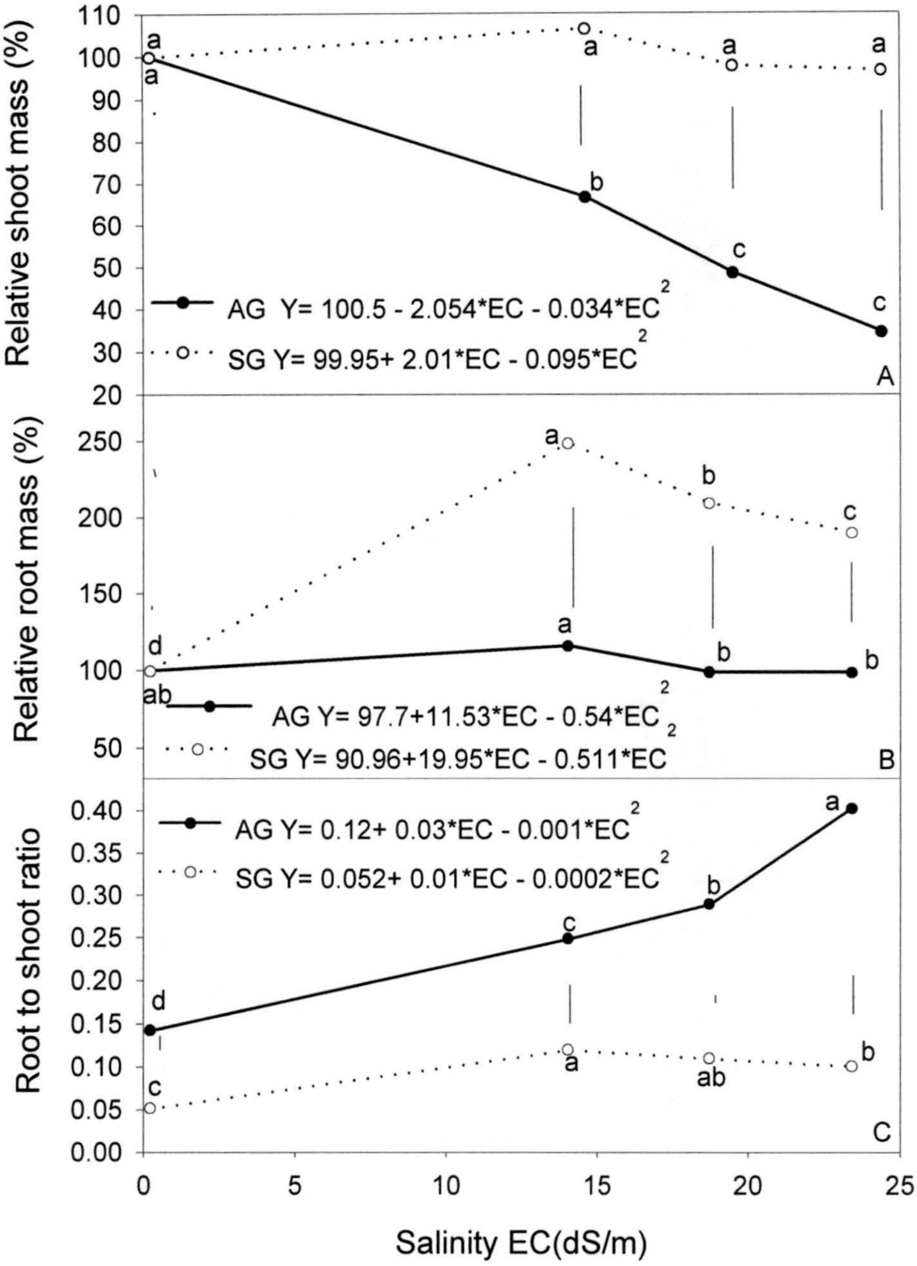

Fig. 1.3. Relative shoot and root growth and root to shoot ratio of alkaligrass (AG) and saltgrass (SG) irrigated with saline solution at 0.2, 14.1, 18.8, and 23.5 dS/m. Vertical bars indicate least significant difference (*P* =0.05) between species within a given salinity level. Different letters indicate significant difference (*P* =0.05) among salinity treatments for each species.

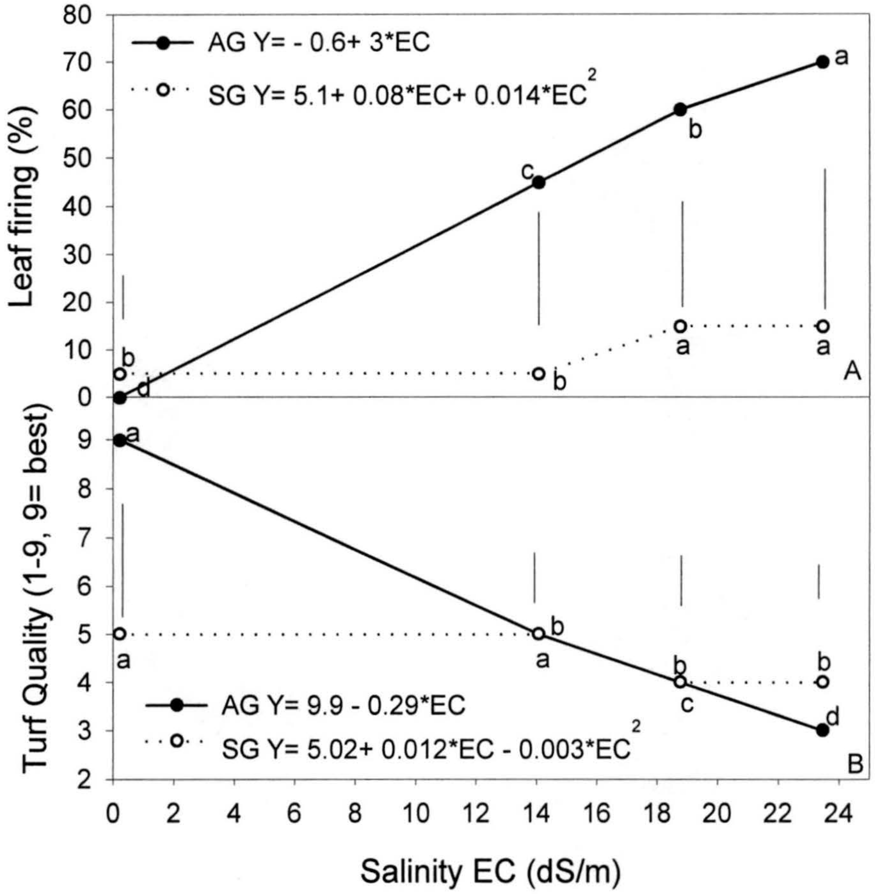

Fig. 1.4. Turf quality and leaf firing of alkaligrass (AG) and saltgrass (SG). irrigated with saline solution at 0.2, 14.1, 18.8, and 23.5 dS/m. Vertical bars indicate least significant difference (*P* =0.05) between species within a given salinity level. Different letters indicate significant difference (*P* =0.05) among salinity treatments for each species.

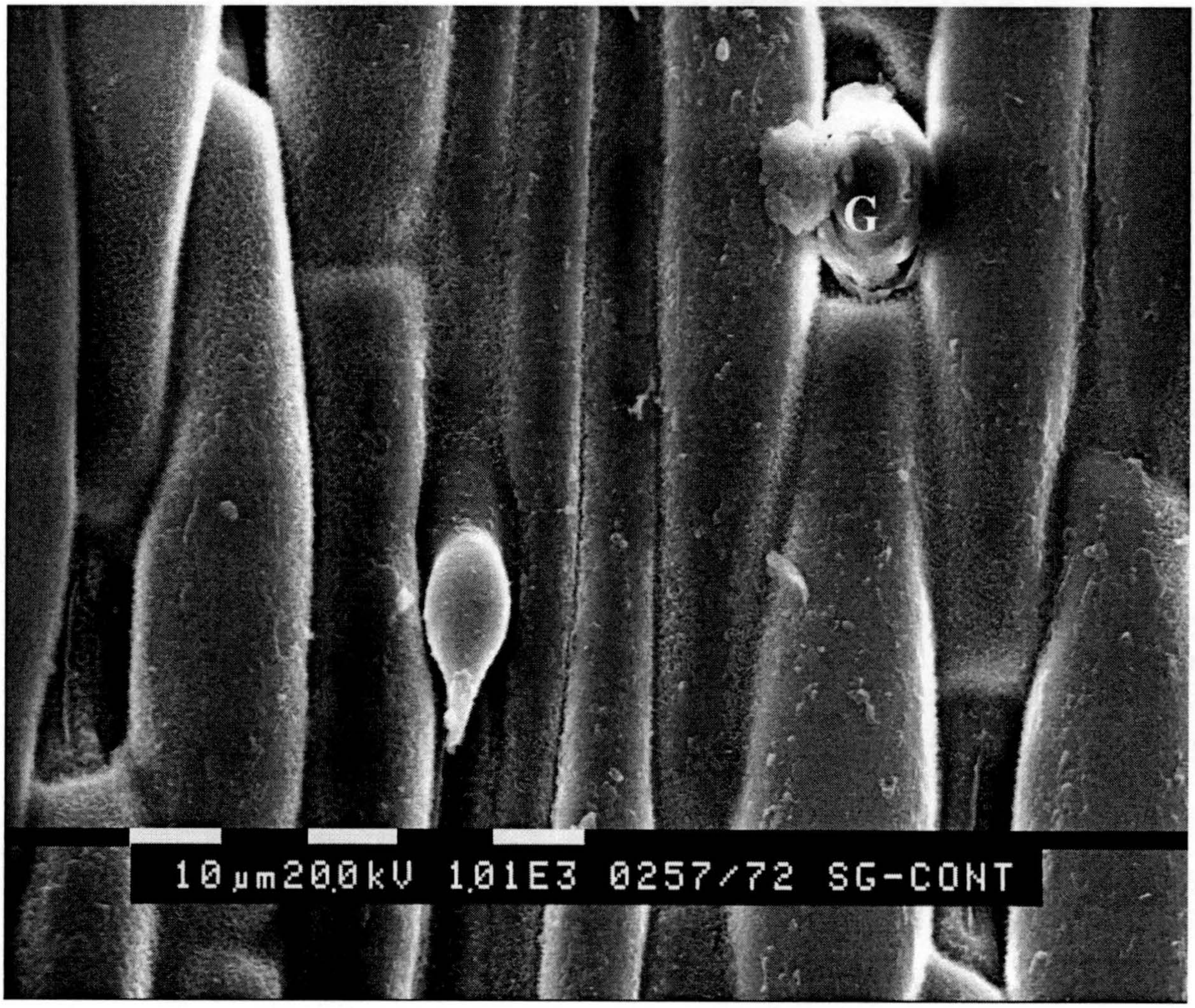

Fig.1.5. Scanning electron microscopy photographs showing salt glands (G) on the leaf surface of saltgrass.

Hydroponic Experiment

Increasing salinity linearly reduced hydroponic shoot growth in KBG, TF, and AG, but not in SG; growth of SG was maintained at a constant level across salinity levels (Table 1.1). Root growth and root length of SG were significantly stimulated under all levels of salinity, whereas root growth and root length of AG were increased at 4.7, 9.4, and 14.1 dS/m, then subsequently decreased at 18.8, and 23.5 dS/m. Tall fescue maintained a stable root mass as salinity increased from control to 14.1 dS/m, then decreased significantly at 23.5 dS/m. Root growth of KBG reduced dramatically with increasing salinity. Regression analysis predicted that KBG, TF, AG, and SG experienced a 50% shoot growth reduction at 5.5, 14.2, 23.0, and 34.5 dS/m, respectively, and a 50% root growth reduction at 7.9, 21.5, 30.4 and 40.8 dS/m, respectively (Table 1.1).

Root growth of all species except KBG was less adversely affected by salinity than that of shoots, leading to significant increase in root to shoot ratio (Table 1.1). Root to shoot ratio of KBG remained almost constant at all salinity levels, whereas root to shoot ratio of TF, AG, and SG were increased significantly at all salinity levels. Root to shoot ratio of TF and AG were consistently higher than that of KBG and SG.

Leaf firing of KBG, TF and AG increased with increasing salinity, reaching 95%, 70%, and 45%, respectively, at 18.8 dS/m. However, there was almost no salinity injury noticeable in SG at all levels of salinity (Table 1.1). Turf quality of KBG, TF and AG was significantly decreased with increasing salinity. In contrast, turf quality of SG was unaffected under all salinity levels, although it produced poorer turf quality than other grasses under non- saline or low salinity conditions, in the systems used in this study.

With scanning electron microscopy, cell damages in the root cortex were observed. These damages may result from root cortex cell collapse. Some KBG cortical cells collapse in the 14.1dS/m treatment, and this condition became severe at 18.8 dS/m (Fig. 1.6). Tall fescue showed severe cortical cell collapse at 23.5 dS/m (Fig. 1.7), but AG (Fig. 1.8) and SG (Fig. 1.9) had few collapsed cells, and only in the 23.5 dS/m treatment.

Table 1.1. Relative shoot, and root growth, relative root length, root/shoot ratio, leaf firing, and turf quality of alkaligrass, saltgrass, tall fescue, and Kentucky bluegrass exposed to salinity stress (dS/m) in the Hydroponic Study.

Salinity level	Shoot growth (%)	Root growth (%)	Root length (%)	Root to shoot ratio	Leaf firing (%)	Turf quality
			Alkaligrass			
2.0	100 aA[Z]	100 abA	100 cA	0.086 b A	0 f B	9 a A
4.7	80.4 bB	111.4 aB	121 a B	0.117abA	5 e C	8 b A
9.4	73.8 c B	110.4 aB	119.2ab A	0.127 aB	25 d B	7 c A
14.1	67.7 d B	105.8abB	113 abc B	0.133aB	35 c C	6 d A
18.8	54.8 e B	90.4 abB	100.7 bcB	0.133aA	45 bC	5 e AB
23.5	48.9 f B	76.5 b B	95.3 c B	0.133aA	55 a B	4 f B
Significance[x]	L***	Q***	Q***	Q**	L***	L***
			Saltgrass			
2.0	100 a A	100 b A	100 d A	0.050 b A	5 a A	6 a B
4.7	101.7 aA	147.3 aA	130.1 a A	0.073 a B	5 a C	6 a B
9.4	100.3 aA	142.1 aA	120.7 c A	0.071 a C	5 a C	6 a B
14.1	101.8 aA	149.3 aA	127.3abA	0.074 a C	5 a D	6 a A
18.8	100.3 aA	146.9 aA	126abc A	0.073 a B	5 a D	6 a A
23.5	97.8 a A	129.7 aA	122.7bcA	0.067 a B	5 a C	6 a A
Significance	Q***	Q***	Q***	Q**	ns	ns
			Tall fescue			
2.0	100 a A	100 aA	100 a A	0.078 c A	0 f B	9 a A
4.7	79.4 b B	99.1 aC	97.2 a C	0.098 bcA	10 eB	7.5 b A
9.4	55.6 c C	97.4 a B	95.9 a C	0.136 a A	25 d B	6 c B
14.1	50.5 c C	93.77 aC	93.8 a C	0.145 a A	45 c B	4.5 d B
18.8	33.8 d C	62.7 b C	84.7 a C	0.144 aA	70 b B	3 e C
23.5	23.9 e C	39.5 c C	61.4 b C	0.123 abA	80 a A	2 f C
Significance	L***	L***	L***	Q***	L***	L***
			Kentucky bluegrass			
2.0	100 a A	100 a A	100 a A	0.064 a A	0 e B	9 a A
4.7	51.2 b C	57.5 b D	70.1 b D	0.072a B	20 d A	6 b B
9.4	41.7 c D	46 c C	51.1 c D	0.071 a C	45 c A	4 c C
14.1	20.4 d D	24 d D	26.3 d D	0.076 a C	70 b A	2 d C
18.8	9.1 e D	9.5 e D	13.3 e D	0.067 a B	95 a A	1 f D
Significance	L***	L***	L***	ns	L***	L***

[Z] Lowercase letters indicate significant differences (P =0.05) among salinity treatments for each species.

Uppercase letters indicate significant differences (P =0.05) among species within a given salinity level.

[x] ns, *, **, and *** indicate non-significant or significant linear (L) or quadratic (Q) regression at P = 0.05, 0.01, and 0.001 level, respectively.

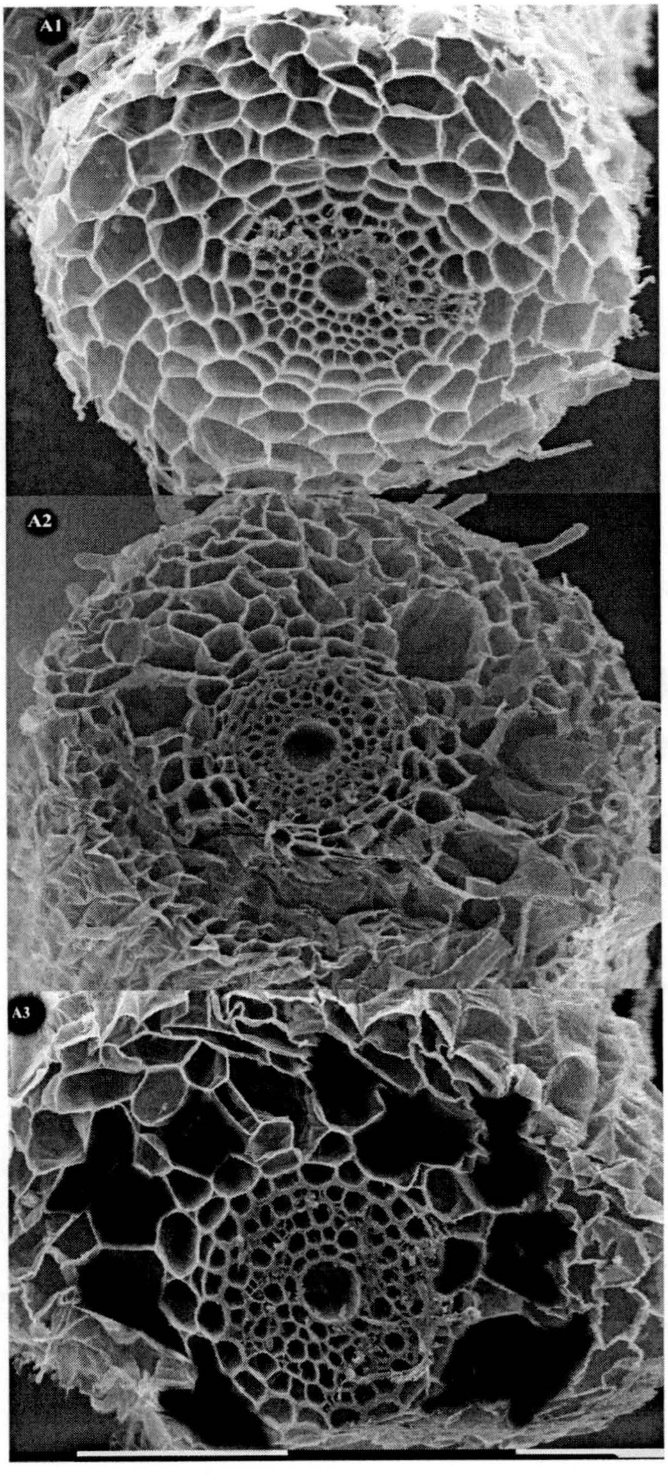

Fig. 1.6. Scanning electron microscopy photographs showing root cortical tissue of Kentucky bluegrass under control (A1), 14.1 dS/m (A2), and 18.8 dS/m (A3) salinity levels.

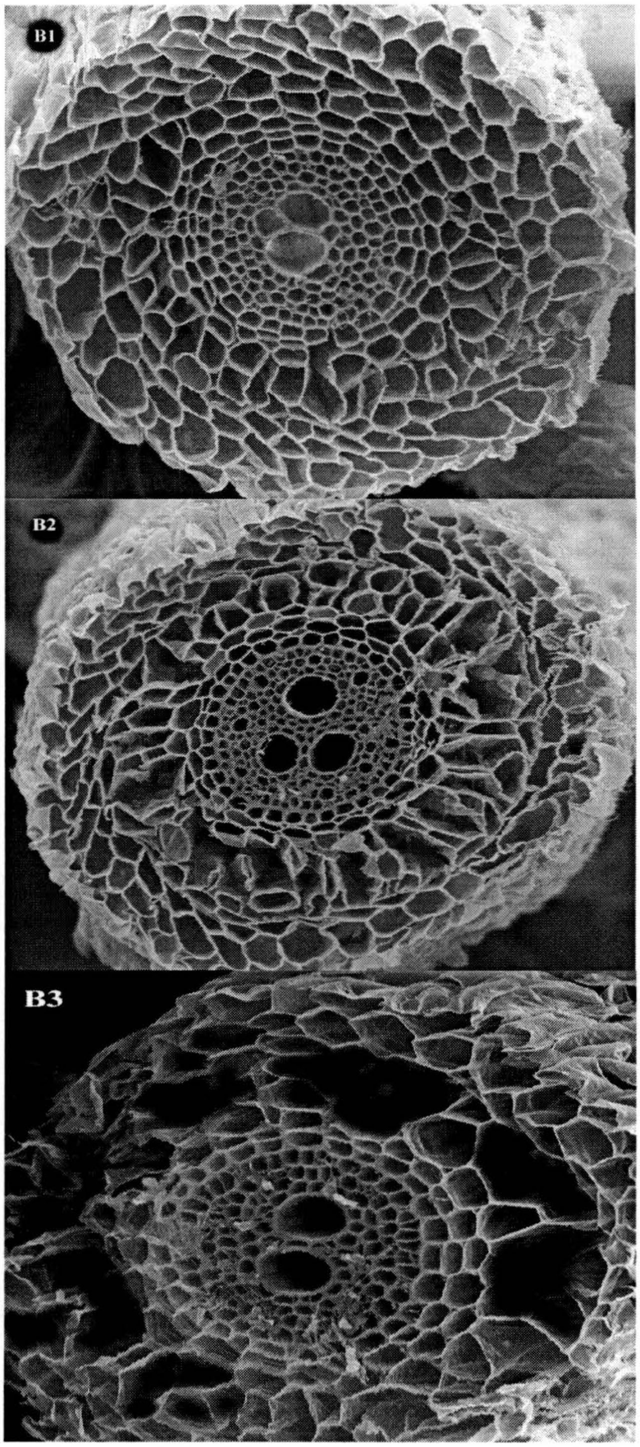

Fig. 1.7. Scanning electron microscopy photographs showing root cortical tissue of tall fescue under control (B1), 14.1 dS/m (B2), and 23.5 dS/m (B3) salinity levels.

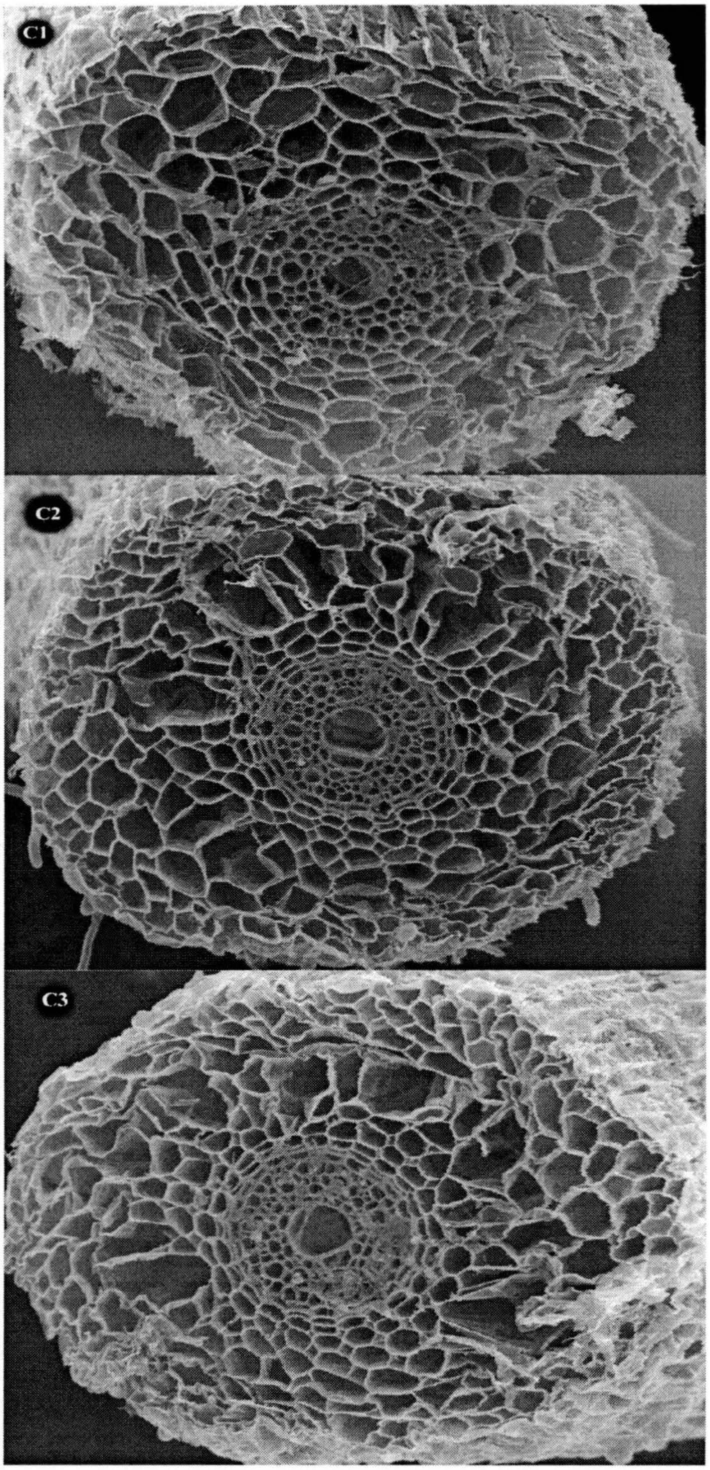

Fig. 1.8. Scanning electron microscopy photographs showing root cortical tissue of alkaligrass under control (C1), 14.1 dS/m (C2), and 23.5 dS/m (C3) salinity levels.

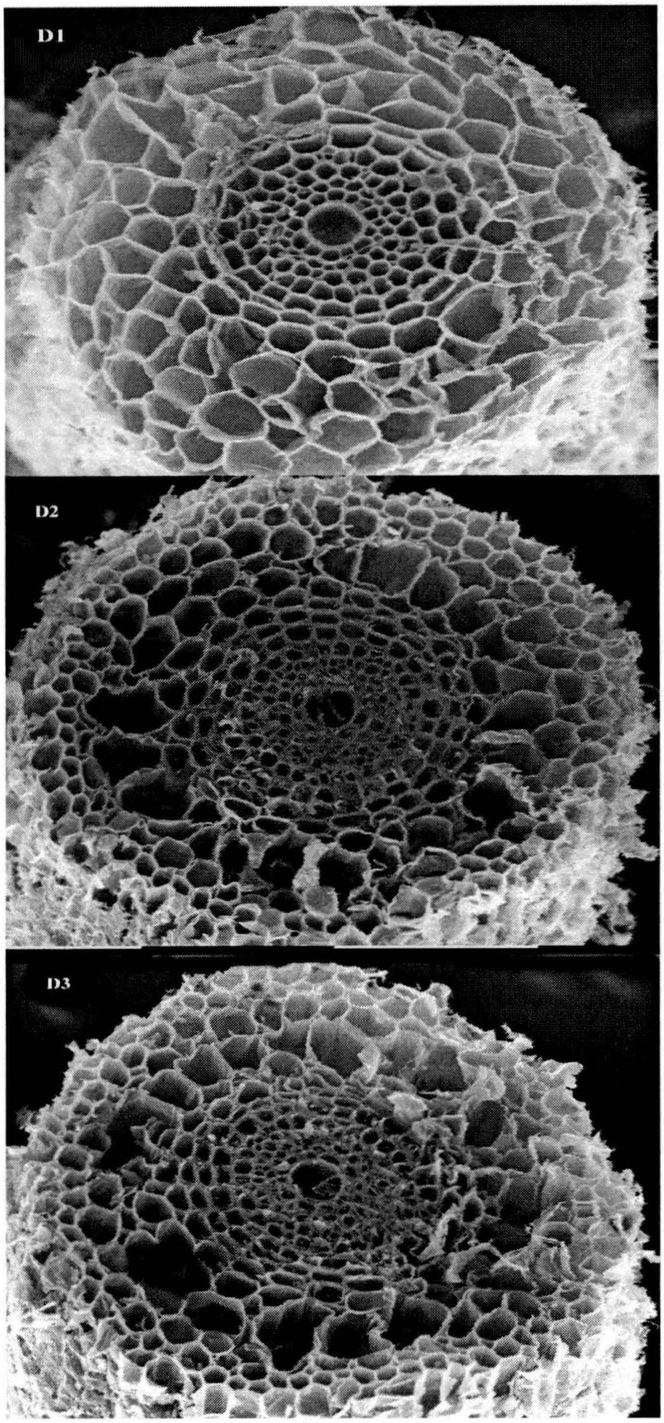

Fig. 1.9. Scanning electron microscopy photographs showing root cortical tissue of saltgrass under control (D1), 14.1 dS/m (D2), and 23.5 dS/m (D3) salinity levels.

Table 1. 2. The salinity level (dS/m) that caused 50 % shoot, and root growth reduction, and 50 % leaf firing of alkaligrass (AG), saltgrass (SG), tall fescue (TF), and Kentuky bluegrass (KBG) in the Hydroponic, and Container Studies.

Parameter	KBG		TF		AG		SG	
	Hydroponic	Container	Hydroponic	Container	Hydroponic	Container	Hydroponic	Container
50% shoot growth reduction	5.5	4.9	14.2	10.0	23.0	20.0	34.5	34.9
50% root growth reduction	7.9	5.8	21.5	19.6	30.4	24.9	40.8	41.0
50% leaf firing	10	9.4	15.28	15.5	21.0	15.4	N/A	54.5

DISCUSSION

Growth parameters, such as shoot growth (Francois, 1988; Marcum and Murdoch, 1990 b), root mass, root length (Marcum and Kopec, 1997; Marcum, 1999), and turf quality (Dean et al., 1996, Marcum and Kopec, 1997; Marcum, 1999) have been reported to be excellent criteria to determine salinity tolerance among turfgrasses. Based on data on growth parameters (relative shoot growth, 50% shoot growth reduction, leaf firing, and turf quality) we ranked the salinity tolerance of selected grasses as: SG > AG > TF> KBG.

From a greenhouse study, Adams (1963) reported that growth of SG was not reduced by salinity up to 2% NaCl (~ 31.3 dS/m). Pasternak et al. (1993) indicated that dry matter yields of SG increased with increasing salinity ranging from 3 to 14 dS/m, and that SG was more salt tolerant than seashore paspalum (*Papsalum vaginatum* Swartz) and bermudagrass (*Cynodon dactylon* L.). Shoot growth and leaf firing results of SG in our studies were also in agreement with the findings of Marcum and Kopec (1997) and Marcum (1999).

Horst and Taylor (1983) studied the effect of salinity (a mixture of NaCl and $CaCl_2$) on 44 cultivars of Kentucky bluegrass and reported that growth was reduced 50% at a salt concentration of 7,500 ppm (about 11 dS/m), which was higher than the value generated in this study. Qian et al (2001) reported that a 2 dS/m difference in salinity caused 50% shoot growth reduction in two Kentucky bluegrass cultivars.

Bower et al. (1970) indicated that yield production of TF was reduced significantly when the salinity level increased from 4 to 12 dS/m. Dean et al. (1996)

found that shoot growth and turf quality of TF were reduced as the salinity level of irrigation water increased from 1.1 to 6 dS/m.

Several studies have found that, among cool season turfgrass species, AG is the most tolerant, TF intermediate, and KBG the least salt tolerant species (Lunt et al., 1961; Harivandi et al., 1982; Torello and Symington, 1984; Rose-Fricker and Wipff, 2001). In general, our findings were in agreement with the results of these studies.

Growth limitation at high salinity may be due to: 1) a depletion of energy that is needed for growth and 2) the loss of turgor. Energy loss can result from diversion of photosynthates from growth functions to active ion transport and uptake (McCree, 1986; Munns and Termaat, 1986; Greenway and Munns, 1983; Yeo, 1983). Under high salinity, cell expansion could be reduced by accumulation of salts in cell walls that would effectively reduce cell turgor and consequently reduce growth (Oertli, 1968; Flowers and Yeo, 1986).

Root growth stimulation under saline conditions has been observed in bermudagrass (Dudeck et al.,1983), St. Augustinegrass and seashore paspalum (Dudeck and Peacock, 1993), salt marsh grass (*Sporobolus virginicus* L.*)* (Marcum and Murdoch, 1992), SG, and alkali sacaton (*Sporobolus airoides* Torr.) (Marcum, 1999). Root growth stimulation of SG and AG, and the extensive root system of TF observed in our studies may be adaptive mechanisms enabling these plants to maintain water balance. The lack of ability to adjust root/shoot ratio in KBG may result in its poor salinity tolerance.

Poljakoff-Mayber (1988) demonstrated that exposure of plant roots to salinity altered the dimensions, shape and volume of epidermal and cortical cells; they became bigger and more isodiametric, with numerous intercellular spaces. These changes were often observed similarly in halophytes and glycophytes, but the levels of salinity that caused such changes were usually two to ten times higher for halophytes than for glycophytes. Our studies indicated that salinity damaged root structure as a result of cortical cell collapse in KBG and TF. The structure damage in cortical tissue could interrupt radial water movement in the roots (Huck et al., 1970), thus limiting water uptake.

Salt glands have been discovered in over thirty species of the tribes *Aeluropodeae, Chlorideae, and Sporoboleae* (Liphschitz and Waisel, 1974). Many members of the *Chlorideae* subfamily have salt glands (Oross and Thomson, 1982; Gould and Shaw, 1983; Oross et al., 1985; Marcum and Murdoch, 1990 a; Marcum et al., 1998). In our study salt glands were observed by scanning electron microscopy on leaves of SG, which is a member of *Chlorideae,* but not in the other grasses, which are members of the *Festucoideae* subfamily.

Data on salinity levels that caused 50% shoot and root growth reductions and 50% leaf firing were generated from both container and hydroponic studies (Table 1.2). Results of t test comparisons indicated that the salinity levels caused 50% growth reduction were lower in the container study than those in the hydroponic study. This may have been because water and nutrients are more readily available in solution culture conditions than in soil culture conditions. Therefore, the results of hydroponic experiments may overestimate plants' salinity tolerance.

In conclusion, with the data described in this paper, we documented the relative salinity tolerance of KBG, TF, AG, and SG. Root growth, root structure, and root to shoot ratio were closely associated with the salinity tolerance of the grasses used in this experiment. In addition, the salt glands present in leaves of SG may contribute to the superior salt tolerance of SG.

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Chapter Two

Effect of Salinity on Water Relations of Four Turfgrasses

ABSTRACT

Little information is available concerning turfgrass water relations characteristics in response to salinity. Greenhouse container and hydroponic experiments were conducted to determine if water relations characteristics were associated with salt tolerance of Kentucky bluegrass (*Poa pratensis* L.) (KBG), tall fescue (*Festuca arundinacea* Schreb) (TF), alkaligrass (*Puccinellia distans* (L.) Parl.) (AG), and saltgrass (*Distichlis spicata* (Torr.) Beetle) collection (SG). In the container experiments, irrigation water at different salinity levels was applied to plants grown in plastic pots filled with a mix of sand and Isolite. In the hydroponic experiment, grasses were grown in saline solution at 2.0, 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m. In the container experiments, TF exhibited slower reductions in leaf water content (LWC) and pressure potential (Ψ_P) than KBG as salinity increased. Although AG had higher LWC, it had lower Ψ_P than SG. In the hydroponic study, salinity reduced LWC of all species, but the degree of LWC reduction were KBG > TF = AG > SG as salinity increased from 2.0 to 23.5 dS/m. Pressure potential (Ψ_P) of KBG, TF, and AG decreased with increasing salinity, whereas SG Ψ_P did not change. Osmotic adjustment (OA) occurred in all grass species under salinity stress, even in salt sensitive KBG. However, in KBG and TF, the contribution of Na^+ and Cl^- to OA increased and these ions became the major contributors at high levels of salinity, whereas Na^+ and Cl^- contributions to OA in SG were maintained

at stable levels (15% for Na and 13% by Cl) as salinity increased from 4.7 to 23.5 dS/m. Saltgrass showed the highest proportion of K^+ at all levels of salinity than other grasses. The proportion of K^+ to OA in AG and TF was lower than in SG but higher than in KBG. As salinity increased, the contribution of unidentified osmolytes to OA increased in SG and decreased in KBG and TF. These interspecific differences in water relations may be, in part, attributed to the different salinity tolerance observed in these grasses.

INTRODUCTION

The need for salt tolerant turfgrass has increased (Harivandi et al., 1992) because of salt accumulation in soil (Hoos, 1981), increased restrictions on the use of potable water for landscape irrigation, saltwater intrusion in the groundwater (McCarty and Dudeck, 1993; Murdoch, 1987), and the use of salt for deicing roadways (Hanes et al., 1970). Turfgrass species and cultivars differ in their responses to salinity (Lunt et al., 1961; Harivandi et al., 1982; Dudeck et al., 1983; Horst and Taylor, 1983; Horst and Beadle, 1984; Dudeck and Peacock, 1985; and Dudeck et al., 1993).

In response to salinity, some plants can adjust osmotically to maintain growth and turgor. Osmotic adjustment under saline conditions can occur in plants by the uptake of inorganic ions from the medium, compartmentalizing ions in the cell vacuole, and balancing osmotic potential in vacuoles by the synthesis of compatible organic solutes in the cytoplasm (Rains, 1972; Hellebust, 1976; Flowers et al., 1977; Zimmermann, 1978; Raven, 1985; Gorham, 1995; Paleg et al., 1985; Jacoby, 1999). Compatible solutes have been identified and characterized as having properties of low polar charges, high solubility, and large hydration shell (Paleg et al., 1985). Polyols (glycerol and sorbitol), amino acids (proline and asparatic acid), methylated quaternary ammonium compounds (glycinebetaine and alaninebetaine) and reducing sugars (such as glucose and sucrose) have been reported to serve as compatible solutes and protect cytoplasmic enzymes against inactivation by inorganic ions (Pollard and Wyn Jones, 1979; Wyn Jones, 1984). Proline in many halophytes, in particular grasses, has been suggested to be a compatible solute under saline conditions (Stewart and Lee, 1974). Gorham et al. (1980) reported that the common salt marsh (*Puccinllia martima* (Huds) Parl.) accumulated a high level of proline as a response to salt stress, and red fescue (*Festuca rubra* L.) and common salt marsh accumulated sugars and proline under salt stress (Briens and Larher, 1982).

In Chapter One, we reported that salinity levels that caused 50% shoot growth reduction were 5.5, 14.2, 23.0, and 34.5 dS/m for KBG, TF, AG and SG, respectively, indicating significant differences in salt tolerance among these species. We also found that SG and AG showed significant root growth stimulation under moderate salinity levels. Root to shoot ratio of TF and AG were about two times higher than SG and KBG under saline conditions. It is possible that different salinity tolerance among these grasses is associated with their water relation characteristics. Information is lack concerning the potential relationships between water relations and salinity tolerance of these selected grasses. Hence, the major objectives of this study were to (1) examine water relations of KBG, TF, AG, and SG in response to salinity, and (2) determine if water relation characteristics were associated with salt tolerance for the selected grasses.

MATERIALS AND METHODS

Plant Materials, Growth Conditions, and Salinity Treatments

Container and hydroponic experiments were carried out in a greenhouse at Colorado State University. 'Challenger' Kentucky bluegrass (*Poa pratensis* L.) (KBG), 'Arid' tall fescue (*Festuca arundinacea* Schreb) (TF), 'Fults' alkaligrass (*Puccinellia distans* (L.) Parl.) (AG), and a saltgrass (*Distichlis spicata* (Torr.) Beetle) collection (SG) from Fort Collins, CO were transplanted and grown for 8 weeks with non-saline irrigation water (0.2 dS/m). Saline water (a mixture of 1NaCl:1$CaCl_2$) at 0.2(control), 1.7, 4.7, and 9.4 dS/m were applied to KBG and TF (Container study I), whereas saline water at 0.2 (control), 14.1, 18.8, 23.5 dS/m were applied to AG and SG (Container study II). Salinity treatments applied in hydroponic experiments were 2.0(control), 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m. Salinity treatments were continued for 6 and 8 weeks in the container and hydroponic experiments, respectively.

Data Collection

Leaf water content. Leaf water content (LWC) was measured 5 and 7 weeks after salinity treatments in the container and hydroponic experiments, respectively. To determine LWC, leaves were cut from plants and quickly placed in a clean, dry glass vial and capped. After determining fresh weight (FW), leaves were dried in an oven at 70°C for 24 hr to determine dry weight (DW). Leaf water content was calculated as:

LWC (%) =[FW- DW/ FW] X 100.

Leaf water, osmotic, and pressure potentials. Leaf water potential (Ψ_W) and leaf osmotic potential (Ψ_O) were measured 5 weeks after salinity treatments in the container experiments, and 7 weeks after salinity treatments in the hydroponic experiments. To determine Ψ_W, leaf pieces along the mid-section of the leaf blades were cut and placed directly into clean and dried psychrometer sample cups. The sample cups were immediately sealed with parafilm and brought to the laboratory in a cooler. In the laboratory, the sample cups were loaded into the psychrometer wells (model SC10 A, Decagon Devices Inc., Pullman, WA), and Ψ_W was measured after 2 hr vaporpressure equilibration (Brown and Collins, 1980). To determine $\Psi_{O,}$ the sample cups containing leaf samples were unloaded from psychrometer wells, sealed with a parafilm and frozen at –20°C for at least 24 hr (Volaire and Thomas, 1995). After being removed from freezer and thawed for 30 min, the sample cups were immediately loaded into the psychrometer wells, and Ψ_O was determined after 2 hr of equilibration. Leaf pressure (turgor) potential (Ψ_P) was calculated as the difference between Ψ_W and Ψ_O.

Osmotic adjustment. Osmotic adjustment (OA) was calculated as the difference in leaf osmotic potential at full turgor ($\Psi^{100}{}_O$) between non-stressed (one day prior to salinity treatment) and stressed leaves (5-7 weeks after salinity treatment). To determine $\Psi^{100}{}_O$, leaves were brought to full hydration by submerging them in distilled water for 4 hr (Babu et al., 1999). Samples were gently wiped to remove external moisture. Fully hydrated samples were placed in microcentrifuge tubes and frozen at –20°C for at least 24 hr. Frozen samples were thawed and crushed in a hydraulic press to release plant sap, and $\Psi^{100}{}_{O}$ was measured by a vaporpressure osmometer (Wescor model 5520, Wescor, Inc., Logan, UT).

Proline determination. Shoot proline content was determined in both experiments 5-7 weeks after the initiation of salinity treatments. Based on the method described by Bates et al. (1973), about 0.5 g leaf tissue was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. After filtration, 2 ml of extract was reacted with 2 ml glacial acetic acid and 2 ml acid-ninhydrin in a test tube for 1 hr at 97°C. The reaction mixture was cooled in an ice bath, 4 ml toluene were added, and it was mixed vigorously. The separated top toluene layer was used for proline measurement with a spectrophotometer (Beckman DU-50, Beckman Instruments, Inc. Fullerton, CA); proline concentration was presented as μmole proline/g FW.

Osmotic adjustment contributions. The relative contributions of ions and proline to whole cell OA were estimated using data on leaf sap ion concentrations, proline content, and sap Ψ_o as described in the Van't Hoff equation (Salisbury and Ross, 1992), assuming an osmotic coefficient of 1 for proline and 0.9 for ions (Lang, 1967). Leaf sap ion concentrations were determined 7 days prior to experiment termination. Intact leaves were thoroughly rinsed with distilled water to remove all external salt. After air drying, leaf samples were clipped and frozen with dry ice. Leaf samples were later thawed, and sap expressed using a hydraulic press. Sap was analyzed for Na, Ca, Mg, and K by inductively coupled plasma atomic emission spectrometry (ICP-AES). Chloride was determined with a Cl-selective electrode (Orion Ionplus Chloride combination electrode). Concurrent with leaf sap ion concentration sampling, leaves were sampled to determine sap Ψ_o. To determine sap Ψ_o, leaf pieces along the mid-section of the leaf blades were cut and placed directly into microcentrifuge tubes and frozen at –20°C for at least 24 hr. Frozen samples were thawed and crushed in a hydraulic press to release plant sap, and sap Ψ_O was measured with a vaporpressure osmometer (Wescor model 5520, Wescor, Inc., Logan, UT).

Data Analyses

In the container experiments, a completely randomized design was used with three replications for each treatment. In the hydroponic experiments, a split plot design was used with salt treatment (tank) being the main plot, and grass species within each tank being the subplot. Each treatment had 3 replications. Since experiment (time) X treatment interactions were not significant, data from both experiments were combined; therefore the number of replications was doubled. Combined data were analyzed by analysis of variance (SAS Institute 1989). Treatment means were separated by Fisher's protected LSD. Regression analysis was used to determine the relationships between each variable and the salinity level.

RESULTS

Container Experiment

Study I (Kentucky Bluegrass and Tall Fescue)

Under non-saline condition, LWC was 87% for both KBG and TF (Fig. 2.1). As salinity increased LWC decreased for both KBG and TF, but TF had higher LWC than KBG; this difference became greater as salinity increased. At 9.4 dS/m, LWC of TF was 81%, whereas LWC of KBG dropped to 71%.

Under the non-saline condition, TF and KBG had similar Ψ_W (–0.3 MPa); as salinity increased, Ψ_W of both KBG and TF decreased (Fig. 2.2). Differences between species were not apparent at salinities less than 9.4 dS/m, at which point the Ψ_W of KBG was 0.3 MPa lower than that of TF. Leaf osmotic potential of both KBG and TF were decreased with increasing salinity. Kentucky bluegrass had lower Ψ_O than that of TF at all salinity levels except 4.7 dS/m.

Under non-saline and low saline conditions, KBG exhibited higher Ψ_P than TF (Fig. 2.2). Leaf pressure potential of KBG decreased as salinity level increased, whereas Ψ_P of TF did not change significantly. Therefore, Ψ_P of KBG and TF were not different at salinity levels > 4.7 dS/m.

Osmotic adjustment (OA) of KBG and TF increased with increasing salinity (Fig. 2.1). Osmotic adjustment of KBG and TF increased 1.1 MPa, as salinity increased from 0.2 to 9.4 dS/m.

Under non-saline condition, KBG shoot proline content was lower than that of TF (Table 2.1). However, proline content of KBG increased more rapidly as salinity increased. For example, at 9.4 dS/m, proline content of KBG was 16.3 μmole/g FW, which was 62% higher than TF.

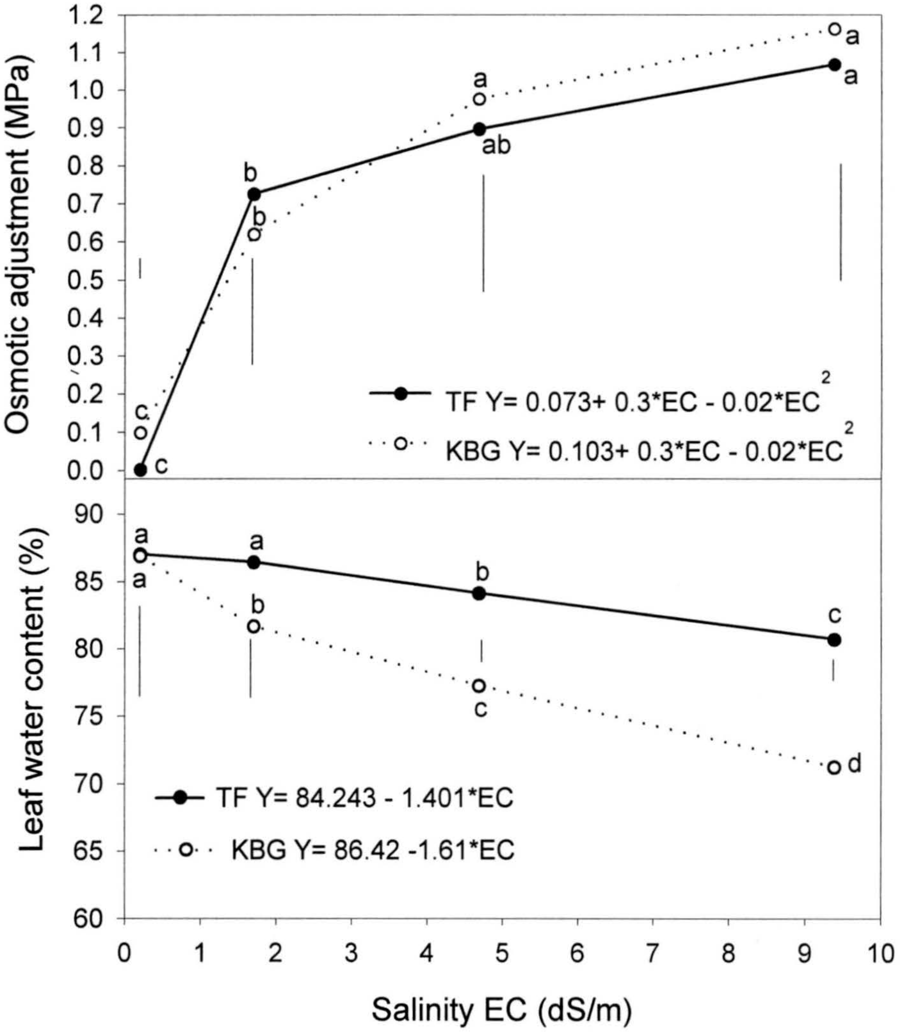

Fig. 2.1. Leaf water content and osmotic adjustment of tall fescue (TF) and Kentucky bluegrass (KBG) irrigated with saline solution at 0.2, 1.7, 4.7, and 9.4 dS/m. Vertical bars indicate least significant difference (*P* =0.05) between species within a given salinity level. Different letters indicate significant difference (*P* =0.05) among salinity treatments for each species.

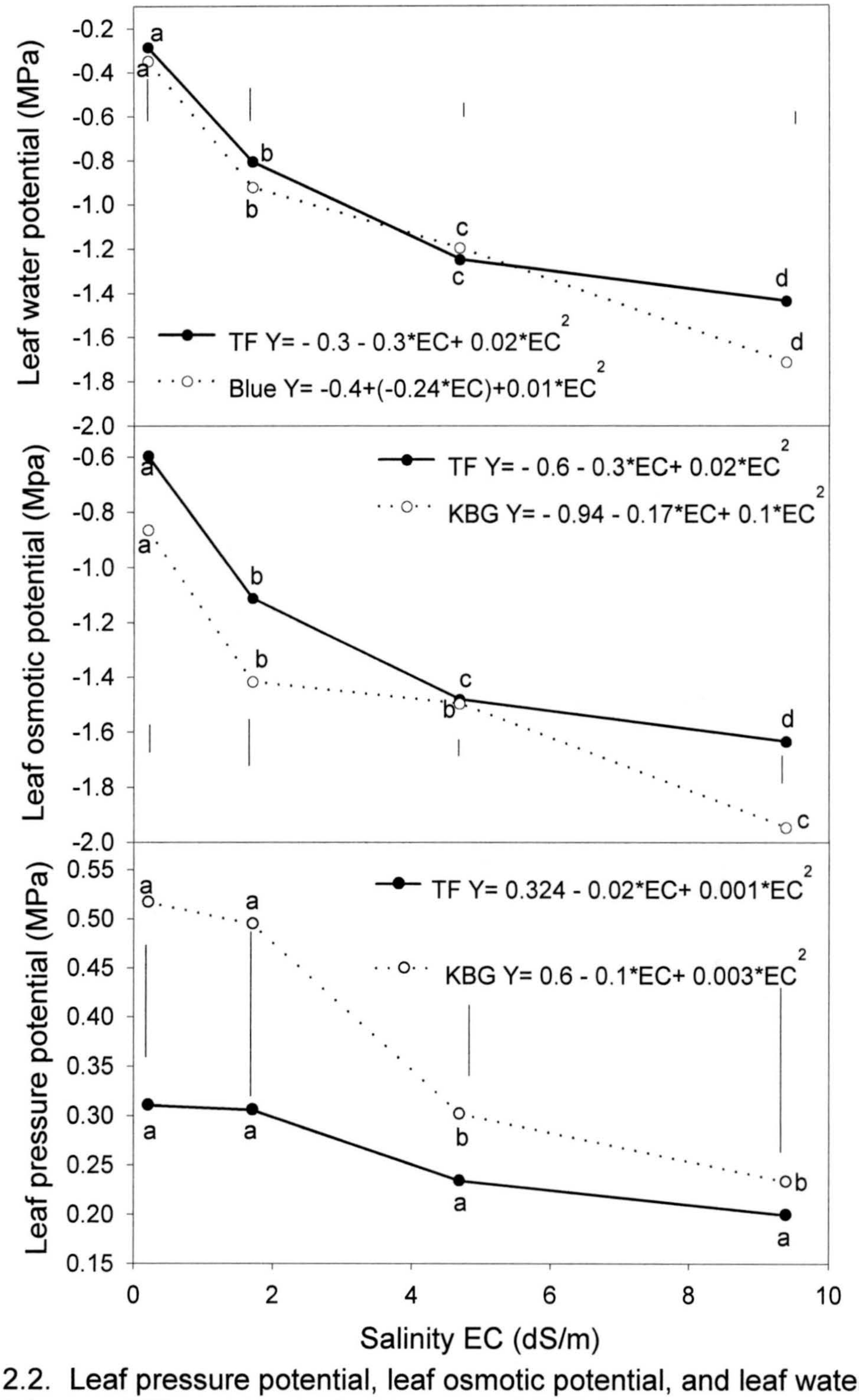

Fig. 2.2. Leaf pressure potential, leaf osmotic potential, and leaf water potential of tall fescue (TF) and Kentucky bluegrass (KBG) irrigated with saline solution at 0.2, 1.7, 4.7, and 9.4 dS/m. Vertical bars indicate least significant difference (*P* =0.05) between species within a given salinity level. Letters indicate significant difference (*P* =0.05) among salinity treatments for each species.

Table 2.1. Shoot proline contents (*u*mole/g FW) for alkaligrass, saltgrass, tall fescue, and Kentucky bluegrass exposed to different salinity levels in the Hydroponic and Container Studies.

Salinity (dS/m)	Alkaligrass	Saltgrass	Tall Fescue	Kentucky bluegrass
		Container		
0.2	2.99 dA^Z	1.11 d B	1.64 d A	0.55 d B
1.7	-	-	2.02 c B	3.00 c A
4.7	-	-	5.18 b B	7.97 b A
9.4	-	-	10.17 a B	16.26 aA
14.1	25.18 cA	7.37 c B	-	-
18.8	34.46bA	10.26 b B	-	-
23.5	39.99aA	13.03aB		
Significance^x	L***	L***	Q***	L***
		Hydroponic		
2.0	2.98 fA^Z	1.41 f B	2.14 e B	0.48 d C
4.7	8.76 e A	2.54 e C	4.27 e B	4.40 c B
9.4	14.66dA	3.76 d C	9.23 d B	10.18bB
14.1	24.89 cA	6.88 c C	20.32c B	23.37aA
18.8	31.17bA	9.45 b C	24.09bB	-
23.5	38.42aA	12.79aC	26.68aB	-
Significance^x	L***	L***	L***	L**

---

^{Z} Lowercase letters indicate significant differences (P =0.05) among salinity treatments for each species.

Uppercase letters indicate significant differences (P =0.05) among species within a given salinity level.

^{x} *, **, and *** indicate significant of linear (L) or quadratic(Q) relationship at $P = 0.05$, 0.01, and 0.001 level, respectively.

Study II (Alkaligrass and Saltgrass).

Under non-saline conditions, LWC of SG was 83.2%, which was lower than that of AG 86.6% (Fig. 2.3). Although LWC of both AG and SG decreased significantly with increasing salinity, AG maintained higher LWC than SG (67.5% vs. 61.3%) at 23.5 dS/m.

Without salinity treatment, Ψ_W of SG was – 0.71 MPa, which was 0.32 MPa lower than that of AG (Fig. 2.4). As salinity increased, both SG and AG exhibited reduced Ψ_W and the difference between SG and AG diminished. Similar to the Ψ_W response, Ψ_O of SG and AG also decreased as salinity increased. However, SG constantly maintained lower Ψ_O than AG at all salinity levels.

Salinity significantly reduced Ψ_P of AG whereas Ψ_P of SG did not change significantly as salinity increased from control to 23.5 dS/m (Fig. 2.4).

Osmotic adjustment of SG and AG increased with increasing salinity, reaching 1.08 and 1.11 MPa for SG and AG, respectively, at 23.5 dS/m (Fig. 2.3).

Shoot proline content of SG and AG increased linearly with increasing salinity (Table 2.1). Proline content of alkaligrass was about 3 times that of SG at all salinity levels. At 23.5 dS/m, proline content of AG and SG was 40.0 and 13.0 μmole/g FW, respectively.

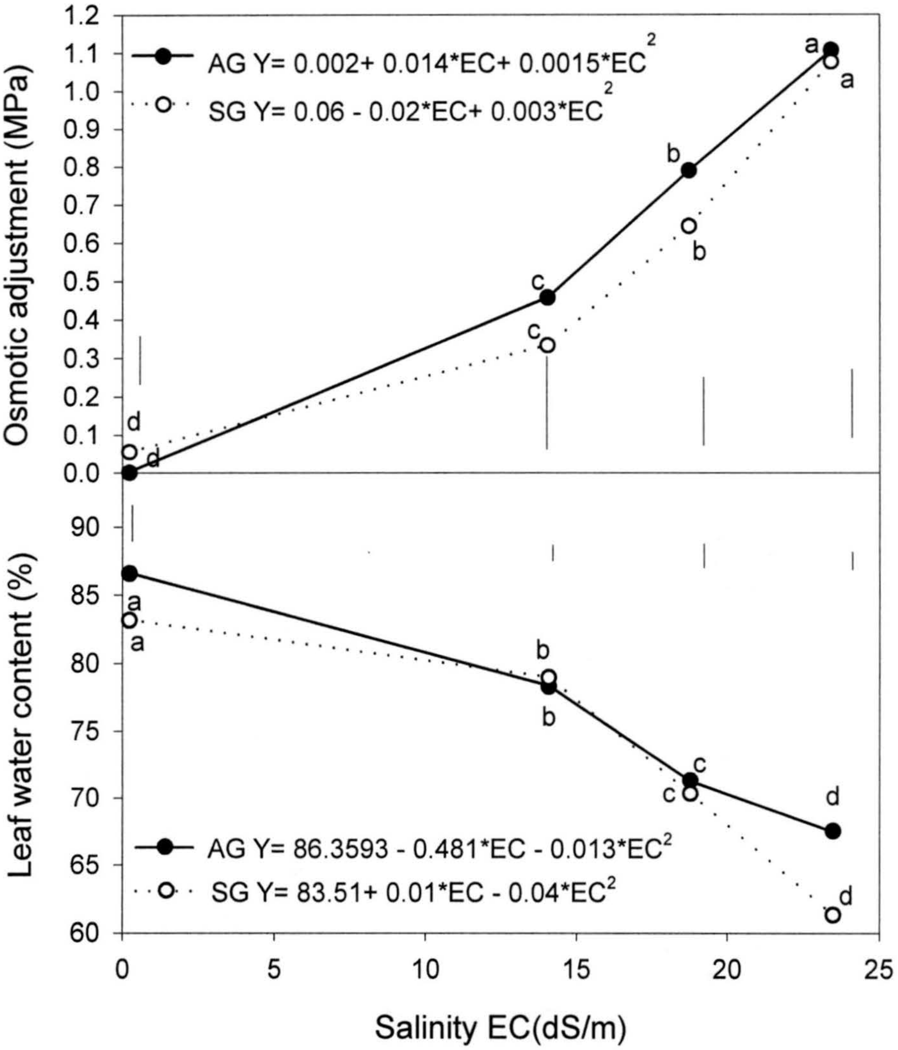

Fig. 2.3. Leaf water content,and osmotic adjustment of alkali grass (AG) and saltgrass (SG) irrigated with saline solution at 0.2, 14.1, 18.8, and 23.5 dS/m. Vertical bars indicate least significant difference (P =0.05) between species within a given salinity level. Different letters indicate significant difference (P =0.05) among salinity treatments for each species.

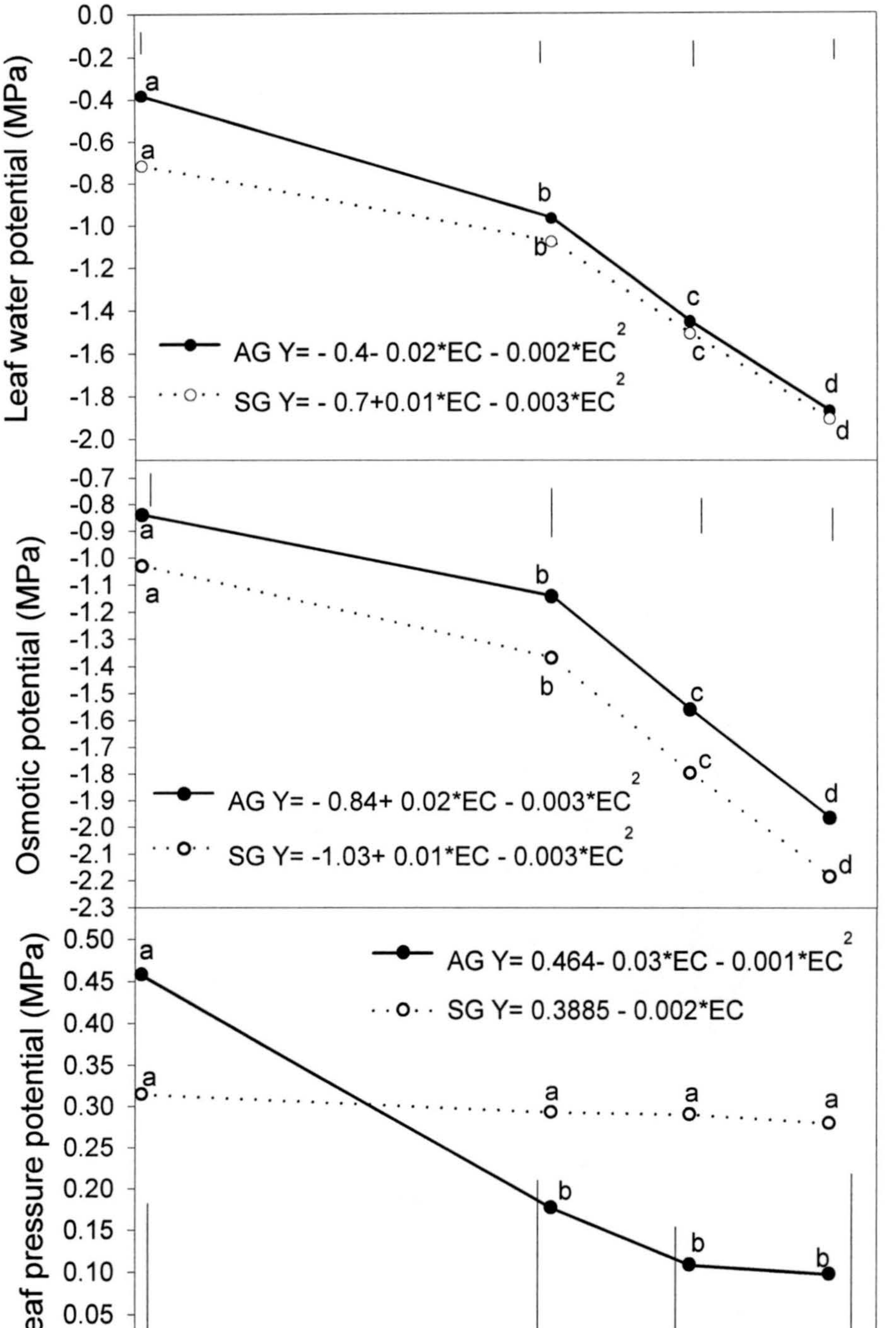

Fig. 2.4. Leaf pressure potential, osmotic potential, and leaf water potential of alkali grass (AG) and saltgrass (SG) irrigated with saline solution at 0.2, 14.1, 18.8, and 23.5 dS/m. Vertical bars indicate least significant difference (*P* =0.05) between species within a given salinity level. Different letters indicate significant difference (*P* =0.05) among salinity treatments for each species.

Hydroponic Experiment

Although increasing salinity reduced LWC for all grasses (Fig. 2.5). Alkaligrass and SG maintained a stable LWC as salinity increased from control to 9.4 dS/m, whereas LWC of TF decreased at salinity ≥ 4.7 dS/m. Leaf water content of KBG dramatically decreased with increasing salinity. At ≥ 4.7 dS/m, SG, AG, and TF had significantly higher LWC than KBG. At 23.5 dS/m, AG had a higher LWC (69.4%) than SG (63.4%). Tall fescue maintained an intermediate LWC and was not statistically different from AG or SG at 23.5 dS/m.

Leaf water potential and Ψ_O of all species decreased as salinity level increased (Fig. 2.6). However, the reduction rates were AG < TF = SG < KBG for Ψ_W and AG < TF < SG = KBG for Ψ_O.

Salinity reduced Ψ_P of KBG, TF, and AG in relative to control, but Ψ_P of SG did not change as salinity level increased from control to 23.5 dS/m (Fig. 2.6). At ≥ 14.1 dS/m, Ψ_P of KBG was lower than those of TF, SG, and AG; leaf pressure potential of TF and AG was lower than that of SG.

Osmotic adjustment of all grasses increased significantly with increasing salinity (Fig. 2.5). At 4.7 dS/m, TF exhibited greater OA than AG, and OA of SG and KBG was not different from that of either AG or TF. However, at ≥ 14.1 dS/m, OA in SG (1.17 MPa) was higher than in AG (0.93 MPa), TF (0.84 MPa), and KBG (0.79 MPa).

Shoot proline content of all grasses increased with increasing salinity (Table 2.1). When salinity was ≤ 9.4 dS/m, AG had 68 to 222% higher mean proline content than all other grasses. At 14.1 dS/m, AG had 262% and 23% higher proline than SG and TF, respectively, but did not differ from KBG for proline content. Saltgrass produced the lowest proline content under salinity treatments. Kentucky bluegrass exhibited lower proline content than other grasses under control conditions, but it increased almost 50-fold as salinity level increased from control to 14.1 dS/m, the relative contributions of proline to whole cell OA were estimated to be 3.8%, 2.7%, 2.5%, 0.8% in AG, KBG, TF, and SG, respectively, at 14.1 dS/m, and 4.7%, 2.8%, and 1.3% in AG, TF, and SG, respectively, at 23.5 dS/m (Fig. 2.7 and 2.8).

In this experiment, we also determined leaf sap ion content (data presented in Chapter 3). The concentration of Na^+ and Cl^- in the shoot sap increased rapidly as salinity increased. In KBG and TF, the contribution of Na^+ and Cl^- to osmotic adjustment increased as the level of salinity increased, and became the major contributors at high levels of salinity. The contribution of Na^+ and Cl^- in KBG was 58% at 14.1 dS/m and 46% in TF at 23.5 dS/m. In SG, however, Na^+ and Cl^- contributions to OA were stable (15% by Na and 13% by Cl) as salinity increased from 4.7 to 23.5 dS/m.

Under non-saline condition, potassium was the major contributor compared with other ions in all grasses, but its contribution declined as salinity increased. Saltgrass had the highest proportion of K^+ at all levels of salinity than did other grasses. The proportion of K^+ to OA in AG and TF was lower than in SG but higher than KBG. As salinity increased, the contribution of unidentified osmolytes to OA increased in SG and decreased in KBG and TF. The trend of the change in OA contribution by the unidentified osmolytes in AG was not clear.

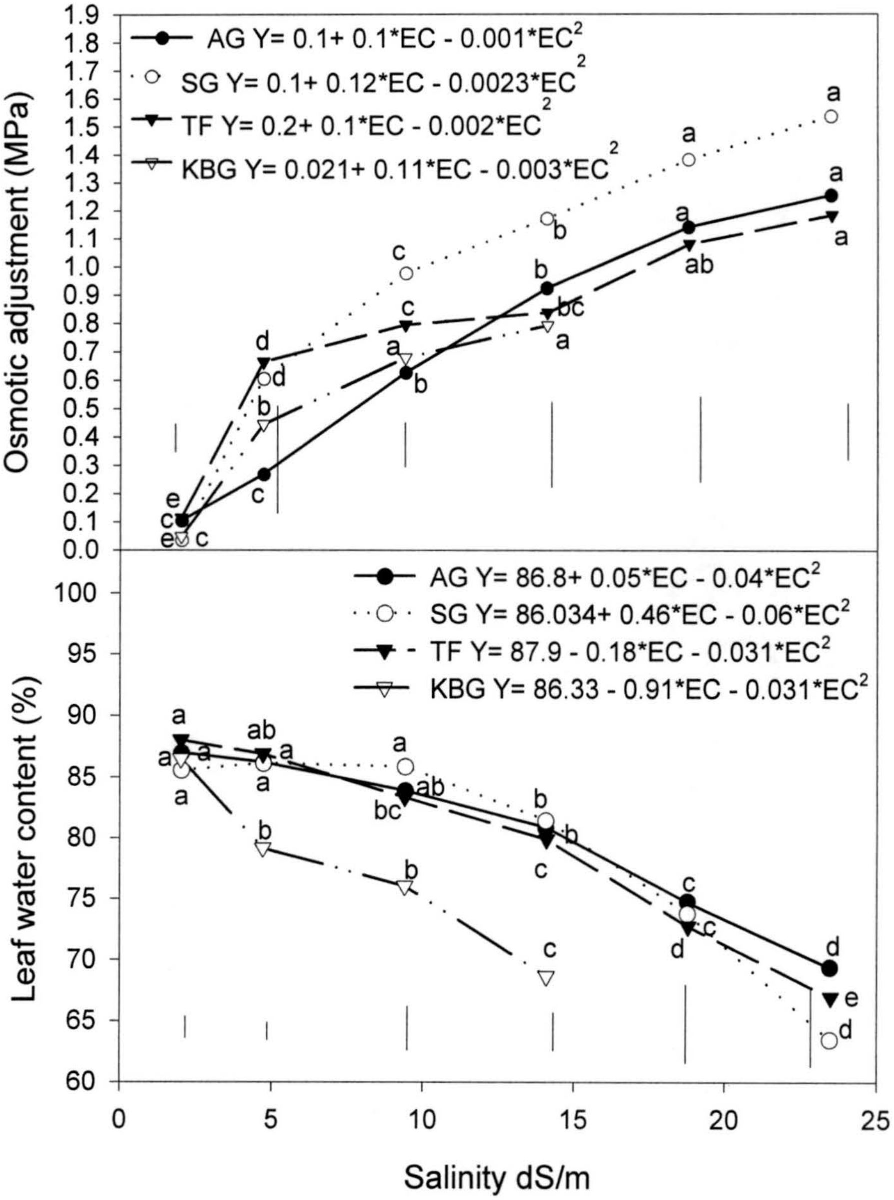

Fig. 2.5. Leaf water contents of alkaligrass (AG), saltgrass (SG),tall fescue (TF) and Kentucky bluegrass (KBG) grown in saline solution at 2.0, 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m in the hydroponic study. Vertical bars indicate least significant difference (*P* =0.05) among species within a given salinity level. Letters indicate significant difference (*P* =0.05) among salinity treatments for each species.

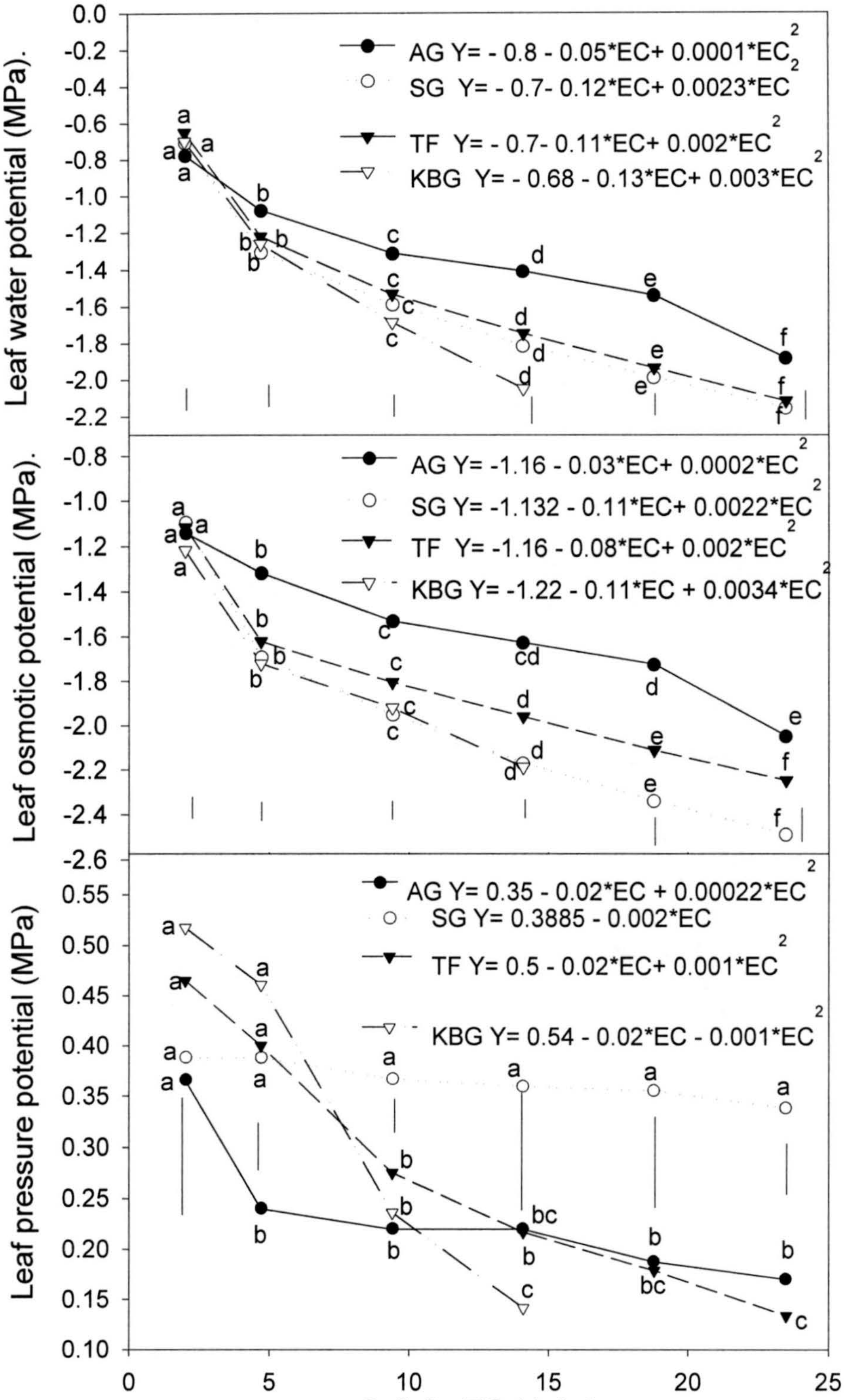

Fig. 2.6. Leaf pressure potential, osmotic potential, and water potential of alkaligrass (AG), saltgrass (SG), tall fescue (TF) and Kentucky bluegrass (KBG) grown in saline solution at 2.0, 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m in the hydroponic study. Vertical bars indicate least significant difference (*P* =0.05) among species within a given salinity level. Letters indicate significant difference (*P* =0.05) among salinity treatments for each species.

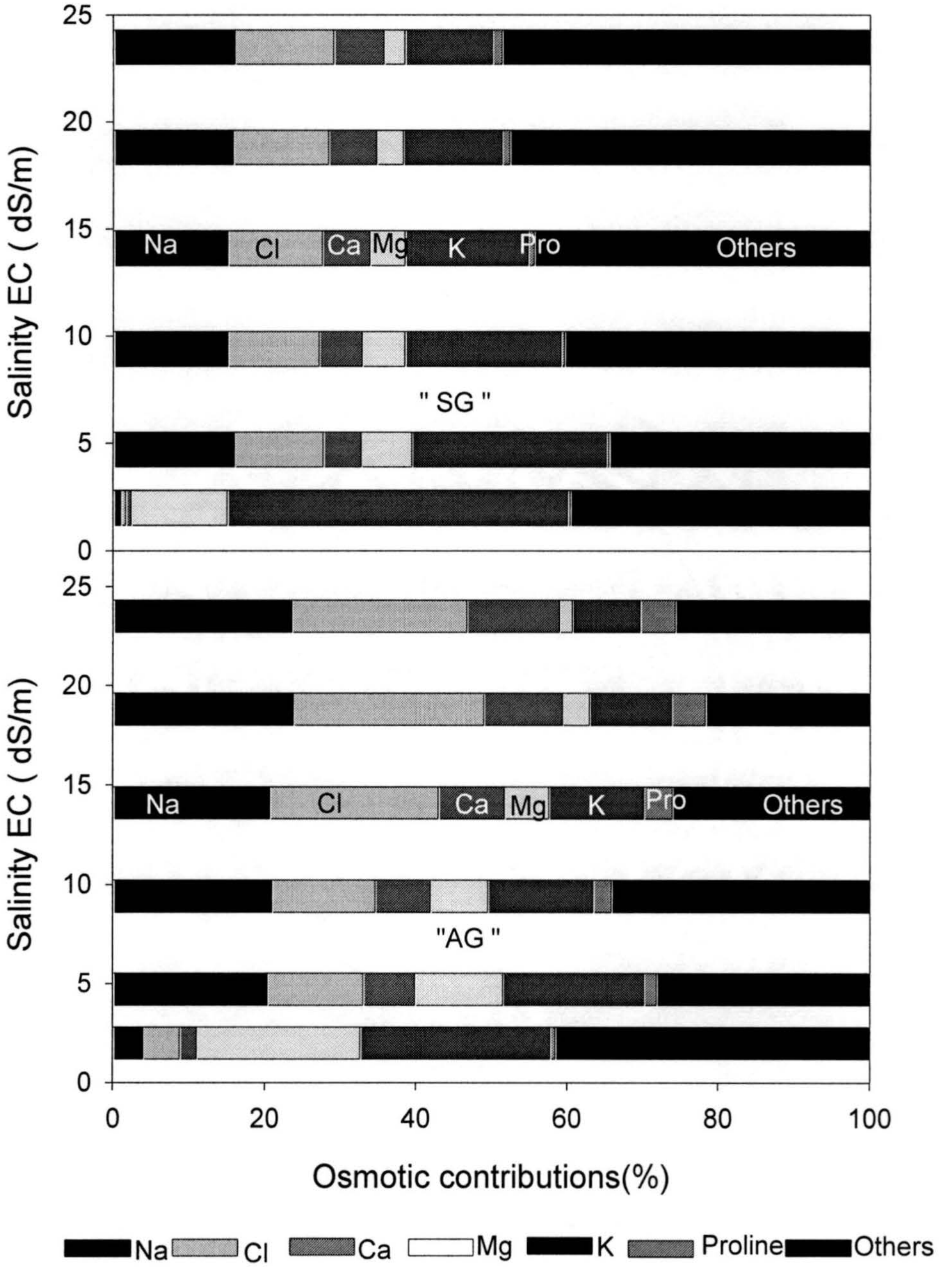

Fig. 2.7. Contributions of ions, proline, and other solutes to the osmotic adjustment of alkaligrass (AG) and saltgrass(SG) shoots at 2.0, 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m in the Hydroponic Study.

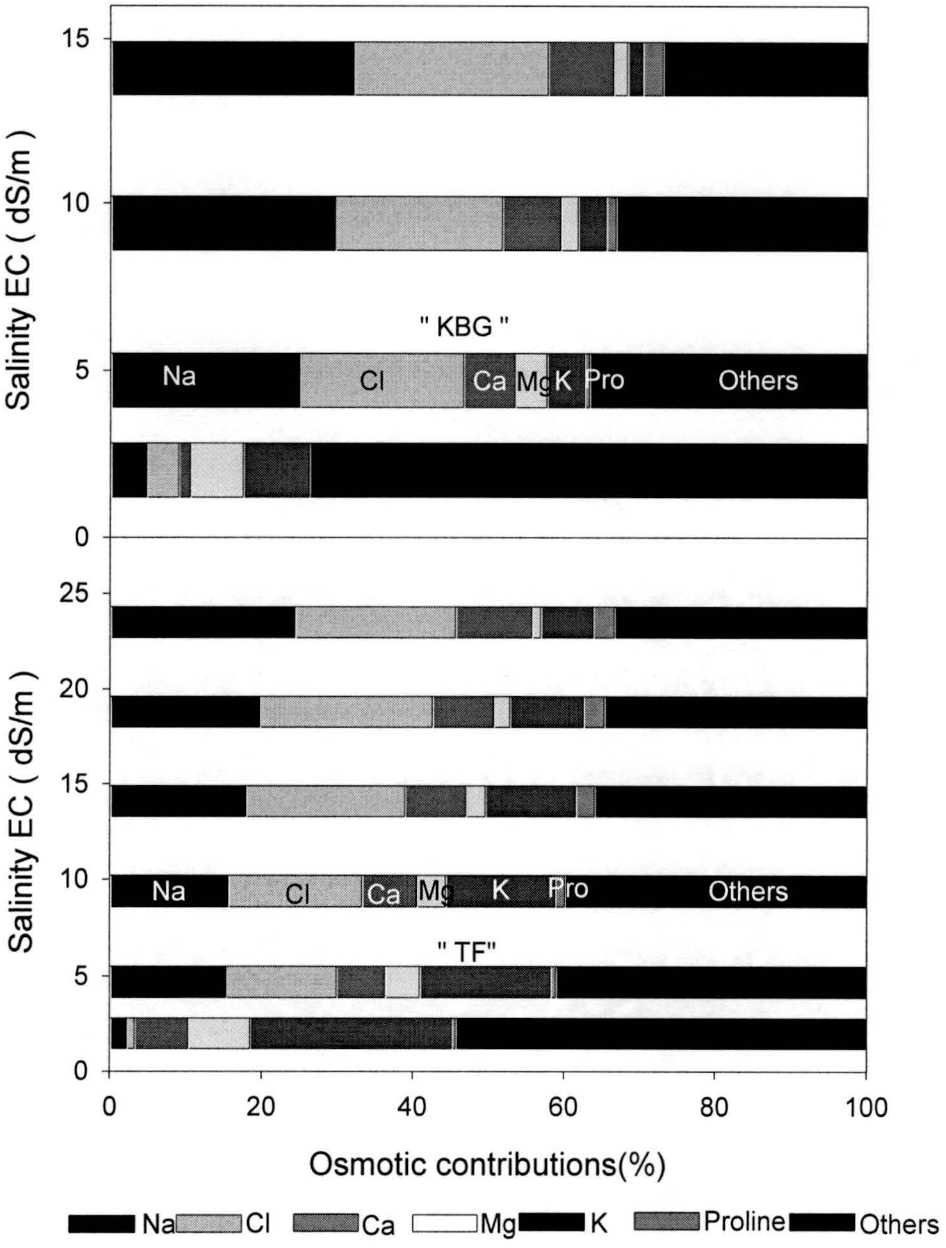

Fig. 2.8. Contributions of ions, Proline, and other solutes to the osmotic adjustment of tall fescue (TF) and Kentucky bluegrass (KBG) shoots at 2.0, 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m in the Hydroponic Study.

Table 2.2. Pearson correlation coefficients for leaf proline (PR), relative shoot dry weight (SH), relative root dry weight (RO), relative root length (RL), root to shoot ratio (R/S), leaf firing (LF), turf quality (TQ), leaf water content (WC), leaf water potential (WP), leaf osmotic potential (OP), leaf pressure potential (PP), and osmotic adjustment (OA) in the Hydroponic Study.

Parameter	WC	WP	OP	PP	OA	LF	TQ	SH	RO	RL	R/S
PR	-0.75***	-0.68***	-0.54***	-0.87***	0.58***	0.88***	-0.75***	-0.78***	-0.51***	-0.36*	0.68***
WC		0.88***	0.81***	0.65***	-0.78***	-0.76***	0.84***	0.65***	0.46***	0.54***	-0.29*
WP			0.98***	0.66***	-0.96***	-0.65***	-0.85***	0.58***	0.25 ns	0.21 ns	-0.37*
OP				0.48***	-0.95***	-0.59***	0.79***	0.52***	0.12 ns	0.14 ns	-0.15 ns
PP					-0.56***	-0.87***	0.74***	0.74***	0.47***	0.39**	-0.64***
OA						0.57***	-0.74***	-0.43**	-0.02 ns	-0.05 ns	0.28*
LF							-0.95***	-0.96***	-0.88***	-0.79***	0.39**
TQ								0.92***	0.67***	0.76***	-0.14 ns
SH									0.92***	0.85***	-0.44**
RO										0.89***	-0.11 ns
RL											0.08 ns

ns, *, **, and *** indicate non-significant or significant of correlations at $P = 0.05$, 0.01, and 0.001 level, respectively (n= 132).

DISCUSSION

Shoot dehydration and loss of turgor are common responses of plants to salinity (Neumann et al., 1988; Wyn Jones, 1981; Marcum and Murdoch, 1994). In this study we found that, the slope of LWC decrease was greatest for KBG > TF = AG > SG as salinity increased from 2.0 to 23.5 dS/m. The relative slope of LWC reduction was associated with the relative effect on shoot growth reduction (r = 0.65), indicating salinity tolerance may, in part, attributed to the ability of plants to maintain a desired tissue hydration level. Leaf water content was also negatively correlated with leaf firing (r = - 0.76) and positively correlated with turf quality (r = 0.84).

Reductions of Ψ_W and Ψ_O in our study agree with results of Ackerson and Youngner (1975) with Bermudagrass (*Cynodon dactylon* L.); Peacock and Dudeck (1985) with Seashore papsalum (*Papsalum vaginatum* Swartz); Dudeck et al. (1993) with St. Augustinegrass (*Stenotaphrum secundatum* Walt.) cultivars; and Qian et al. (2001) with two Kentucky bluegrass cultivars.

The Ψ_W of KBG decreased more than Ψ_O, resulting in a loss of Ψ_p ; at 14.1 and 23.5 dS/m, SG maintained the highest; AG and TF the intermediate, and KBG had the lowest Ψ_P. The greater ability to maintain LWC and Ψ_P in SG, AG, and TF than in KBG could result from root growth stimulation of SG and AG and the extensive root system of TF under saline conditions, which likely contributed to more efficient water and nutrient uptakes. The severe shoot growth reduction of KBG under high salinity may be associated with the greater degree of tissue dehydration and the loss of turgor. Leaf turgor or cell volume maintenance has been suggested to be essential for sustained growth and development (Greenway and Munns, 1980; Munns and Termaat, 1986).

Rajasekaran et al., (2000) stated that there is a significant correlation between Ψ_P and salt tolerance index in *Lycopersicon* spp. exposed to saline conditions.

As a response to salinity, plants in saline environments must adjust osmotically (Yeo, 1983). Osmotic adjustment is defined as the net accumulation of solutes in a cell in response to a fall in Ψ_w, independent of the effect of solute concentration due to tissue water loss (Turner and Jones, 1980). Osmotic adjustment under saline conditions can occur in plants due to the uptake of inorganic ions and compartmentalize them in the cell vacuole (this is important to avoid enzyme deactivation), or by internal synthesis of organic solutes (compatible solutes) in the cytoplasm, or more generally by a combination of both. Osmotic adjustment solves several problems for cells (Morgan, 1984) because it helps to maintain turgor and cell volume, and maintains enzymes function due to accumulation of compatible solutes in the cytoplasm. Our results, however, indicated that OA occurred in all grass species under salinity stress, even in salt sensitive KBG and the relative OA was negatively associated with salinity tolerance based on relative growth reduction and turf quality rating (Table 2.2).

Estimation of various components of whole cell OA indicated that in the most salt tolerant grass in our study, SG, the OA contributed by Na^+ and Cl^- was stable as salinity increased from 4.7 to 23.5 dS/m. However, the proportion contributed by unidentified osmolytes increased with increasing salinity in SG. This result indicated that the ion homeostasis was maintained for up to 23.5 dS/m in SG. It seems that one of the major advantage of the more salt tolerant SG and AG over salt sensitive KBG in this study were not because of the degree of OA per se, but because of the more responsive control of Na^+ and Cl^- uptake and/or partition, and the more effective capacity to coordinate the increasing Na^+ and Cl^- with processes of synthesis of compatible solutes, which do not inhibit normal metabolic reaction. The accumulation of nontoxic ions (K^+) and organic solutes (not identified in our study) in the cytoplasm can balance cytoplasmic Ψ_O with that of the vacuole, and to protect cytoplasmic enzymes against inactivation by inorganic ions (Pollard and Wyn Jones, 1979; Wyn Jones, 1984).

Proline is the most frequently reported solute accumulated in salt stressed plants (Wyn Jones, 1984; Gorham et al., 1985). The physiological significance of proline accumulation is controversial; some reported that it represents a form of injury, while others suggested proline acts as a compatible solute. Although shoot proline content of KBG, TF, SG, and AG significantly increased with increasing salinity (Table 2.1), results of OA measurement indicate that proline made insubstantial cytoplasmic osmotic contributions in all grass species. Furthermore, proline was positively correlated with leaf firing (r = 0.88) and negatively correlated with turf quality (r = -0.75) (Table 2.2), and the most salt tolerant SG exhibited the lowest proline content in comparison with other grasses. Similar findings were reported by Huang and Redman (1995), i.e. proline appeared to be insufficient to influence leaf osmolality of barley cultivars since it accounted for less than 1% of the total osmotica. These results suggesting that proline accumulation may be a result of salt injury. Alkaligrass is an apparent exception in that salt injury was minimal under conditions leading to high proline content. Torello and Rice (1986) have been reported that among cool season turfgrass species, AG had the highest proline content than did red fescue and KBG. Therefore, proline accumulation is likely stress consequences in KBG, TF and SG, but may play a role as a compatible solute in AG.

In summary, this chapter has demonstrated different effects on water relations among the four turf grass species used here. The differences in salt tolerance among these species may attribute to maintenance of high LWC, Ψ_P, and accumulation of compatible solute. These water relation characteristics may play significant role for plants to cope with high salt concentration.

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Chapter Three

Effect of Salinity on Ion Relations of Four Turfgrasses

ABSTRACT

Information on the degrees and patterns of ion accumulation in various parts of a plant is important in evaluating salinity tolerance, as well as salinity tolerance mechanisms. 'Challenger' Kentucky bluegrass (*Poa pratensis* L.) (KBG), 'Arid' tall fescue (*Festuca arundinacea* Schreb) (TF), 'Fults' alkaligrass (*Puccinellia distans* (L.) Parl.) (AG), and a saltgrass (*Distichlis spicata* (Torr.) Beetle) (SG) collection were grown under various salinity levels in greenhouse container and hydroponic experiments. Salinity treatments were applied using $1NaCl:1CaCl_2$ solution at different concentrations. Ion concentrations in shoots and roots of each grass grown at different salinity levels were examined. As salinity increased, Na^+, Cl^-, and Ca^{++} concentrations increased linearly, while Mg^{++} and K^+ concentrations decreased in shoots, sap, and roots of all species. Salinity tolerance of these species was associated with their ability to exclude Na^+ and Cl^- from shoots and maintain a relatively high level of K^+. Potassium to sodium ratio in all species was higher in shoots than in roots, indicating these plants achieved K^+ selectivity via multiple processes in multiple locations. Maintenance of a K^+/Na^+ ratio close to or above 1 was important for plant survival. Combining data generated from both container and hydroponic experiments, our results indicated that, SG, AG, TF, and KBG could maintain a shoot K^+/Na^+ ratio of 1 when salinity levels were less than 22.3,

13.6, 7.4, and 3.7 dS/m, respectively. Leaf ion secretion rate of SG increased as salinity level increased, reaching 5.05, 6.86, and 1.22 mg/ g dw/day for Na^+, Cl^-, and Ca^{++}, respectively, at 23.5 dS/m. No Mg^{++} or K^+ were secreted. Regulation of ion concentrations (via ion restriction, exclusion or secretion) and maintenance of higher K^+/Na^+ ratio in shoots appeared to be important mechanisms contributing to the differences in salinity tolerance observed in these species.

Introduction

Plants grown under saline conditions accumulate large quantities of inorganic ions for osmotic adjustment. This may lead to specific ion effects or ion excesses (Greenway and Munns, 1980; Lauchli, 1986; Cheeseman, 1988; Cramer, 1997). Plants differ in the accumulation of specific ions. Sodium, K^+, and Cl^- contribute significantly to cell osmotic potential, depending on species and age of the plant. Other ions, such as Ca^{++}, PO_4^{--}, and NO_3^- contribute relatively little to whole cell osmotic potential (Flowers et al., 1977, Zimmermann, 1978).

Marshes and swamps along coastal areas contain high levels of Na^+ and Cl^-, which create ion imbalances that may impair the selectivity of root membranes (Bohra and Dorffling, 1993). Excessive Na^+ may result in the inhibition of K^+, Ca^{++}, and Mg^{++} uptake because of the competition of Na^+ with K^+, Ca^{++}, and Mg^{++}. Likewise, excessive Cl^- may cause deficiencies of N, S, and P due to the competition of Cl^- with NO_3^-, SO_4^{--} and $P_2O_4^{--}$.

However, salinity tolerant plants can maintain a cellular ion composition very different from that of the medium by the regulation of ion transport across cell membranes (Flowers et al., 1977; Greenway and Munns, 1980; Leidi and Sarz, 1997). Salt tolerance in many plants has been correlated to their ability to exclude Na^+ and Cl^- from the shoots, maintaining low Na^+ and Cl^- levels (Wyn Jones, 1981; Hajibagheri et al., 1989; Cerda et al., 1995).

Potassium, the most dominant cellular cation, as well as the only monovalent cation known to be essential for all higher plants (Epstein, 1972), has biophysical effects associated with osmotic and cation/anion balances and biochemical functions, such as enzyme activation (Marschner, 1995). Greenway and Munns (1980) and Chow et al. (1990) found that maintenance of a high cytoplasmic K^+ level and K^+/Na^+ ratio was essential for plant survival in saline habitats. Others have also shown this in wheat (*Triticum aestivum* L.) (Omielan et al., 1991; Schachtman and Munns, 1992) and maize (*Zea mays* L.) (Cerda et al., 1995).

Calcium is important for normal plant function. It is involved in cell wall extensibility and membrane integrity and function, and acts as a second messenger for many biochemical processes within the cytosol (Hanson, 1984). Calcium readily enters the apoplast and provides stable intermolecular linkages in cell walls and at the exterior surface of plasma membrane. The Ca^{++}-mediated linkages are one of the control mechanisms for growth and developmental processes (Mengel and Kirkby, 1987; Marschner, 1995). In addition, Ca^{++} is a non-toxic nutrient that is very effective in amelioration of Na^+ toxicity in plants (Greenway and Munns, 1980; Reid and Smith, 2000). Research has shown that calcium plays an important role in ion homeostasis and growth under saline conditions (Cramer et al., 1985, 1986 and 1987; Cheeseman, 1988; Subbarao et al., 1990; Garg, 1998).

Plants mechanisms that regulate internal ion concentrations include ion compartmentalization (Gorham et al.,1985), ion exclusion in the root systems (Leonard, 1983), ion absorption by specialized xylem parenchyma cells (Yeo et al., 1977), redistribution of ions to old leaves, petioles, and stems (Yeo and Flowers, 1984), and ion secretion through salt excreting structures (salt glands) on leaf epidermal surfaces (Liphschitz and Waisel, 1974; Oross et al., 1985; Marcum et al., 1998).

In previous chapters, we reported that KBG, TF, AG, and SG exhibited significant differences in salinity tolerance. More information is needed concerning ion relations in these four species. as may be related to these tolerances differences. Therefore, the major objective of this study was to examine ion concentrations in shoots and roots of four turfgrasses grown under various salinity levels.

Materials and Methods

Plant Materials, Growth Conditions and Salinity Treatment

Detailed information on plant materials, growth conditions and salinity treatments were discussed in Chapter One. Container and hydroponic experiments were carried out in a greenhouse at Colorado State University. 'Challenger' Kentucky bluegrass (*Poa pratensis* L.) (KBG), 'Arid' tall fescue (*Festuca arundinacea* Schreb) (TF), 'Fults' alkaligrass (*Puccinellia distans* (L.) Parl.) (AG), and a saltgrass (*Distichlis spicata* (Torr.) Beetle) (SG) collection were initially grown without salinity treatments. After grasses were well-established, saline water (a mixture of $1NaCl:1CaCl_2$) at 0.2 (control), 1.7, 4.7, and 9.4 dS/m was applied to KBG and TF (Container Study I), whereas saline water at 0.2 (control), 14.1, 18.8, 23.5 dS/m was applied to AG and SG (Container Study II). Salinity treatments in the hydroponic experiments were 2.0 (control), 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m. Salinity treatments were continued for a period of 8 wks.

Data Collection - Container Experiments

During the 8 wk salinity treatment period, shoots were clipped weekly, washed with deionized water, and dried at 70°C for 24 hr. At the termination of the experiment, roots were clipped, washed with deionized water, and dried at 70°C for 24 hr. Accumulated shoots and dried roots were ground in a Wiley mill to pass through a screen with 425-µm openings. Approximately 1 g screened and dried sample were weighed and ashed for 7 hr at 500°C. Ash was dissolved in 10 ml of 1N HCl and diluted with deionized water. Solution aliquots were analyzed for Na^+, Ca^{++}, Mg^{++}, and K^+ by inductively-coupled plasma atomic emission spectrophotometry (ICP-AES) (Model 975 plasma Atomcomp, Thermo Jarrell Ash Corp., Franklin, MA 02038). To determine Cl^- content, shoot and root samples (200 mg) were dissolved in 50 ml of 2% acetic acid and filtered. Chloride was analyzed by a Cl-selective electrode (Orion Ionplus Chloride combination electrode).

Data Collection - Hydroponic Experiment

Shoot and root samples were collected, and ion content was analyzed as described in the container experiment. In addition, leaf sap mineral content was determined 7 days prior to experiment termination. Intact leaves were thoroughly rinsed with distilled water to remove all external salts. Air dried leaf samples were clipped, immediately placed in zip plastic bags, and frozen with dry ice. Leaf samples were later thawed and leaf sap expressed using a hydraulic press. Leaf residue was dried at 70°C for 24 hr to determine dry weight. Sap was diluted with distilled water prior to analyzing Na^+, Ca^{++}, Mg^{++}, and K^+ by ICP-AES. Chloride was determined with a Cl-selective electrode.

Since salt glands were only observed on leaf surfaces of SG (details described in Chapter One), we measured Na^+, Cl^-, Ca^{++}, Mg^{++}, and K^+ secretion rates of SG 7 days prior to experiment termination. Intact leaves were thoroughly rinsed to remove all external salts. Plants were allowed to grow under different salinity conditions for an additional 24 hr. Then, 7 fully mature leaves were cut, immediately placed in 10 ml distilled water in a scintillation vial, sealed, and shaken for ten seconds to dissolve external secreted salt crystals. Leaves were then removed and dried at 70°C for 24 hr to determine dry weight. Vials were resealed, frozen, and subsequently analyzed for Na^+,

Cl^-, Ca^{++}, Mg^{++}, and K^+ contents. Ion secretion rates were expressed as mg ion/g leaf dw/day.

Data Analysis

In the container experiments, a completely randomized design was used with three replications for each treatment. In the hydroponic experiments, a split plot design was used with salt treatment (tank) as the main plot factor, and grass species within each tank as the subplot factor. Each treatment had 3 replications. Since experiment (time) X treatment interactions were not significant, data from both experiments were combined. Therefore, the number of replications was doubled. Combined data were analyzed by analysis of variance (SAS Institute 1989). Treatment means were separated by Fisher's protected LSD. Regression analysis was used to determine relationships between each variable and the salinity level.

RESULTS

Container Experiments

Study I (Kentucky Bluegrass and Tall Fescue)

Sodium, Cl^-, and Ca^{++} concentrations in shoots and roots of both species increased significantly as salinity level increased (Table 3.1). Sodium and Cl^- concentrations of TF were higher in shoots than in roots. By contrast, KBG shoots had higher Na^+ and Cl^- content than roots. In shoots and roots of both species, Ca^{++} concentration was significantly lower than Na^+ and Cl^-.

Sodium and Cl^- levels were similar between KBG and TF under non-saline conditions (Table 3.1). However, as salinity increased, KBG shoots and roots accumulated more Na^+ and Cl^- than TF. For example, at the highest salinity level (9.4 dS/m) KBG shoot Na^+ and Cl^- concentrations were 96.6% and 66.4% higher, respectively, and root concentrations were 21.2% and 14.01% higher, respectively, than TF.

Increasing salinity reduced Mg^{++} and K^+ concentrations in both KBG and TF (Table 3.1). Tall fescue maintained a higher level of K than KBG in both shoots and roots under non-saline conditions and in all salinity treatments. Tall fescue also had 33.3% higher Mg^{++} than KBG in roots, but there was no difference in Mg^{++} concentrations between KBG and TF in shoots.

Under non-saline conditions, TF exhibited higher K^+/Na^+ ratio than KBG. Although increased Na^+ content and decreased K^+ content under saline conditions resulted in decreases in K^+/Na^+ ratios in both speacies, TF consistently produced a higher K^+/Na^+ ratio than KBG at all salinity levels (Table 3.1). Regression analysis predicted that the

K^+/Na^+ ratio decreased to 1 at 4.8 and 1.9 dS/m in TF and KBG shoots, respectively. In roots, K^+/Na^+ ratio decreased to 1 at 3.65 dS/m in TF and 2.45 dS/m in KBG.

Table 3.1. Shoot and root mineral concentration (mg/g. dw) of Kentucky bluegrass (KBG) and tall fescue (TF) exposed to salinity stress(dS/m) in Container Study I.

Salinity	Na$^+$		Cl$^-$		Ca^{++}		Mg^{++}		K$^+$		K$^+$ / Na$^+$	
level	KBG	TF	KBG	TF	KBG	TF	KBG	TF	KBG	TF	KBG	TF
						Shoot						
0.2	0.61dA^z	0.46 dA	0.69 dA	1.19 dA	0.35 dA	0.33 dA	1.39 aB	2.10 aA	1.50 aB	5.59 aA	2.51 aB	12.29 aA
1.7	4.29 cA	0.67 cB	5.53 cA	4.44 cB	1.32 cB	1.67 cA	1.25 abA	1.62 abA	0.79 bB	4.86 bA	0.19 bB	7.36 bA
4.7	6.76 bA	3.24 bB	8.68 bA	5.90 bB	3.91 bA	2.43 bB	1.13 bA	1.21 bcA	0.42 cB	3.88 cA	0.06 bB	1.21 cA
9.4	8.75 aA	4.45 aB	12.03 aA	7.23 aB	4.84 aA	2.91 aB	0.75 cA	0.89 cA	0.29 cB	3.65 cA	0.03 bB	0.82 cA
Salinity effect												
Linear	***	***	***	***	***	***	***	***	***	***	*	***
Quadratic	***	***	***	***	***	***	***	***	***	***	ns	***

Table 3.1. Continued.

Salinity	Na^+		Cl^-		Ca^{++}		Mg^{++}		K^+		K^+ / Na^+	
level	KBG	TF	KBG	TF	KBG	TF	KBG	TF	KBG	TF	KBG	TF
						Root						
0.2	0.85dA[z]	0.61 dB	1.30 dA	1.48 dA	0.41 dB	0.69 dA	1.94 aB	2.63 aA	2.53 aB	4.96 aA	3.04 aB	8.09 aA
1.7	5.95 cA	3.48 cB	7.74 cA	5.27 cB	2.64 cA	2.58 cA	1.49 bB	2.27 bA	1.69 bB	4.58 aA	0.28 bB	1.32 bA
4.7	6.95 bA	4.53 bB	10.21 bA	7.63 bB	3.02 bB	3.58 bA	1.08 cB	1.80 cA	1.08 cB	3.24 bA	0.16 bB	0.72 cA
9.4	7.43 aA	6.13 aB	11.72 aA	10.28 aB	3.89 aB	4.63 aA	0.84 cB	1.26 dA	0.38 dB	3.09 bA	0.05 bB	0.50 cA
Salinity effect												
Linear	**	***	***	***	***	***	***	***	***	***	*	*
Quadratic	**	***	***	***	***	***	***	***	***	***	ns	*
Contrast												
shoot vs roo	*	*	*	*	*	*	ns	*	*	ns	ns	*

[z] Lowercase letters indicate significant differences (*P* =0.05) among salinity treatments for each species.

Uppercase letters indicate significant differences (*P* =0.05) between species within a given salinity level.

[x] ns, *, **, and *** indicate non-significant or significant of linear or quadratic regression at *P* = 0.05, 0.01, and 0.001 level, respectively.

Study II (Alkaligrass and Saltgrass)

Without salinity treatment, SG and AG exhibited similar concentrations of Na^+ and Cl^-. Shoot and root Na^+, Cl^-, and Ca^{++} concentrations in both AG and SG increased with increasing salinity, with Na^+, Cl^-, and Ca^{++} concentrations lower in shoots than in roots for both species (Table 3.2). Alkaligrass exhibited higher Na^+, Cl^-, and Ca^{++} content than did SG at all salinity levels. At 23.5 dS/m, AG had shoot Na^+, Cl^-, and Ca^{++} concentrations 47.4%, 53.7%, and 38.1% higher, respectively, and roots Na^+, Cl^-, and Ca^{++} concentrations 76.7%, 41.8%, and 39.3% higher, respectively, than SG.

Although increasing salinity reduced shoot and root Mg^{++} concentrations of both AG and SG (Table 3.2), Mg^{++} reduction was greater in AG than in SG. At 23.5 dS/m, Mg^{++} concentration in SG shoots and roots were 17.7% and 206.3% higher than that of AG, respectively. Shoots had higher Mg^{++} concentrations than roots in both species.

Potassium concentrations in AG shoots and roots decreased as salinity level increased (Table 3.2). Although SG shoot K^+ concentrations decreased significantly with increasing salinity, SG roots maintained a stable K^+ content up to 18.8 dS/m. Under nonsaline and low saline conditions, AG roots contained higher K^+ content than SG, however, at 23.5 dS/m, SG roots had 157% higher K^+ content than that of AG. Saltgrass shoots exhibited higher K^+ than AG across all salinity treatments.

The changes in Na^+ and K^+ resulted in decreases in the K^+/Na^+ ratios in shoots and roots of both species (Table 3.2). Regression analysis predicted that shoot K^+/Na^+ ratio decreased to 1 at 24.2 and 16.4 dS/m, and root K^+/Na^+ ratio decreased to 1 at 14.64 and 14.1 dS/m, for SG and AG, respectively.

Table 3.2. Shoot and root mineral concentration (mg/g. dw) of alkaligrass (AG) and saltgrass (SG) exposed to salinity stress (dS/m) in Container Study II.

Salinity	Na$^+$		Cl$^-$		Ca^{++}		Mg^{++}		K$^+$		K$^+$ / Na$^+$	
level	AG	SG	AG	SG	AG	SG	AG	SG	AG	SG	AG	SG
						Shoot						
0.2	0.36dA^z	0.33 cA	0.77 dA	0.41 dB	0.18 dB	0.20 cA	1.59 aA	1.34 aA	3.69 aB	4.63 aA	10.41 aA	15.25 aA
14.1	2.13 cA	1.24 bB	6.50 cA	3.55 cB	1.03 cA	1.30 bA	0.99 bB	1.21 aA	2.80 bB	3.68 bA	1.32 bB	3.06 bA
18.8	2.79 bA	2.15 aB	7.54 bA	4.26 bB	2.13 bA	1.72 abB	0.85 cB	1.14 abA	2.57 cB	3.10 cA	0.93 bcB	1.45 bA
23.5	3.64 aA	2.47 aB	8.33 aA	5.42 aB	2.54 aA	1.84 aB	0.79 cB	0.93 bA	2.30 dB	2.95 cA	0.63 cB	1.20 bA
Salinity effect												
Linear	***	***	***	***	***	***	***	*	***	***	***	***
Quadratic	***	***	***	***	***	***	***	**	***	***	***	***

Table 3.2. Continued.

Salinity	Na$^+$		Cl$^-$		Ca^{++}		Mg^{++}		K$^+$		K$^+$ / Na$^+$	
level	AG	SG	AG	SG	AG	SG	AG	SG	AG	SG	AG	SG
						Root						
0.2	0.43dA[z]	0.42 cA	0.95 dA	0.46 dB	0.73 dA	0.44 dB	0.85 aA	0.80 aA	4.19 aA	2.18 aB	9.78 aA	5.16 aB
14.1	3.41 cA	2.21 bB	5.35 cA	4.23 cB	2.73 cA	2.62 cA	0.35 bB	0.64 bA	2.42 bA	2.17 aA	0.71 bB	0.98 bA
18.8	4.19 bA	2.80 aB	6.91 bA	5.33 bB	3.65 bA	3.06 bB	0.27 bcB	0.55 bcA	1.53 cB	1.97 abA	0.37 bB	0.70 bcA
23.5	5.99 aA	3.39 aB	10.55 aA	7.44 aB	4.54 aA	3.26 aB	0.16 cB	0.49 cA	0.72 dB	1.85 bA	0.12 bB	0.55 cA
Salinity effect												
Linear	***	***	***	***	***	***	***	***	***	*	***	***
Quadratic	***	***	***	***	***	***	***	***	***	*	***	***
Contrast												
shoot vs roo	*	*	*	*	*	*	*	*	*	*	*	*

[z] Lowercase letters indicate significant differences (*P* =0.05) among salinity treatments for each species.
Uppercase letters indicate significant differences (*P* =0.05) between species within a given salinity level.

[x] ns, *, **, and *** indicate non-significant or significant of linear or quadratic regression at *P* = 0.05, 0.01, and 0.001 level, respectively.

Hydroponic Experiment

Sodium, Cl^-, and Ca^{++} concentrations in shoots, sap, and roots of all species were increased linearly as salinity level increased. Shoot and sap Na^+, Cl^-, and Ca^{++} concentrations in TF, AG, and SG were lower than those in roots (Table 3.3, 3.4 and 3.5). In contrast, KBG shoots had higher Na^+, Cl^-, and Ca^{++} contents than its roots (Table 3.3 and 3.5). Kentucky bluegrass died when grown at 23.5 dS/m.

Although shoot Na^+ and Cl^- contents increased linearly with increasing salinity for all species, the extent of increase ranked as: KBG > TF > AG > SG. Likewise, Na^+ and Cl^- concentrations of KBG leaf sap were higher than other grasses. Tall fescue had higher Na^+ and Cl^- content than AG and SG at 23.5 dS/m (Table 3.3, 3.4 and 3.5).

Shoot and root Ca^{++} concentrations of AG were higher than other grasses when salinity level exceeded 9.4 dS/m (Table 3.3 and 3.5). In the sap, however, KBG had more Ca^{++} than other grasses at 14.1 dS/m (Table 3.4). At 23.5 dS/m, AG and TF sap had higher Ca^{++} concentration than SG (Table 3.4).

Increased salinity reduced Mg^{++} and K^+ concentrations in shoots, sap, and roots of all grasses (Table 3.3, 3.4 and 3.5). Shoot K^+ content of TF, and shoot Mg^{++} and K^+ contents of SG and AG were higher than these of roots (Table 3.3). In contrast, Mg^{++} and K^+ contents of KBG did not differ between roots and shoots, and Mg^{++} content of TF was higher in roots than shoot (Table 3.3). Sap Mg^{++} and K^+ concentrations of SG were higher than that of other grasses at 14.1, and 23.5 dS/m (Table 3.4). Saltgrass shoot, sap, and root had higher K^+ content than other grasses, and KBG had the lowest K^+ concentrations (Table 3.3, 3.4 and 3.5).

Increased salinity reduced K^+/Na^+ ratio in shoots, sap and roots of all species (Table 3.3, 3.4 and 3.5). Regression analysis predicted that shoot K^+/Na^+ ratio decreased to 1 at 10.8, 20.4, 9.9, and 5.5 dS/m for AG, SG, TF, and KBG, respectively. Root K^+/Na^+ ratio decreased to less than 1 when salinity exceeded 9.3, 10.0, 8.4, and 4.2 dS/m for AG, SG, TF, and KBG, respectively.

Leaf ion secretion rate of SG increased as salinity level increased, attaining 5.05, 6.86, and 1.22 mg/ g dw/day for Na^+, Cl^-, and Ca^{++}, respectively, at 23.5 dS/m (Fig. 3.1). Interestingly, almost no Mg^{++} and K^+ were secreted.

Table 3.3. Shoot mineral concentration (mg/g. dw) of alkaligrass, saltgrass, tall fescue, and Kentucky bluegrass exposed to salinity stress (dS/m) in the Hydroponic Study.

Salinity level	Na^+	Cl^-	Ca^{++}	Mg^{++}	K^+	K^+/Na^+
Alkaligrass						
2.0	0.64fAB[z]	0.83 f AB	0.42 eA	2.72 a A	6.73 a B	10.53 a B
4.7	2.48 e B	4.66 e B	2.17 dB	1.14 b B	6.11 b B	2.47bAB
9.4	3.63 d C	6.78 d B	3.39 cA	0.90 bc B	5.75 c B	1.59 c B
14.1	5.26 c B	7.97 c B	4.70 bA	0.82 bc B	5.41cdB	1.03 cd B
18.8	6.05 b C	9.02 b C	5.77 aA	0.58 cd B	5.36 d B	0.89 cd B
23.5	6.80 a B	9.68 a B	6.31 aA	0.39 d B	4.79 e B	0.71 d B
Significance[x]	L***	L***	L***	Q*	L***	Q***
Saltgrass						
2.0	0.44 e B	0.58 f B	0.37 f A	1.82 a B	8.93 a A	22.16 a A
4.7	1.71 d B	3.51 e C	1.32 e C	1.56 b A	6.80 b A	4.78 b A
9.4	2.93 c D	4.52 d C	2.20 d B	1.33 c A	6.62 c A	2.26 b A
14.1	3.48 bc C	5.23 c C	2.76 c D	1.28 cd A	6.27 d A	1.80 b A
18.8	3.99 ab D	6.30 b D	3.51 bD	1.18 d A	6.06 e A	1.52 b A
23.5	4.42 a C	6.85 a C	3.99 a C	1.04 e A	5.85 f A	1.32 b A
Significance	L***	L***	L***	Q*	Q***	Q**
Tall fescue						
2.0	0.84 f A	0.71 f AB	0.31 e A	0.85 a C	5.90 a C	7.05 a B
4.7	2.56 e B	3.58 e C	3.19 dA	0.68 ab C	5.16 b C	2.05 b B
9.4	4.94 d B	6.54 d B	3.50 dA	0.63abcC	4.52 c C	0.92 c C
14.1	6.03 c B	8.35 c B	4.26 c B	0.62abcCB	4.22 cdC	0.70 c C
18.8	6.81 b B	10.30 b B	5.07 b B	0.47 bc B	4.16 cdC	0.61 c C
23.5	8.60 a A	11.63 a A	5.69 a B	0.36 c B	3.72 d C	0.43 c C
Significance	L***	L***	L***	Q ns	L***	Q***
Kentucky bluegrass						
2.0	0.75 d A	1.05 d A	0.36 dA	0.90 a C	2.67 a D	3.80 a B
4.7	5.10 c A	6.92 c A	2.18 c B	0.59 b C	1.58 b D	0.31 b B
9.4	8.02 b A	12.28 b A	3.52 bA	0.59 b C	0.81 c D	0.10 b D
14.1	9.43 a A	13.25abA	3.92 a C	0.44 bcC	0.74 cdD	0.08 b D
18.8	9.89 a A	13.95 a A	4.08 a C	0.28 c C	0.54 d D	0.06 b D
Significance	L***	L***	L***	L**	Q***	Q*

[z] Lowercase letters indicate significant differences (P =0.05) among salinity treatments for each species.

Uppercase letters indicate significant differences (P =0.05) among species within a given salinity level.

[x] ns, *, **, and *** indicates non-significant or significant linear (L) or quadratic (Q) regression at P = 0.05, 0.01, and 0.001 level, respectively.

Table 3.4. Sap mineral concentration (mg/g. dw) of alkaligrass, saltgrass, tall fescue, and Kentucky bluegrass exposed to salinity stress (dS/m) in the Hydroponic Study.

Salinity level	Na^+	Cl^-	Ca^{++}	Mg^{++}	K^+	K^+ / Na^+
Alkaligrass						
2.0	0.46 eA[z]	0.87 dA	0.45 f B	2.70 a A	5.03 a B	10.85 aC
4.7	2.76 d B	2.66 cC	1.63 e BC	1.67 b A	4.32 b C	1.56 b C
9.4	3.30 c B	3.33 cC	2.01 d B	1.27 c A	3.75 c C	1.13 bc C
14.1	3.46 c C	5.78 bCB	2.57 c C	1.04 d A	3.59 cd C	1.04 bc B
18.8	4.23 b B	6.92 abB	3.23 b A	0.66 e B	3.31 cd C	0.78 bc C
23.5	4.97 a B	7.53 aB	4.53 a A	0.38 f B	3.25 d B	0.65 c B
Significance[x]	L***	L***	L***	Q*	L***	Q***
Saltgrass						
2.0	0.11 f B	0.12 eB	0.14 e D	1.51 a B	8.64 a A	76.51 aA
4.7	2.78 e B	3.16 dC	1.53 d C	1.23 ab B	7.64 b A	2.75 b A
9.4	3.03 d B	3.75 cC	2.01 c B	1.20 b A	7.07 c A	2.33 b A
14.1	3.37 c C	4.32 bC	2.47 bC	1.10 bc A	6.20 d A	1.84 b A
18.8	3.80 b C	4.69 abC	2.68 ab B	0.89 cd A	5.37 e A	1.41 b A
23.5	4.10 a C	5.20 aC	2.96 a B	0.75 dA	5.07 e A	1.24 b A
Significance	L***	L***	L***	Q*	Q***	Q**
Tall fescue						
2.0	0.25 f B	0.18 eB	1.41 f A	1.00 a C	5.23 a B	20.80 a B
4.7	2.55 e B	3.78 dB	1.87 e BA	0.81 b C	4.94 b B	1.94 b B
9.4	2.90 d B	5.09 cB	2.35 d BA	0.75 c B	4.59 c B	1.59 bc B
14.1	3.74 c B	6.79 bB	2.95 c B	0.58 d B	4.25 d B	1.14 cd B
18.8	4.41 b A	7.88 aA	3.19 b A	0.50 e B	3.71 e B	0.84 de B
23.5	5.99 a A	8.04 aA	4.34 a A	0.30 f B	2.90 f C	0.49 e C
Significance	L***	L***	L***	Q***	L***	Q***
Kentucky bluegrass						
2.0	0.60 d A	0.81 dA	0.33 d C	0.93 a C	1.82 a C	3.30 a D
4.7	4.43 c A	5.93 cA	2.13 c A	0.79 b C	1.50 b D	0.34 b D
9.4	5.88 b A	6.75 bA	2.66 b A	0.48 c C	1.25 c D	0.21 b D
14.1	7.11 a A	8.79 aA	3.36 a A	0.43 c B	0.76 d D	0.11 b C
Significance	L***	L***	L***	L***	Q***	Q*

[z] Lowercase letters indicate significant differences (P =0.05) among salinity treatments for each species.

Uppercase letters indicate significant differences (P =0.05) among species within a given salinity level.

[x] *, **, and *** indicates significant linear (L) or quadratic (Q) regression at P = 0.05, 0.01, and 0.001 level, respectively.

Table 3.5. Root mineral concentration (mg/g. dw) of alkaligrass, saltgrass, tall fescue, and Kentucky bluegrass exposed to salinity stress (dS/m) in the Hydroponic Study.

Salinity level	Na^+	Cl^-	Ca^{++}	Mg^{++}	K^+	K^+ / Na^+
			Alkaligrass			
2.0	0.64 f AB[z]	0.97 e B	0.93 f A	1.78 a A	5.15 a A	8.15 a AB
4.7	1.77 e C	4.19 d B	2.57 e A	1.01 bAB	3.93 b B	2.36 b A
9.4	5.72 d A	8.58 c A	5.41 d A	0.57 bcB	3.14 c B	0.55 c B
14.1	6.88 c A	10.68 bA	6.51 c A	0.41 c B	2.47 b C	0.36 c B
18.8	7.78 b B	11.87 abA	7.38 b A	0.20 c C	1.67 e C	0.22 c B
23.5	8.78 a B	13.20 a B	8.22 a A	0.15 c C	0.89 f C	0.10 c C
Significance[x]	L***	L***	L***	Q***	L***	Q***
			Saltgrass			
2.0	0.50 f B	0.91 f B	0.65 f B	1.25 a B	4.52 a A	9.13 a A
4.7	2.64 e BC	3.38 e B	2.57 e A	1.15 abAB	4.52 a A	1.71b AB
9.4	3.78 d B	5.52 d B	3.99 d B	0.96 bcA	4.52 a A	1.20 b A
14.1	4.85 c B	6.61 c B	4.45 c C	0.83 c A	4.33 a A	0.89 b A
18.8	5.50 b C	7.41 b B	5.33 b C	0.51 d B	4.11 a A	0.75 b A
23.5	6.15 a C	8.33 a C	5.92 a C	0.42 d B	4.03 a A	0.66 b A
Significance	L***	L***	L***	L***	Q ns	Q*
			Tall fescue			
2.0	0.87 f A	1.82 e A	0.45 f C	1.75 a A	5.07 a A	5.92 a B
4.7	3.22 e B	6.44 d A	2.28 e A	1.33 b A	3.70 b B	1.18 b B
9.4	5.80 d A	8.95 c A	3.49 d B	0.96 cA	3.15 c B	0.54 bc B
14.1	7.02 c A	10.44 c A	5.47 c B	0.91 cd A	2.86 c B	0.41 c B
18.8	8.19 bAB	12.03 b A	5.99 b B	0.84 cd A	2.31 d B	0.28 c B
23.5	10.53 a A	15.10 a A	7.16 a B	0.74 d A	1.93 d B	0.18 c B
Significance	L***	L***	L***	Q***	L***	Q**
			Kentucky bluegrass			
2.0	0.84 f A	1.18 f AB	0.34 e C	0.82 a C	2.23 a B	2.72 a C
4.7	4.84 e A	6.62 e A	1.89 d A	0.65 b B	1.42 b C	0.29 bC
9.4	5.73 d A	8.79 d A	2.65 c C	0.51 cB	0.58 c C	0.10 b C
14.1	6.85 c A	10.17 c A	3.06 b D	0.46 cd B	0.27 d D	0.04 b C
18.8	8.55 b A	11.77 b A	3.66 a D	0.39 d B	0.19 d D	0.02 b C
Significance	L***	L***	L***	Q***	Q***	Q**

[z] Lowercase letters indicate significant differences (P =0.05) among salinity treatments for each species.

Uppercase letters indicate significant differences (P =0.05) among species within a given salinity level.

[x] ns, *, **, and *** indicates non-significant or significant linear (L) or quadratic(Q) regression at $P = 0.05$, 0.01, and 0.001 level, respectively.

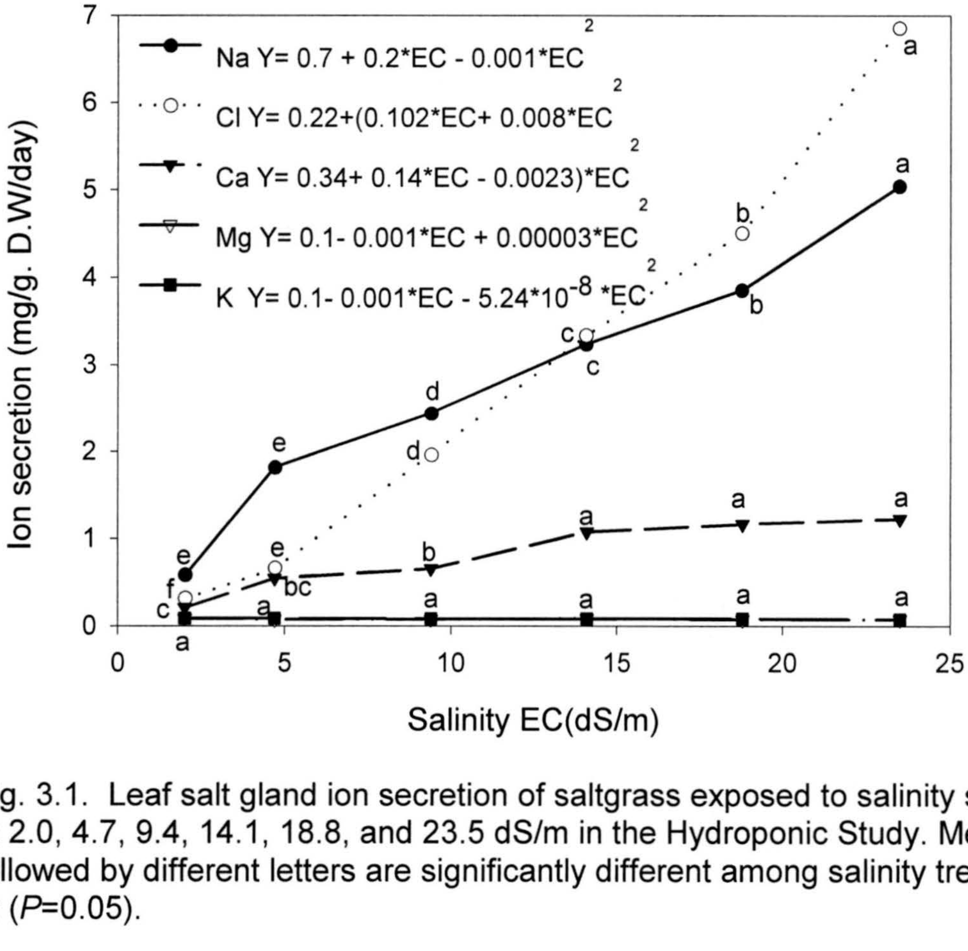

Fig. 3.1. Leaf salt gland ion secretion of saltgrass exposed to salinity stress at 2.0, 4.7, 9.4, 14.1, 18.8, and 23.5 dS/m in the Hydroponic Study. Means followed by different letters are significantly different among salinity treatments at (*P*=0.05).

Table 3.6. Pearson correlation coefficients for leaf proline (PR), relative shoot dry weight (SH), relative root dry weight (RO), relative root length (RL), leaf firing (LF), turf quality (TQ), leaf water content (WC), osmotic adjustment (OA), Sap Na, Cl, Ca, Mg, K, and K/Na ratio in hydroponic study.

Parameter	WC	OA	SH	RO	RL	LF	TQ	Na	Cl	Ca	Mg	K	K/Na
PR	-0.75***	0.58***	-0.78***	-0.51***	-0.36*	0.88***	-0.75***	0.76***	0.87***	0.80***	-0.51***	-0.42***	-0.38**
WC		-0.78***	0.65***	0.46***	0.54***	-0.76***	0.84***	-0.82***	-0.87***	-0.89***	0.66***	0.54***	0.39*
OA			-0.43**	-0.02 ns	-0.05 ns	0.57***	-0.74***	0.78***	0.82***	0.85***	-0.65***	-0.13ns	-0.54***
SH				0.92***	0.85***	-0.96***	0.84***	-0.82***	-0.89***	-0.75***	0.69***	0.77***	0.42**
RO					0.89***	-0.82***	0.66***	-0.59***	-0.61***	-0.42**	0.53***	0.87***	0.05 ns
RL						-0.78***	0.69***	-0.55***	-0.52***	-0.38**	0.56***	0.78***	0.07 ns
LF							-0.95***	0.84***	0.89***	0.83***	-0.69***	-0.65***	-0.32*
TQ								-0.92***	-0.95***	-0.81***	0.73***	0.41**	0.15 ns
Na									0.95***	0.89***	-0.76***	-0.66***	-0.65***
Cl										0.92***	-0.78***	-0.62***	-0.58***
Ca											-0.77***	-0.54***	-0.56***
Mg												0.55***	0.39*
K													0.65***

ns, *, **, and *** indicate non-significant or significant of correlations at $P = 0.05$, 0.01, and 0.001 level, respectively (n= 132).

Discussion

Along with Lunt et al.(1961); Torello and Rice (1986); Marcum (1999); and Qian et al.(2001), our results indicated that increasing Na^+, Cl^-, and Ca^{++} in the growth medium increased Na^+, Cl^-, and Ca^{++} concentrations and decreased Mg^{++} and K^+ concentrations in shoots, sap, and roots of all species. Information on ion accumulation differences among different species and the patterns of accumulation of ions in various parts of a plant is very important in evaluation of salinity tolerance, and to understanding salinity tolerance mechanisms employed by the plant.

From both the container and hydroponic experiments we concluded that the selected species were quite different in their ability to exclude Na^+ and Cl^- from their shoots. Shoot and sap Na^+ and Cl^- concentrations were positively correlated with leaf firing (r = 0.84 to 0.89), negatively correlated with turf quality (r= - 0.92 to – 0.95), shoot growth (r = - 0.82 to – 0.89), and root growth (r = - 0.59 to – 0.61) (Table 3.6). Therefore, accumulation of Na^+ and Cl^- under saline conditions was one of the major causes of growth reduction observed in our study. Salinity tolerance of these grasses was associated with their ability to exclude Na^+ and Cl^- from the shoots, thus maintaining a moderate ion content. Similar results were observed in a number of other studies, especially in grasses (Weimberg 1986). Among cool season turfgrass species, salt tolerant AG and 'Dawson' red fescue (*Festuca rabra* L.) restricted shoot Na^+ accumulation compared to less tolerant 'Jamestown' red fescue and 'Adelphi' and 'Ram I' KBG (Torello and Rice, 1986). 'Limousine', a more salt tolerant KBG cultivar, accumulated less Na^+ and Cl^- than 'Kenblue', a more salt sensitive KBG (Qian et al., 2001). When exposed to NaCl up to 400 mM (~ 36.56 dS/m), salt tolerant 'Tifway' bermudagrass (*Cynodon dactylon* L.), Manilagrass (*Zoysia matrella* L.), St. Augustinegrass (*Stenotaphrum secundatum* Walt.), and seashore paspalum (*Papsalum vaginatum* Swartz) restricted shoot Na^+ and Cl^- accumulation relative to salt sensitive Japanese lawngrass (*Zoysia japonica* Steud.) and centipedegrass (*Eremochloa ophuriodes* (Munro) Hack.) (Marcum and Murdoch, 1994). Flowers and Hajibagheri (2001) suggested that ion transport to the shoot reflects cytosolic ion concentrations, with a more sensitive cultivar of barley (*Hordeum vulgare* L.) having a higher sodium concentration in its cytoplasm than a more resistant cultivar.

Several possible mechanisms may be involved in excluding Na^+ and Cl^- from shoots, including ion absorption by specialized xylem parenchyma cells (in the proximal root region) (Yeo et al., 1977), ion restriction because of ion selectivity during ion transportation (Hajibagheri et al., 1989; Datta et al., 1996), and ion secretion through salt glands on the leaf epidermal surfaces (Liphschitz and Waisel, 1974; Oross et al., 1985; Marcum et al., 1998).

Our results showed that Na^+ and Cl^- concentrations in TF, AG, and SG were lower in shoots than in roots, indicating that ion absorption and ion restriction may play significant role in salinity tolerance in these grasses. The fact that KBG shoots had higher Na^+ and Cl^- than its roots suggested that KGB had a limited capacity for those responses.

Shoot and sap of SG had the lowest concentrations of Na^+, Cl^-, and Ca^{++} among the grasses we tested. The low ion concentrations of SG were likely associated with efficient Na^+, Cl^-, and Ca^{++} secretion as salinity levels increased. The secretion rates of

Na^+, and Cl^- were 5.05, and 6.86 mg/g dw/day at 23.5 dS/m, respectively. Similar results have been reported by Marcum (1999).

Along with Na^+ and Cl^- exclusion from shoots, selectivity of K^+ over Na^+ is critical in turfgrass salinity tolerance. Our results indicated that SG shoots, sap, and roots had higher K^+ content than other grasses, and KBG shoots and roots had the lowest K^+ concentrations. Shoot K^+ content was positively correlated with shoot and root growth and turf quality, and negatively correlated with leaf firing (Table 3.6). Marcum and Murdoch (1994) reported that among warm season turfgrass species, salt tolerant Manilagrass and seashore paspalum maintained higher shoot K^+ than salt sensitive Japanese lawngrass and centipedegrass. Salt tolerance has been associated with high shoot K^+ concentrations in relation to Na^+ and Cl^- in barley (Storey and Wyn Jones, 1978) and tall wheatgrass (*Agropyron elongatum* Host Beauv.) (Shannon, 1978).

Datta et al. (1996) suggested that restriction or exclusion of Na^+ and higher K^+/Na^+ ratio contributed to the higher degree of salt tolerance in sudan grass (*Sorghum Sudanese* Stapf.) compared to teosinte (*Euchlaena maxicana* Schard.) and maize. Flowers and Hajibagheri (2001) found that between two cultivars of barley the K^+/Na^+ ratio of salt resistant 'Gerbel' was twice of that in salt sensitive 'Triumph'.

Wyn Jones et al. (1979) suggested a threshold K^+/Na^+ ratio of 1 for normal growth of plants subjected to salinity. Shannon (1978) studied salt tolerance of 32 lines of tall wheatgrass; he found that salinity tolerance was associated with maintenance of K^+/Na^+ ratio more than 1. Combining data generated from both the container and hydroponic experiments, results indicated that, SG, AG, TF, and KBG could maintain a shoot K^+/Na^+ ratio of 1 when salinity level was less than 22.3, 13.6, 7.4, and 3.7 dS/m, respectively.

This indicated that SG had the highest selectivity of K^+ over Na^+ while KBG had the lowest selectivity of K^+ when Na^+ concentrations were high. Interestingly, K^+/Na^+ ratio in all species was higher in shoots than in roots, possibly because these plants achieve the K^+ selectivity via multiple processes in multiple locations.

The difference in K^+/Na^+ ratio of SG, AG, TF, and KBG may associate with Ca^{++} concentrations in their roots. In the container study, TF roots had higher Ca^{++} than that of KBG (Table 3.2). In the hydroponic study, Ca^{++} content in roots ranked as AG > TF > SG > KBG. Higher Ca^{++} in roots may have benefited AG and TF by helping to maintain the proper function of biological membranes under saline conditions (Hanson, 1984; Kent and Lauchli, 1985). Calcium is an important factor in the maintenance of membrane integrity and ion uptake and transport regulation; therefore, Ca^{++} is essential for K^+/Na^+ selectivity (Cramer et al., 1985, 1986 and 1987; Cheeseman, 1988; Subbarao et al., 1990; Cachorro et al., 1994; Garg, 1998). He and Cramer (1992) indicated that changes in tissues Ca^{++} concentration in *Brassica* were correlated with the relative salt tolerance of the species.

In summary, this chapter has documented differential ion relations of four turfgrasses in response to different salinity levels. Regulation of ion concentrations (via ion restriction, exclusion or secretion) and maintenance of higher K^+/Na^+ ratio in shoots were important mechanisms attributed to the differences in salinity tolerance observed in these species.

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