Nutrient Uptake Responses and Inorganic Ion Contribution to Solute Potential under Salinity Stress in Halophytic Seashore Paspalums

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ABSTRACT

There is increasing interest in superior halophytes for use on saline turfgrass, forage, and land reclamations sites. We investigated halophytic seashore paspalum (*Paspalum vaginatum* Swartz) ecotype responses for inorganic ion uptake in shoot tissues and to identify total and individual inorganic ion contributions to total solute potential (Ψ_s) adjustment under increasing salinity. In a greenhouse study, nine ecotypes varying substantially in salinity tolerance were grown in nutrient/sand culture with six salinity levels up to 49.7 dS m^{-1}. Increasing salinity reduced uptake of K, Ca, and Mg, with Mg most affected. Sodium tissue content increased with salinity, but Cl increased and then declined as salinity increased. The least salt- tolerant ecotype, 'Adalayd', exhibited lower Cl uptake at high salinity compared to the most salt-tolerant types (SI 93-2, HI 101). Shoot and root growth were positively correlated to K tissue content and K was the primary ion for solute potential (Ψ_s) adjustment. Inorganic ions contributed 57 to 97% to Ψ_s adjustment with salt-tolerant ecotypes exhibiting less dependence on inorganic ions for Ψ_s adjustment apparently due to their ability to maintain synthesis of organic osmolytes under high salinity. In terms of physiological traits adapted for salt-screening protocols the following were not useful: tissue nutrient/element content; K/Na and Ca/Na tissue content ratios; and K or other ion contributions to total Ψ_s in percent or MPa units. Shoot tissue content relationships of K, Mg, and Ca to increasing salinity indicate the importance of nutritional programs for these nutrients on salt-affected sites.

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Salinity stresses are major factors limiting plant growth and productivity in many areas of the world and include imposition of ion toxicities (e.g., Na and Cl), ionic imbalances, osmotic stress, and soil permeability problems (Epstein et al., 1980; Flowers, 1999). Salinity tolerance differs among crop species with growth of most crops adversely affected when electrical conductivity of soil (saturated paste extract, EC$_e$) is 1 to 3 dS m^{-1}, while moderately salt-tolerant crops may continue to grow up to 8 dS m^{-1} (Maas, 1987). Only halophytes, however, can survive at higher salinity ≥30 dS m^{-1} (Flowers et al., 1977; Flowers and Yeo, 1986; Maas, 1987).

Seashore paspalum (*Paspalum vaginatum* Swartz), a halophytic warm season grass, has recently gained interest for use on saline turfgrass and forage sites, drainage water reuse schemes, and land reclamation under saline conditions (Duncan and Carrow, 2000; Semple et al., 2003; Grattan et al., 2004; Rogers et al., 2005). Seashore paspalum exhibits a wide range in salinity tolerance among ecotypes (Lee et al., 2004b, 2005). For several halophytic grasses, Hester et al. (2001) reported a wide intraspecific variation in salinity tolerance that was as great as the interspecific variation observed.

Genotypes within a species that exhibit superior salinity tolerance must possess different mechanisms or degree of expression of mechanisms compared to the least tolerant types. Knowledge of these mechanisms can assist in developing physiological-based screening protocols in traditional breeding programs and focus biotechnologi-

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cal approaches toward enhancement of specific biochemical traits contributing to superior salt tolerance within the species (Bohnert and Jensen, 1996; Duncan and Carrow, 1999; Hester et al., 2001; Ashraf and Harris, 2004).

When salt-tolerant plants are exposed to a more negative water potential (Ψ_w) in saline soil conditions, they are able to maintain water uptake, growth, and persistence through solute potential (Ψ_s) adjustment primarily accomplished by uptake of inorganic ions and/or synthesis of organic osmolytes (Bohnert and Shen, 1999). Halophytes that are ion includers often adapt to low water potentials by accumulation of inorganic solutes to maintain turgor pressure and total water potential (Glenn, 1987; Flowers et al., 1990; Glenn et al., 1992). To avoid toxicity of Na, Cl, and other inorganic ions within the cytosol and cell walls, these ions are compartmentalized mainly into the vacuoles of shoot and root cells (Flowers et al., 1977; Lerner et al., 1994). Compatible osmolytes (ones that do not disturb intracellular biochemistry), such as organic solutes and K to some extent, must also be present in the cytosol for osmotic adjustment and osmoprotection (Sairam et al., 2006). Generally, salinity tolerance is related to maintaining higher contents of K and Ca, because these ions are involved in turgor control and cell wall integrity, respectively, under saline conditions (Cramer et al., 1985; Flowers and Yeo, 1986; Wolf et al., 1991).

Genotypic differences in ion accumulation have been noted among four seashore paspalum ecotypes, where the most salt-tolerant type (FSP-1) exhibited the lowest shoot tissue concentrations of Na and Cl as salinity (sea salt) increased above 30 dS m^{-1} (Dudeck and Peacock, 1985). Increasing salinity reduced plant tissue K content in all ecotypes. In a study with FSP-1 and another more salt-tolerant type, FSP-3, increasing salinity caused Na to increase in both grasses, but to a lesser extent in FSP-3, while Cl tissue content did not change with salinity and K content decreased (Dudeck and Peacock, 1993). Contributions of inorganic ions to osmotic adjustment were not reported in these studies, but osmotic adjustment through inorganic ion uptake and/or synthesis of organic compounds has been postulated to have a significant role in salt tolerance in seashore paspalum by Marcum and Murdoch (1994).

In addition to inorganic ion contributions to osmotic adjustment, genotypic differences in nutrient and element uptake under salinity have implications for maintaining adequate nutrition and for optimizing nutrient/element related salinity tolerance mechanisms. Uptake of essential cations and anions including K^+, Ca^{2+}, Mg^{2+}, NH_4^+, and NO_3^- have been reported to be suppressed in various species by high concentrations of Na^+ and Cl^-, such as occurs in saline soils and irrigation waters (Grieve and Fujiyama, 1987; Awad et al., 1990; Rubinigg et al., 2003). Increasing salinity resulted in reduced Ca and Mg tissue contents within all four seashore paspalum ecotypes studied by Dudeck and Peacock (1985). In a study using two paspalum ecotypes, increasing salinity did not alter Ca, Cl, or Mn shoot tissue content, while Mg decreased with increasing salinity for the most salt-tolerant type (Dudeck and Peacock (1993). For both types, increasing salinity resulted in reduced levels of K, P, and Zn, while Na increased. The studies involving seashore paspalum have included a limited number of ecotypes and with a relatively narrow range of salinity tolerance.

Lee et al. (2004b, 2005) reported the salinity tolerance of nine seashore paspalum ecotypes; and salinity effects on total water potential, solute potential, and turgor pressure. Within the current paper, these nine ecotypes differing substantially in salinity tolerance were used to investigate under increasing salinity up to EC_w 50 dS m^{-1}: (i) differential ecotype nutrient and element uptake in the shoot tissues and their implications; and (ii) differential ecotype response for total inorganic and specific inorganic ion contributions to solute potential, $\Psi_{s,}$ at the highest salinity level; and the implications of these responses.

MATERIALS AND METHODS

Nine seashore paspalum ecotypes were selected for this study based on salinity tolerance, which included the most (SI 93-2, HI 101), intermediate ('Sea Isle 2000', TCR 1, TCR 6, 'Sea Isle 1', HI 34, SI 90), and least tolerant ('Adalayd') (Lee et al., 2005). The Sea Isle cultivars were released in 1999 from the University of Georgia program of Dr. Ron Duncan (Georgia-turf, 2007) while Adalayd is an older cultivar introduced from Australia in 1975 (Duncan and Carrow, 2000). In this paper, all grasses are referred to as ecotypes since they are all selections from various harsh environments except Adalayd. This study was conducted using a solution/sand culture under controlled greenhouse conditions at the Griffin Campus in Griffin, GA from May to November 1998. On cloudy days and to extend the daylength, supplemental light with 400W metal halide lights were used to maintain light intensity in the range of 500 to 900 μmol m^{-2} s^{-1}. Temperature in the greenhouse was 30 ± 2/27 ± 2°C (day/night) with a 14 h photoperiod. Plugs of each grass were grown using a greenhouse mix in 2 cm square by 3 cm containers in the greenhouse before plugging into larger containers of 13.0 cm long by 10.0 cm wide by 12.5 cm high. Soil was washed from the plugs before transplanting. Five 2-cm plugs of an ecotype were planted into a larger container with six replications of each grass and salinity level combination. Ecotypes were maintained under cutting practices of 2.5 cm (once a week) throughout the study. Each large container with nine holes at the bottom was filled with washed sand. The culture solution was prepared using a half-strength of Hoagland and Arnon's (1950) nutrient solution (#2), modified with Fe-EDTA as an iron source to give 5mg L^{-1} of Fe (Sprint 138, 6% Fe, Becker-Underwood, Ames, IA). Nine containers, each with an ecotype, were held in a wooden frame and placed in a 28-L container of nutrient solution (1.2 dS m^{-1} and pH = 6.3 ± 0.5) formulated with deionized water.

After a 4-wk acclimation period, the nutrient solution was salinized with a sea salt mixture to give salinity levels of 10.3, 20.5, 30.7, 39.5, and 49.7 dS m^{-1} (designated as EC_w0, EC_w10, EC_w20, EC_w30, EC_w40, and EC_w50, respectively). To minimize salt shock, EC_w (electrical conductivity of the nutrient solution) levels were gradually increased by adding 6.9 g kg^{-1} sea salt everyday until the final target salinity was achieved (Dudeck and Peacock, 1985). The nutrient solution was replaced weekly, aerated continuously, and refilled to constant volume using deionized water at approximately every 2 d. Electrical conductivity of the nonsaline solution was 1.2 dS m^{-1} and pH was 6.3 ± 0.5 during the experiment. Salinity levels were monitored by measuring the EC_w twice a week at 25°C with an Orion 160 conductivity meter (Orion, Boston, MA).

Shoot clippings were collected weekly and combined into 2-wk intervals to provide four clipping periods during the experiment. The excised clippings were immediately oven-dried at 70°C for 48 h and the samples from the last collection period at the end of the study were ground for tissue analyses. For mineral analyses of K, Na, Ca, Mg, Mn, Fe, B, Cu, and Zn, the microwave-assisted acid digestion method was used (Binstock et al., 1991). The homogenized ground samples (0.25 g) were extracted by heating with 10 mL concentrated nitric acid. Extractable chloride with 100 mg ground tissue was assayed using a chlorimetric autoanalyzer method according to Zali et al. (1956).

The contribution of inorganic ions to solute potential was determined using the van't Hoff equation as reported by Alarcon et al. (1993), where the calculated contribution of individual solutes to measured solute potential, $\Psi_{s,}$ was based on relative dry weight at saturation [dry weight/(saturated weight-dry weight)] and solute concentration on a dry weight basis. Solute potential of leaf tissue sap was determined as reported by Lee et al. (2005). We assumed that solutes behaved as ideal osmotica as noted by Alarcon et al. (1993). Ideally the solute contribution of the sap should be determined, but with fine-textured grasses this is very difficult. Some minor overestimation of solutes may occur from the use of solute tissue content, but we used the highest salinity level where inorganic solute concentration in the vacuoles would be very high for an ion accumulator halophyte such as seashore paspalum, which would minimize error.

The experimental design was a split-plot design with six replications where salinity level and ecotypes were the main and subplot, respectively. Six salinity levels (one salt level per container) were arranged randomly within each replication. Contents of inorganic ions were statistically analyzed using least significant difference (LSD) to separate means of grass ecotypes at each salinity level and among salinity levels for each ecotype (SAS Institute, 2001). Multiple regression analysis was used to determine the most significant inorganic ions that correlated with variation in shoot and root growth (dependent variables) across all salinity treatments. The unit used for regression was each ecotype-salinity replication. All independent variables significant at $p \leq 0.05$ level were included in the forward selection model, and partial R^2 and coefficient values were assessed for the relationship of variables.

RESULTS

Nutrient and Element Uptake in the Shoot Tissue

Seashore paspalum ecotypes are listed in the tables in declining order of salinity tolerance based on responses of shoot, root, and total growth (shoot, root, crown), where SI 93-2 and HI 101 were most tolerant, followed by six entries, and then Adalayd, which exhibited the least tolerance (Lee et al., 2005). Full data for P, Fe, Mn, Cu, Zn, and B are not presented since the effects of salinity on shoot tissue contents were small in magnitude as were ecotype differences. As salinity increased, ecotype responses for tissue content of these nutrients were generally classified into three response types as salinity increased: decreasing (P and Cu), initially decreasing followed by increasing (Fe and B), and initially increasing followed by decreasing (Cl, Mn, and Zn) tissue ion concentrations. The range of shoot tissue contents across all salinity levels and ecotypes was 3.7 to 6.1 mg g^{-1} P, and for the micronutrients in mg kg^{-1} values were: 75.1 to 107.0 Fe; 59.2 to 176.7 Mn; 49.7 to 78.0 Zn; 17.1 to 23.3 Cu; and 14.6 to 23.1 B.

Table 1. Potassium content (K) of nine seashore paspalum ecotypes under different salinity levels.

Entry	Potassium content (mg g^{-1} dry weight) at						F-test†	LSD (0.05)†
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	43.7 a‡	40.6 ab	39.4 bc	38.3 bc	35.8 cd	33.8 a-c	**	3.6
HI 101	42.1 ab	40.9 ab	40.1 bc	39.2 b	38.2 ab	35.9 a	*	2.9
Sea Isle 2000	41.4 ab	39.7 ab	38.8 cd	35.4 d	34.5 d	31.2 d	**	3.8
TCR 1	40.0 b	41.8 a	41.8 ab	41.6 a	40.1 a	33.3 b-d	**	3.7
TCR 6	42.6 ab	42.0 a	43.4 a	38.9 bc	37.6 bc	35.9 a	***	2.8
Sea Isle 1	43.6 a	39.1 b	39.6 bc	36.9 cd	35.4 d	36.1 a	**	4.0
HI 34	42.2 ab	40.5 ab	40.8 a-c	39.2 d	37.8 bc	35.8 a	***	1.8
SI 90	42.3 ab	40.0 ab	39.5 bc	37.4 b-d	36.2 b-d	34.8 ab	**	3.5
Adalayd	42.6 ab	39.7 ab	41.0 a-c	36.1 d	35.8 cd	32.0 cd	**	4.1
F-test†	0.28	0.36	**	***	***	**		
LSD (0.05)†	2.8	2.6	2.9	2.2	2.0	2.5		

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†F-test and LSD test (0.05) for a column are to compare mean performances among entries within a salt level at the 0.001 (***), 0.01 (**), and 0.05 (*) levels. The LSD (0.05) for a row is for paired comparisons to the control salinity level.

‡Means within a column followed by the same letter are not significantly different based on LSD (0.05).

Potassium was the most abundant nutrient in shoot tissue, and ranged from 40.0 to 43.76 mg g^{-1} for the nine ecotypes at EC_w0, and from 31.2 to 36.1 mg g^{-1} at EC_w50 (average of 19% decrease from control) (Table 1). Potassium uptake decreased with increasing salinity for all seashore paspalums and there were significant differences among ecotypes at salinity levels ≥ EC_w20.

Sodium uptake was enhanced with increasing salinity for all ecotypes with the greatest increase between EC_w0 and EC_w10 (Table 2). Average Na content ranged from 2.0 to 11.0 mg g^{-1} at control and EC_w50, respectively (5.5-fold increase). Differences in Na tissue content among the seashore paspalums were evident with increasing salinity, but small in magnitude and without an apparent relationship to salinity tolerance.

Average Cl content increased from the control 1.3 times to an average of 23.5 mg g^{-1} at EC_w30 but decreased slightly at salinity levels ≥ EC_w40. As with Na, Cl concentrations exhibited the greatest increase between EC_w0 (18.1) and EC_w10 (22.9 mg g^{-1} average) for all grasses except SI 90. Differences in Cl content among the seashore paspalums were evident with salinity treatment, but only at EC_w50 did the more salt-tolerant SI 93-2 (22.2) and HI 101 (21.0) exhibit higher shoot Cl contents than Adalayd (15.8 mg g^{-1}) (Table 3).

SI 93-2, TCR 6, and SI 90 exhibited a decrease in Ca content from EC_w0 to EC_w10. As salinity increased to EC_w20 or EC_w30, Ca content for the various ecotypes tended to increase and then decrease again (Table 4). Average Ca content at EC_w0 was 1.8 and at EC_w50 it was 1.6 mg g^{-1}. While significant differences among ecotypes were evident, they were not great in magnitude.

Shoot tissue Mg content decreased as salinity increased with the highest at EC_w0 (average 3.9 mg $g^{-1)}$ and the lowest at EC_w50 (average 2.1 mg g^{-1}) (Table 5). Ecotypes differences were small in magnitude.

Table 2. Sodium content (Na) of nine seashore paspalum ecotypes under different salinity levels.

Entry	Sodium content (mg g^{-1} dry weight) at						F-test†	LSD (0.05)†
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	1.5 d‡	7.1 a-d	8.4 bc	9.0 ef	9.6 c-e	10.3 cd	***	0.9
HI 101	2.0 b-d	7.6 ab	8.5 bc	9.5 d-f	9.5 c-e	10.9 bc	***	1.0
Sea Isle 2000	1.8 b-d	7.3 a-c	9.6 a	11.3 a	10.3 a	12.4 a	***	1.2
TCR 1	2.3 a-c	7.8 a	9.1 ab	10.9 ab	11.4 ab	11.9 ab	***	1.2
TCR 6	2.4 ab	7.1 b-d	9.1 ab	10.2 a-d	11.1 ab	10.5 cd	***	1.6
Sea Isle 1	2.0 b-d	7.5 a-c	9.0 a-c	10.8 a-c	10.2 b-d	11.2 bc	***	1.1
HI 34	1.5 d	6.9 cd	8.7 a-c	9.8 c-e	9.1 de	10.5 cd	***	1.1
SI 90	1.6 d	7.5 a-c	8.8 a-c	10.0 b-e	10.7 bc	11.6 ab	***	1.0
Adalayd	2.9 a	7.5 a-c	8.1 cd	9.5 d-f	10.4 bc	10.3 cd	***	1.2
F-test†	**	*	**	**	***	**		
LSD (0.05)†	0.6	0.7	1.0	1.1	1.2	1.0		

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†F-test and LSD test (0.05) for a column are to compare mean performances among entries within a salt level at the 0.001 (***), 0.01 (**), and 0.05 (*) levels. The LSD (0.05) for a row is for paired comparisons to the control salinity level.

‡Means within a column followed by the same letter are not significantly different based on LSD (0.05).

Table 3. Chloride content (Cl) of nine seashore paspalums under different salinity levels.

Entry	Chloride content (mg g^{-1} dry weight) at						F-test†	LSD (0.05)†
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	17.0 a‡	22.9 ab	22.7 a-c	22.9 a-c	20.8 b-d	22.2 a-c	0.08	4.3
HI 101	16.1 a	23.4 a	22.4 bc	23.4 ab	21.9 a-d	21.9 bc	**	3.1
Sea Isle 2000	15.7 a	20.7 a	23.6 ab	24.7 a	22.9 a-c	21.4 bc	***	2.3
TCR 1	19.9 a	23.7 a	23.7 ab	25.6 a	23.6 a-c	24.9 a	0.45	6.1
TCR 6	16.1 a	22.4 ab	24.9 a	25.9 a	24.7 a	19.9 c	**	3.7
Sea Isle 1	17.5 a	24.2 a	23.7 ab	23.8 ab	23.5 a-c	22.8 ab	***	2.3
HI 34	16.2 a	21.9 ab	23.5 ab	23.9 ab	22.8 a-c	20.2 bc	***	2.1
SI 90	24.9 a	23.4 a	24.2 ab	23.5 ab	22.4 a-c	21.7 bc	0.99	6.7
Adalayd	21.0 a	22.3 ab	20.5 c	20.0 c	19.9 cd	15.8 d	0.51	7.3
F-test†	0.59	0.17	**	*	*	***		
LSD (0.05)†	10.1	2.7	2.2	3.3	3.0	2.7		

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†F-test and LSD test (0.05) for a column are to compare mean performances among entries within a salt level at the 0.001 (***), 0.01 (**), and 0.05 (*) levels. The LSD (0.05) for a row is for paired comparisons to the control salinity level.

‡Means within a column followed by the same letter are not significantly different based on LSD (0.05).

For the grasses used in this study, the influence of increasing salinity on shoot and root growth responses as well as shoot water responses (water potential, solute potential, turgor pressure, relative water content) were reported in the paper by Lee et al. (2005). To determine the most important inorganic nutrients contributing to shoot and root growth responses of sea-shore paspalums as salinity increased, multiple regression analysis was used (Table 6). The following equations demonstrate shoot and root growth effect from nutrient concentrations, which included all variables significant at $p \leq 0.05$ level:

$$\text{Shoot growth} = -1.6 + 1.2\ (\text{K}) - 0.1\ (\text{B}) + 3.7\ (\text{Mg}) - 0.02\ (\text{Fe}),\ R^2 = 0.88$$

$$\text{Root growth} = 0.1 + 0.1\ (\text{K}) - 0.003\ (\text{Fe}) + 0.002\ (\text{Mn}) - 0.01\ (\text{Zn}) + 0.6\ (\text{P}) + 0.1\ (\text{Cl}),\ R^2 = 0.67.$$

Four nutrients (K, B, Mg, and Fe) in the shoot tissues accounted for 88% of the variation for shoot growth, while six nutrients (K, Fe, Mn, Zn, P, and Cl) accounted for 67% of root growth variation. Among the nutrients, K accounted for 76 and 30% of the total variation in shoot and root growth, respectively. Also, shoot growth was correlated with K and Mg uptake positively, and Fe and B uptake

Table 4. Calcium content (Ca) of nine seashore paspalums under different salinity levels.

Entry	Calcium content (mg g^{-1} dry weight) at						F-test†	LSD (0.05)†
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	2.0 b-d‡	1.6 b	2.0 b-d	1.9 d-f	1.6 cd	1.4 de	**	0.2
HI 101	2.0 cd	1.7 b	2.1 bc	2.0 c-e	1.7 b-d	1.5 c-e	**	0.3
Sea Isle 2000	2.5 a	2.3 a	2.6 a	2.5 a	2.1 a	1.8 a	***	0.2
TCR 1	2.2 b	1.7 b	1.8 d	1.9 ef	1.7 b-d	1.5 c-e	***	0.2
TCR 6	2.1 bc	1.6 b	1.8 d	1.9 d-f	1.6 b-d	1.5 de	***	0.2
Sea Isle 1	1.7 e	1.7 b	2.0 b-d	2.1 b-d	1.8 bc	1.8 ab	0.26	0.4
HI 34	2.0 b-d	1.7 b	2.1 bc	1.9 ef	1.5 cd	1.5 cd	**	0.3
SI 90	1.9 c-e	1.6 b	1.9 cd	1.8 f	1.5 d	1.4 e	***	0.2
Adalayd	1.7 e	1.8 b	2.1 b	2.1 bc	1.9 ab	1.6 bc	**	0.3
F-test†	***	***	***	***	**	***		
LSD (0.05)†	0.2	0.2	0.2	0.2	0.2	0.2		

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†F-test and LSD test (0.05) for a column are to compare mean performances among entries within a salt level at the 0.001 (***), 0.01 (**), and 0.05 (*) levels. The LSD (0.05) for a row is for paired comparisons to the control salinity level.

‡Means within a column followed by the same letter are not significantly different based on LSD (0.05).

Table 5. Magnesium content (Mg) of nine seashore paspalums under different salinity levels.

Entry	Magnesium content (mg g^{-1} dry weight) at						F-test†	LSD (0.05)†
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	3.8 c‡	3.1 cd	2.5 d	2.1 c	2.0 a	1.9 d	***	0.2
HI 101	4.1 a-c	3.4 bc	2.8 b-d	2.4 b	2.1 a	2.1 bc	***	0.4
Sea Isle 2000	4.2 a	3.7 ab	3.1 a-c	2.7 a	2.2 a	2.1 b-d	***	0.3
TCR 1	4.3 a	3.2 cd	2.7 cd	2.4 b	2.2 a	2.1 b-d	***	0.3
TCR 6	3.2 d	3.1 d	2.8 b-d	2.2 bc	2.2 a	1.9 cd	***	0.3
Sea Isle 1	4.4 a	3.6 ab	3.2 ab	2.7 a	2.2 a	2.4 a	***	0.4
HI 34	3.8 bc	3.1 cd	2.8 b-d	2.4 b	2.0 a	2.1 bc	***	0.4
SI 90	3.4 d	3.0 d	2.7 d	2.3 bc	2.1 a	2.1 bc	***	0.3
Adalayd	4.1 ab	3.8 a	3.4 a	2.7 a	2.2 a	2.1 b	***	0.4
F-test†	***	***	**	***	0.14	***		
LSD (0.05)†	0.3	0.3	0.4	0.2	0.3	0.02		

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†F-test and LSD test (0.05) for a column are to compare mean performances among entries within a salt level at the 0.001 (***), 0.01 (**), and 0.05 (*) levels. The LSD (0.05) for a row is for paired comparisons to the control salinity level.

‡Means within a column followed by the same letter are not significantly different based on LSD (0.05).

negatively. Root growth was related to K, Mn, P, and Cl content positively and Fe and Zn uptake negatively.

Estimated Contributions of Inorganic Osmolytes to Solute Potential (Ψ_s)

At EC_w50, inorganic solutes accounted for an average of 70% (range 57–97%) of the measured total solute potential, Ψ_s; where the total would include inorganic and organic osmolytes in both the vacuole and cytosol (Table 7). Inorganic ion contribution to measured Ψ_s was 57 and 71% for the salt-tolerant SI 93-2 and HI 101, respectively, and 82% for Adalyad. Sea Isle 1 exhibited a very strong capacity for inorganic solute contribution to total Ψ_s. In terms of solute potential, estimated inorganic solute contributions to Ψ_s averaged −1.76 MPa (range −1.41 to −2.42), while the total measured Ψ_s averaged −2.52 MPa (range −2.43 to −2.70).

The major contributions to estimated inorganic osmolytes were by K (average of 29.3%), Na (17.7%), and Cl (19.9%) and these three ions accounted for 93 to 95% of the inorganic osmolytes. For SI 93-2 and HI 101, K accounted for 23.6 (i.e., −0.59/−2.50 MPa) and 30.5%, respectively, of the ion contribution to the estimated inorganic solute adjustment, while for Adalyad, K contributed 36.3%. Sodium accounted for 12.4 and 15.8% of the total osmolyte adjustment for SI 93-2 and HI 101, respectively, but for Adalayd the Na contribution was 19.7%. Chloride accounted for 17.2, 20.5, and 19.8% of total Ψ_s for SI 93-2, HI 101, and Adalayd, respectively. Since halophytic seashore paspalum is an ion includer, it is assumed that the Na and Cl were sequestered in the vacuole, while K can be within the cytosol and chloroplast as a compatible ion as well as in the vacuole contributing to osmotic adjustment.

DISCUSSION

Nutrient and Element Shoot Tissue Responses to Increasing Salinity

While tissue sufficiency ranges have not been published for seashore paspalum, the shoot tissue content of seashore paspalum ecotypes across all salinity levels were within or greater than the general sufficiency ranges for other grasses with the exception of Ca which was lower (Carrow et al., 2001). Nutrients that decreased somewhat with increasing salinity were K, Ca, Mg, P, and Cu with average shoot tissue content at EC_w50 compared to EC_w0 levels of 81 (34.2 mg g^{-1}), 78 (1.6 mg g^{-1}), 53 (2.1 mg g^{-1}), 71 (4.1 mg g^{-1}), and 89% (18.6 mg kg^{-1}), respectively. Of particular note are the relatively high K tissue content and low Ca tissue content relative to other grasses with reported concentration ranges of 10.0 to 30.0 mg g^{-1} for K and 3.0 to 12.5 mg g^{-1} for Ca (Carrow et al., 2001).

The reduction in shoot tissue contents of Ca and Mg as salinity increased was probably due to the interactive substitution with Na (El-Hendawy et al., 2005). A negative correlation between Na and cations were found in this study, which were r^2 = 0.71 (K), 0.26 (Ca), and 0.85 (Mg), indicating existence of Na competition with these cations, especially for K and Mg. Dudeck and Peacock (1985, 1993) also reported that increasing Na affected Mg and K more than Ca tissue content in several paspalums they studied. Calcium plays a role in membrane integrity and maintenance of ion selectivity for plants (Marschner, 1995), but no significant difference in shoot Ca content was found between the most (SI 93-2 and HI 101) and least (Adalayd) salinity tolerant ecotypes in this study.

Table 6. Regression analysis of variables of inorganic nutrients attributing to shoot and root growth under salinity stress.

Shoot growth				Root growth			
Variable†	Coefficient	Partial R^2	*P* value	Variable†	Coefficient	Partial R^2	*P* value
K	1.20	0.76	<0.0001	K	0.08	0.30	0.0242
B	-0.08	0.06	<0.0001	Fe	-0.003	0.14	0.0795
Mg	3.72	0.03	<0.0001	Mn	0.002	0.08	<0.0001
Fe	-0.02	0.03	0.0007	Zn	-0.01	0.07	0.0003
				P	0.57	0.05	0.0013
				Cl	0.06	0.03	0.0320
Overall		0.88***		Overall		0.67***	

***Significant differences at the 0.001 probability level.

†Included all variables to meet 0.05 significance level for entry into the model.

In keeping with observations of Dudeck and Peacock (1985), Mg was generally higher than Ca in shoots of all ecotypes at all salinity levels. This result might be related to higher Mg composition (∼1290 mg L^{-1}) in sea salt mixture compared to ∼++411 mg L^{-1} for Ca, coupled with a strong competition between Mg and Ca at the cation binding sites on the root plasma membrane (Grattan and Grieve, 1999). However, the Ca/Mg ratios averaged across grasses at EC$_w$0 and EC$_w$50 were 0.52 and 0.74, respectively, reflecting the greater reduction in tissue Mg content as salinity increases compared to Ca. Ecotype differences in Ca/Mg were minor. Grieve et al. (2004) reported a Ca/Mg value of 0.60 for paspalum which did not change as salinity increased from 15 to 25 dS m^{-1} using drainage water.

With respect to shoot tissue K content only minor ecotype differences were observed with no apparent relationship to salinity tolerance (Table 1). Comparisons among the most and least tolerant ecotypes revealed some difference in shoot K/Na ratio as salinity level changed. At EC$_w$50, SI 93-2 and HI 101 had the higher K/Na ratio (means ± standard error = 3.3 ± 0.1 and 3.3 ± 0.1, respectively) compared to Adalayd (3.1 ± 0.1). Similarly at EC$_w$40, SI 93-2 and HI 101 had 3.7 ± 0.2 and 4.0 ± 0.2, respectively, while Adalayd had 3.4 ± 0.1. However, the K/Na ratios were all much higher than the >1 noted to be sufficient to supply necessary K for normal metabolic processes and were similar to those reported by Grieve et al. (2004) for Sea Isle 1 of 2.9 to 3.3.

Potassium tissue content, however, was the most important cation related to shoot (partial R^2 = 0.76) and root growth (R^2 = 0.30) in seashore paspalum ecotypes under variable salinity stress (Table 6). This is in agreement with observations for many crops when subjected to saline stress (Marschner, 1995; Grattan and Grieve, 1999). Potassium has been reported to be involved in activation of several enzymes, membrane transport, neutralization of anions, maintenance of cystolic osmotic potential (up to 100–200 mM range of K), and maintenance of osmotic potential in vacuoles and guard cells (Marschner, 1995). Thus, the correlation of K to shoot and root growth may reflect a combination of both osmotic and metabolic K activities.

Sodium and Cl uptake causes a decline in water potential in plant tissues compared to the external solution, resulting in enhancement of water uptake and turgor pressure maintenance and continuing cell growth for halophytes (Reimann and Breckle, 1993; Canny, 1995; Leidi and Saiz, 1997). A dramatic increase in Na shoot tissue content occurred from EC$_w$0 to EC$_w$10 for all grasses with an average of 2.0 and 7.4 mg g^{-1}, respectively (Table 2). Sodium tissue content continued to increase with increasing salinity and averaged 11.1 mg g^{-1} at EC$_w$50. While there were minor differences among ecotypes for Na tissue content at different salinity levels, no apparent relationship to salinity tolerance was observed. Dudeck and Peacock (1985, 1993) also reported an increase in Na content as salinity increased, but with lower shoot tissue levels for the more salt-tolerant types they used.

Chloride shoot tissue content also increased from EC$_w$0 to EC$_w$10 for all grasses except SI 90 but to a much lesser extent than Na with a Cl average of 18.3 and 22.8 mg g^{-1}, respectively (Table 3). As salinity increased, Cl content increased to a maximum at EC$_w$30 (average of 23.7 mg g^{-1}) and then declined to an average of 21.2 mg g^{-1} at EC$_w$50. Comparison of Cl content of SI 93-2 (22.2) and HI 101 (21.9 mg g^{-1}) at EC$_w$50 to the less salt-tolerant Adalayd showed that Cl tissue content of Adalayd was appreciably lower at 15.8 mg g^{-1}. Dudeck and Peacock (1993) observed that increasing salinity resulted in a linear increase in Cl shoot tissue content except for the most salt-tolerant type where the maximum was at 32 dS m^{-1} and then declined.

As noted, shoot tissue concentrations between salt-tolerant vs. less tolerant ecotypes of various inorganic ions were affected by increasing salinity, but comparison of individual ion content (K, Ca, Mg, and Na) or ratios of Ca/Mg and K/Na did not appear to be related to ecotype salinity tolerance except for Cl shoot tissue concentration exhibiting a potential relationship with salinity tolerance. These characteristics, therefore, would not seem to be useful as physiological salt-screening traits. However, several nutritional related implications for K, Mg, and Ca

Table 7. Estimated inorganic solute contribution to total solute potential (Ψ_s) using inorganic osmotica and their individual contribution at the highest salinity level (EC_w50) by ecotype.

Entry	Estimated Ion Contribution (MPa) by†						Inorganic ion contribution to Ψ_s	Total measured Ψ_s§	Contribution¶
	K	Na	Ca	Mg	Cl	Others‡			
							MPa	MPa	%
SI 93-2	−0.59a	−0.31	−0.02a	−0.05a	−0.43ab	−0.005	−1.41	−2.50a	57
HI 101	−0.79abc	−0.41	−0.03ab	−0.07abc	−0.53abc	−0.007	−1.83	−2.59ab	71
Sea Isle 2000	−0.61a	−0.41	−0.04bc	−0.07abc	−0.46ab	−0.006	−1.60	−2.43a	66
TCR 1	−0.73ab	−0.44	−0.03ab	−0.07abc	−0.60bc	−0.007	−1.89	−2.70b	70
TCR 6	−0.63ab	−0.31	−0.03ab	−0.05a	−0.39a	−0.005	−1.41	−2.51a	57
Sea Isle 1	−1.01c	−0.53	−0.05c	−0.11d	−0.70c	−0.010	−2.42	−2.49a	97
HI 34	−0.74abc	−0.37	−0.03ab	−0.07ab	−0.46ab	−0.006	−1.68	−2.49a	68
SI 90	−0.65ab	−0.37	−0.02a	−0.06ab	−0.45ab	−0.006	−1.56	−2.49a	62
Adalayd	−0.90bc	−0.49	−0.04bc	−0.10cd	−0.49ab	−0.008	−2.03	−2.48a	82
F-test#	+	0.20	*	*	+	–	–	*	–
LSD (0.05)#	0.27	0.19	0.01	0.03	0.19	–	–	0.16	–

*Significant differences at the 0.05 probability level.

†van't Hoff equation Ψ_s (MPa) = −csRT, where cs = Osmolarlity (mol L^{-1}), R = 0.0083143 L MPa mol^{-1} K^{-1}, and T = 293 K were considered.

‡Includes P, Fe, Mn, Zn, Cu, and B

§Data from Lee et al. (2005).

¶Contribution = (Ψs calculated/Ψs measured) × 100.

#F-test and LSD test (0.05) for a column are to compare mean performances among entries at the 0.05 (*) and 0.10 (+) levels.

are suggested by the data that would be of importance for managing this species in salt-affected environments.

In terms of K, the shoot tissue data would suggest that K requirement is high and care should be taken to provide ample K in salt-affected situations. The observation that K tissue content was strongly related to shoot growth and moderately related to root growth would support this statement. Paspalum would appear to have active K uptake mechanisms, since in this seawater situation with high levels of other cations (Na, Mg, and Ca), paspalum was able to take up and maintain K concentrations at an average of 81% of the control under the highest salinity and at shoot tissue concentrations above those normally found for other turfgrasses; therefore, when supplemental K is added, it should be readily taken up.

Since Mg tissue concentration was most readily suppressed by increasing salinity relative to K or Ca even when using Mg-rich seawater, it would appear that seashore paspalum does not have aggressive means for active uptake of Mg. Thus, Mg should be carefully monitored and supplemented in salt-affected sites that would contain less Mg. This may be especially a problem on salt-affected sites receiving high Ca additions, such as gypsum, which may further suppress Mg availability.

While Ca shoot tissue content was less affected by increasing salinity than Mg or K, the tendency for low inherent tissue concentrations of Ca in this species relative to other grasses indicates that reduced availability of Ca in the soil may more easily induce a Ca deficiency. Many salt-affected sites receive high levels of supplemental gypsum to alleviate sodic conditions, so adequate Ca may be available in most cases. The authors have observed on seashore paspalum irrigation with highly saline water (9–10 dS m^{-1}) Ca deficiency symptoms and low Ca shoot tissue content (<1.7 mg g^{-1}) when soil Ca was moderately low. Foliar applied Ca increased the shoot tissue levels to >2.0 mg g^{-1} and visual deficiency symptoms disappeared.

Inorganic Osmolyte Relations

Lee et al. (2005) reported in a companion paper that at EC_w ≥ 30 dS m^{-1}, salt-sensitve Adalayd exhibited a turgor pressure (Ψ_p) of 0 to −0.21 MPa, while the other grasses maintained positive $\Psi_{p,}$ and that relative water content (RWC) of Adalayd was lower at EC_w 50 than the salt- tolerant ecotypes. Lee et al. (2005) also noted that shoot growth responses paralleled shoot turgor potential (Ψ_p) with salt-tolerant types (SI 93-2, HI 101) maintaining more favorable Ψ_p vs. the least tolerant (Adalayd). Their results suggest that paspalum ecotypes would differ in inorganic and organic osmolyte responses for maintenance of total solute potential (Ψ_s). In the current study, total inorganic ions and individual ions did influence osmotic adjustment of seashore paspalum with several of the responses potentially related to ecotype salinity tolerance.

Osmotic adjustment of seashore paspalums was achieved more by inorganic solutes than organic osmolytes based on percentage of total contribution, with a 57 to 97% contribution from inorganic ions across the ecotypes. Halophytes, such as an ion-accumulating seashore paspalum, typically use inorganic solutes for a significant component of osmoregulation by solute potential (Ψ_s) adjustment in saline environments (Flowers et al., 1977; Glenn, 1987). Since synthesis of organic osmolytes is a high energy-requiring process, it has been suggested that ion-includer halophytes may depend on inorganic ion uptake preferentially for osmoregulation ((Rains, 1987;

Glenn et al., 1992). However, in our study, the estimated contribution of the total inorganic solutes to solute potential (Ψ_s) adjustment was relatively lower in more salt-tolerant SI 93-2 (57%, −1.41 MPa) and HI 101(71%, −1.83 MPa) ecotypes than in Adalayd (82%, −2.03 MPa). This indicates that inorganic ions are the most important for maintaining total Ψ_s for paspalum as a species, but it does not appear that the most salt-tolerant ecotypes are the most aggressive inorganic ion includers nor is this trait positively related to salinity tolerance within this species. When investigating the photosynthetic responses of these ecotypes under salinity stress, Lee et al. (2004a) reported that the most salt-tolerant ecotypes were able to maintain photosynthetic capacity and, thereby, are able to produce organic compounds under high salinity for osmoregulation, osmoprotection, and growth compared to less salt-tolerant types. The high percentage reliance of Adalayd on inorganic ions for Ψ_s adjustment may be attributed to the inability to produce organic osmolytes in sufficient quantities for total Ψ_s adjustment; therefore, requiring greater inorganic uptake for osmoregulation. Growth responses of these ecotypes confirm the greater photosynthetic capacity of the more salt-tolerant types (Lee et al., 2005).

Sea Isle 1 exhibited the highest contribution of inorganic ion contribution to solute potential at 97% and it had the highest contribution for K, Na, and Cl as individual ions (Table 7). Interestingly, Sea Isle 1 demonstrated the highest numerical tissue K content at EC_w 50 salinity and it was the only ecotype to exhibit a numerical (nonsignificant) increase as salinity increased from EC_w 40 to EC_w 50 (Table 1). These observations suggest that Sea Isle 1 may have a mechanism for enhanced K uptake under severe salinity that is less expressed in other ecotypes.

In agreement with previous reports on halophytes, K and Cl contributed the most to total Ψ_s adjustment (24–41% range for K; 15–28% range for Cl), followed by Na, Mg, and Ca (Table 7) (Flowers et al., 1977; Heslop-Harrison and Reger, 1986). The contributing effects of other nutrients were relatively minimal at 0.2 to 0.4% of the measured total Ψ_s. The contribution of K to total Ψ_s was 24 and 31% for the most salinity tolerant SI 93-2 and HI 101 ecotypes, respectively, while it was 36% for the less tolerant Adalayd. In terms of individual solute potential contribution to total Ψ_s, K accounted for −0.59 and −0.70 MPa for SI 93-2 and HI 101, respectively, while for Adalayd the contribution was −0.90 MPa (Table 7). A similar but less pronounced trend was observed for Na contribution to total Ψ_s with SI 93-2 (12%) and HI 101 (16%) to Adalyad (20%) (Table 7). This was supported by the individual solute potential contribution of Na to total Ψ_s with −0.31, −0.41, and −0.49 MPa, respectively, for SI 93-2, HI 101, and Adalyad.

Maintenance of adequate K in the cystol is essential for metabolic activity, but excess K is toxic and it must be sequestered in the vacuole (Marschner, 1995). Seashore paspalum apparently has very strong means for active uptake K and means to allocate K as a nutrient and osmoregulator, since seawater contains much higher concentrations of Na, Cl, Mg, and Ca. The more tolerant ecotypes would appear to depend less on K and Na for osmotic adjustment, but still actively take up sufficient K for cystolic metabolic requirements for enhancement of shoot or root growth (Leidi and Saiz, 1997, Wei et al., 2003).

In sum, increasing salinity resulted in reduced uptake of K, Ca, and Mg with Mg most affected. Sodium tissue content increased with salinity, while Cl increased but then declined. The least salt-tolerant ecotypes, Adalayd, exhibited lower Cl uptake at high salinity compared to the most salt-tolerant types (SI 93-2, HI 101), but trends of other nutrients and Na under increasing salinity were not related to salinity tolerance of ecotypes. Shoot and root growth were highly correlated to K tissue content. The shoot tissue content relationships of K, Mg, and Ca to increasing salinity provided insight into nutritional programs on salt-affected sites for this species. Inorganic ions contributed 57 to 97% to osmotic adjustment, indicating that seashore paspalum can readily use inorganic ions in a saline site for maintenance of more favorable plant water relations. In terms of physiological traits that may be useful in salt-screening protocols for this species, determination of K contribution to total Ψ_s in either percent or MPa units was not useful and the same conclusion could be made for the other inorganic ions. While it appears that high percent or MPa contributions of K by the least tolerance type may be a negative screen for salt sensitivity, this appears to be a secondary effect related to the inability of salt-sensitive ecotypes to produce organic photosynthates (Lee et al., 2004a). More useful physiological traits for salinity screening than inorganic ion relationships appear to be either photosynthesis characteristics or organic osmolytes responses (Lee et al., 2004a).

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