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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 350 projects at a cost of $27 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Salt Tolerance of Inland Saltgrass

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SUMMARY

Studies are underway at Colorado State University to develop turf-type inland saltgrass [*Distichlis spicata* var. *stricta* (L.) Greene] cultivars for targeted use in the regions where soil and water salinity are high. With such an effort, it is important to understand the genetic variability of salt tolerance of inland saltgrass selections. This study's findings include:

- Following one-month exposure to 12 mmho/cm salinity in hydroponic culture, five selections exhibited acceptable turf quality (quality rating > 6.0). COAZ-01, COAZ-17, COAZ-18, COAZ-02, and COAZ-22 had better quality than other selections.
- Relative leaf firing range was 0-20%, 0-32%, 5-35 %, and 8-63% after one month at 12, 24, 36, and 48 mmho/cm salinity growth solutions, respectively. Selections COAZ-01, COAZ-18, COAZ-02, COAZ-22, COAZ-08, and CO-01 showed less leaf firing than other lines at 48 mmho/cm salinity.
- Root activity (viability) increased as salinity increased from 2 mmho/cm to 36 mmho/cm. As salinity increased further, root viability decreased. COAZ-18 and COAZ-19 exhibited the highest root activity among all experimental lines.
- Based on the number of times in the best statistical category for turf quality, leaf firing, root growth, and root viability in this experiment, selections COAZ-18 and COAZ-01, and COAZ-22 have superior salt tolerance.

Salinity problems are becoming more prevalent in managed turfgrass systems due to: 1) the use of lower-quality water for landscape irrigation in the water-deprived western US, 2) seawater intrusion into turf facilities located on coastal sites, 3) water conservation practices that reduce salt leaching, and 4) road de-icing (3, 16). Turfgrass managers are struggling to produce quality turf on sites with high salinity and that are irrigated with marginal quality water. With increasing salinity problems, there is a great need for the development and use of salt tolerant turfgrasses.

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Inland saltgrass [*Distichlis spicata* var. *stricta* (L.) Greene], native to Western North America, is a dioecious, rhizomatous, perennial, salt tolerant, warm-season grass. It is commonly found in saline environments, including saline/alkali salt flats and basins, where it is often a dominant species (8). Saltgrass is also common on the margin of lakes, riverbanks, and in seepage areas where water salinity is high. Saltgrass also grows along roadsides where the road is subjected to regular winter de-icing. Saltgrass meadows exist in lowland areas that are flat to gentle sloping (9). Saltgrass grows fairly well on sites with salty and alkaline soils that are poorly drained and have a high water table.

Inland saltgrass, native to Western North America, is a perennial, salt tolerant, warm-season grass commonly found in saline environments, including salt flats and basins like the one shown above.

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Saltgrass is also common on the margin of lakes, riverbanks, and in seepage areas where water salinity is high. Saltgrass also grows along roadsides where the road is subjected to regular winter de-icing.

In addition, saltgrass grows in a wide range of soil types and pH levels which makes it one of the most widespread and common halophyte species in the United States (20). Attributes of saltgrass also include excellent wear, compaction, and drought tolerance (10). Genotypes vary from upright and tall types to compacted and dwarf types, and from cold hardy to cold tender types (17, 18). These attributes make saltgrass a great candidate for development and use as a turfgrass.

Although saltgrass has been classified as a facultative halophyte (12), saltgrass genotypes differ in their salinity tolerance. Gallagher (6) found great variation in saltgrass growth response to high salinity among genotypes collected from various locations throughout America. Marcum et al. (11) evaluated the relative salt tolerance of 21 desert saltgrass accessions and found a great range of salinity tolerance among the saltgrass accessions, but all were more salt tolerant than 'Midiron' bermudagrass. Aschenbach (3) found that significant differences in salt tolerance existed among different saltgrass source populations. The author suggested that differences in salinity tolerance among saltgrass accessions should be considered when using plant material for landscape restoration.

Work is in progress at Colorado State University to develop seed- and vegetatively-propagated turf-type saltgrass varieties for targeted use in the regions where soil and water salinity are high. A series of turf-type saltgrass lines have been selected as parental breeding materials. No information is available on the relative salinity tolerance of these elite, turf-type saltgrass selec-

Work is in progress at Colorado State University to develop seed- and vegetatively-propagated turf-type saltgrass varieties for targeted use in the regions where soil and water salinity are high.

tions. The objective of this study was to screen salinity tolerance of these advanced saltgrass selections.

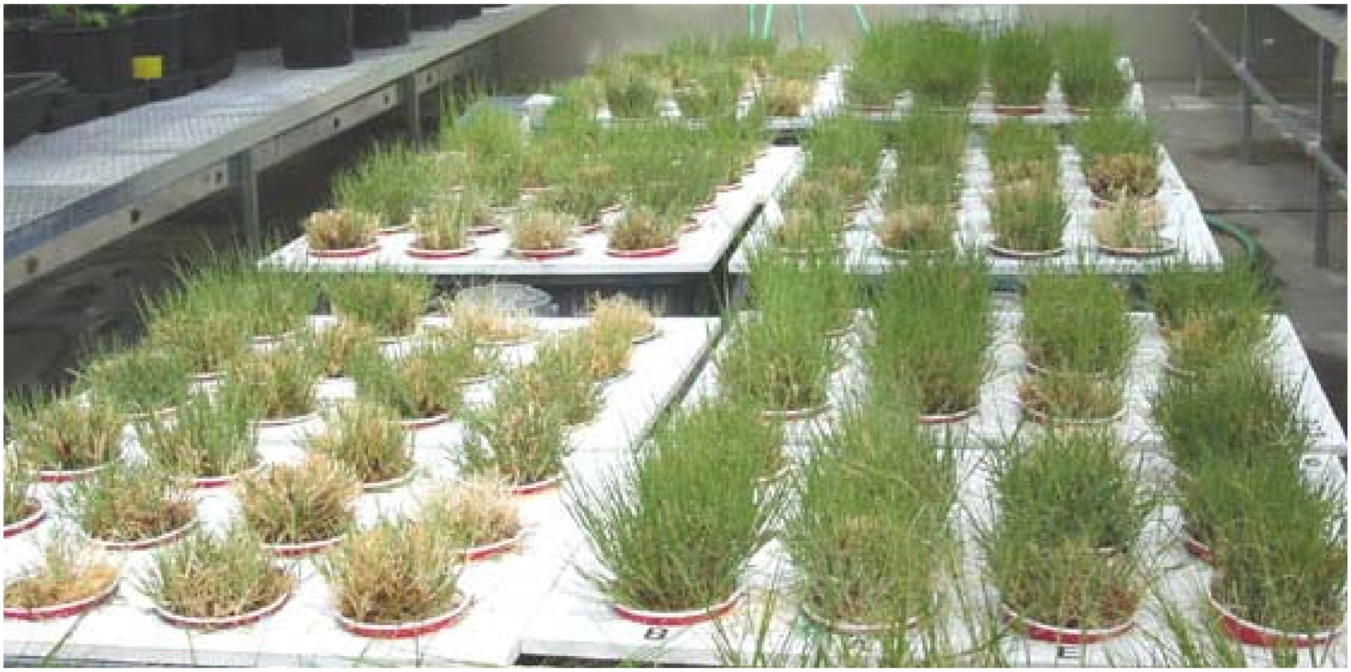
Screening for Salinity Tolerance

To screen for salinity tolerance, a greenhouse hydroponic study was conducted. Rhizomes of 14 saltgrass breeding lines were planted in a solution culture system. Individual lines were planted into shallow pots containing coarse sand. Pots were suspended over tanks containing 10 gallons of constantly aerated and balanced nutrient solution. The pots had coarse nylon-screen bottoms allowing roots to grow into the nutrient solution. A total of eight tanks were used each accommodating 14 cups, representing each of the 14 saltgrass lines.

To ensure complete establishment, plants were grown for six months prior to initiation of salinity treatment. After six months establishment, four tanks were then subjected to salinity treatment while the other four tanks were maintained as the control (electrical conductivity of nutrient solution =2.0 mmho/cm). For salinity treatments, instant ocean salt (Aquarium Systems, Mentor, OH) was gradually added to increase salinity by 2 mmho/cm daily until 12 mmho/cm was reached. The four salinity treatment tanks were held at 12 $dS \cdot m^{-1}$ for a period of one month and data were collected on clipping yield, leaf firing, turf quality, root mass, and root viability for both the 12 mmho/cm salinity treatment and the control.

Turf quality was rated visually on a scale of 0 (brown, dead turf) to 9 (optimum color, density, and uniformity). Leaf firing percentage, an indication of salt injury, was determined by visually estimating the total percentage of bleached leaf area. Root viability was quantified by measuring root dehydrogenase activity using the triphenyltetrazolium chloride reduction technique.

Following data collection, salinity ramping was resumed for salinity treatment until 24 mmho/cm solution salinity was reached, whereas nutrient solution of the control tanks was maintained at 2.0 mmho/cm. Salinity was again held at these levels for one month, and data were collect-



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	Salinity Level [y] (mmho/cm)			
Selections	12	24	36	48
	---Turf quality 0-9, (9=best)---			
CO-01	5.7 bc[z]	6.1 abc	5.5 ab	3.7 b
COAZ-19	5.3 bcd	5.7 bc	3.5 cd	2.0 cde
COAZ-15	3.3 e	2.9 f	1.0 f	0.7 e
COAZ-16	4.3 cde	4.7 cdef	4.2 bc	2.7 bc
COAZ-20	5.7 bc	5.0 cde	2.8 cdef	1.3 cde
COAZ-17	7.7 a	7.0 ab	3.3 cde	2.0 cde
COAZ-06	3.5 de	3.2 ef	1.5 ef	1.0 e
COAZ-08	5.0 bcde	5.3 bcd	3.2 cde	2.0 cde
COAZ-11	5.0 bcde	3.7 def	2.0 def	1.0 de
COAZ-21	5.3 bcd	4.6 cdef	2.2 def	0.7 e
COAZ-18	6.0 abc	7.0 ab	6.7 a	5.3 a
COAZ-01	7.7 a	7.7 a	7.0 a	5.7 a
COAZ-22	6.0 abc	6.0 abc	3.6 cd	2.7 bc
COAZ-02	6.7 ab	5.7 bc	2.8 cdef	1.3 cde
LSD	1.88	1.82	1.93	1.62

[z] Means in the same column followed by the same letter are not significantly different at P = 0.05.

[y] The 2 mmho/cm control was used as the covariate in statistical analysis, so it is not shown in the table.

Table 1. Visual turf quality of 14 saltgrass selections under different salinity levels for one month

Selections	Salinity level[y] (mmho/cm) 12	24	36	48
	--------------------------Leaf firing (%)-------------------------------			
CO-01	8.33 bcde[z]	5.00 bc	8.33 ab	11.67 d
COAZ-19	5.00 cde	6.67 bc	30.00 ab	50.00 abc
COAZ-15	5.00 cde	16.67 ab	23.33 ab	51.67 abc
COAZ-16	15.00 ab	10.00 bc	16.67 ab	33.33 abcd
COAZ-20	15.00 ab	31.67 a	35.00 a	54.33 ab
COAZ-17	5.00 cde	10.00 bc	13.33 ab	36.67 abcd
COAZ-06	0.00 e	1.67 bc	6.67 ab	26.67 bcd
COAZ-08	11.67 abc	10.00 bc	13.33 ab	13.33 d
COAZ-11	20.33 a	30.00 a	30.00 ab	63.33 a
COAZ-21	10.00 bcd	10.00 bc	26.67 ab	61.00 a
COAZ-18	6.00 bcde	6.67 bc	8.33 ab	11.67 d
COAZ-01	1.00 de	3.33 bc	5.00 b	8.33 d
COAZ-22	5.00 cde	0.00 e	13.33 ab	13.33 d
COAZ-02	0.00 e	10.00 bc	16.67 ab	11.67 d
LSD	9.83	15.53	29.86	30.58

[z] Means in the same column followed by the same letter are not significantly different at P = 0.05.
[y] The 2 mmho/cm control was used as the covariate in statistical analysis, so it is not shown in the table.

Table 2. Percent leaf firing (relative to control) of saltgrass selections exposed to different salinity levels for one month

ed. The cycle was repeated until salinity of growth solution reached 36 mmho/cm and then 48 mmho/cm. Selections were maintained at these individual salinity levels for a period of one month, respectively, for data collection. At the end of each month's salinity cycle, all roots were harvested at the base of each cup so that the plants had to re-grow their roots as salinity level was being increased. Roots were harvested in the same manner for the control tanks.

Saltgrass Selections Vary in Salinity Tolerance

In general, turf quality decreased with increasing salinity (Table 1). The comparison of saltgrass selections within individual salinity levels clearly showed differences. Following one-month exposure to 12 mmho/cm growth medium salinity, five selections exhibited acceptable turf quality (quality rating > 6.0). COAZ-01, COAZ-17, COAZ-18, COAZ-02, and COAZ-22 had better quality than other selections. Selection COAZ-15 had the poorest quality among all selections.

After one-month exposure to 24 mmho/cm, experimental lines COAZ-01, COAZ-18, COAZ-17, CO-01, and COAZ-22 produced acceptable quality which was significantly higher than that of COAZ-15, COAZ-16, COAZ-20, COAZ-06, COAZ-11, and COAZ-21. When salinity increased to 36 mmho/cm, only COAZ-01 and COAZ-18 exhibited acceptable quality, and at 48 mmho/cm, quality of all selections declined to unacceptable. However, at the highest salinity, selections COAZ-01 and COAZ-18 exhibited greater turf quality than other lines, followed by

Selections	Salinity level [y] (mmho/cm) 12	24	36	48
	----------------------------------grams per pot--------------------------------			
CO-01	0.0538 ab z	0.1141 abc	0.1242 abc	0.1293 bcd
COAZ-19	0.0423 b	0.0565 abc	0.0441 cde	0.0307 de
COAZ-15	0.0458 b	0.0556 abc	0.0678 bcde	0.0245 e
COAZ-16	0.0387 b	0.0501 bc	0.0285 de	0.0394 de
COAZ-20	0.0524 ab	0.1274 ab	0.1163 abcd	0.1069 cde
COAZ-17	0.0582 ab	0.0699 abc	0.0358 de	0.0287 de
COAZ-06	0.0674 ab	0.01051 abc	0.1539 ab	0.0787 de
COAZ-08	0.0713 ab	0.0684 abc	0.0479 cde	0.0643 de
COAZ-11	0.0413 b	0.0511 bc	0.0274 e	0.0374 de
COAZ-21	0.0426 b	0.0443 c	0.0285 de	0.0199 e
COAZ-18	0.0651 ab	0.1338 a	0.1460 ab	0.1864 abc
COAZ-01	0.0537 ab	0.1015 abc	0.1530 ab	0.2340 ab
COAZ-22	0.0992 a	0.1085 abc	0.1729 a	0.2401 a
COAZ-02	0.0680 ab	0.1186 abc	0.0840 bcde	0.0903 cde
LSD	0.0492	0.0790	0.0883	0.1045

[z] Means in the same column followed by the same letter are not significantly different at P = 0.05.
[y] The 2 mmho/cm control was used as the covariate in statistical analysis, so it is not shown in the table.

Table 3. Effect of salinity levels on root dry weights of 14 saltgrass selections

CO-01, COAZ-22, COAZ-16 and COAZ-19 (Table 1).

Relative leaf firing range was 0-20%, 0-32%, 5-35 %, and 8-63% after one month at 12, 24, 36, and 48 mmho/cm salinity growth solutions, respectively (Table 2). Selections COAZ-01, COAZ-18, COAZ-02, COAZ-22, COAZ-08, and CO-01 showed less leaf firing than other lines at 48 mmho/cm salinity (Table 2). Low leaf firing under high salinity is a good indication of a selection's ability to maintain better turf quality under highly saline conditions.

As salinity increased from 2 mmho/cm to 36 mmho/cm, root growth increased in selections CO-01, COAZ-01, COAZ-18, COAZ-22, and COAZ-06 while these trends were not as clear in other selections (Table 3). At high salinity levels, COAZ-22, COAZ-01, and COAZ-18 exhibited greater root mass than COAZ-21, COAZ-15, COAZ-17, COAZ-06, COAZ-08, COAZ-11, COAZ-16, and COAZ-19.

For most selections, root activity (viability) increased as salinity increased from 2 mmho/cm to 36 mmho/cm (Table 4). As salinity increased further, root viability decreased. COAZ-18 and COAZ-19 exhibited the highest root activity among all experimental lines. We found that moderate levels of salinity increase saltgrass root biomass and root viability.

Clipping yield is one indicator of turf vigor. From a previous study, we have found that the salinity levels that caused a 25% clipping reduction ranged from 21.2 mmho/cm for COAZ-16 to 29.9 mmho/cm for COAZ-18 of saltgrass (15). The salinity that resulted in a 25% clipping yield reduction ranged from 2.3 to 10.0 mmho/cm for Kentucky bluegrass (16, 19) and 5.7 to 14.0 mmho/cm for creeping bentgrass (5, 14). The

Selections	Salinity level[y] (mmho/cm) 12	24	36	48
	-------absorption per gram of root dry weight (gram)-------			
CO-01	31.43 ef [z]	33.41 de	88.50 de	14.97 h
COAZ-19	34.66 def	92.33 ab	115.99 bcd	110.19 b
COAZ-15	28.14 ef	31.41 e	28.65 f	45.56 g
COAZ-16	55.46 cde	41.30 e	94.63 cde	69.00 de
COAZ-20	72.09 bc	70.57 c	106.84 bcde	75.95 cd
COAZ-17	75.08 bc	118.72 a	103.09 bcde	65.90 def
COAZ-06	24.70 f	52.50 c	72.69 e	51.61 efg
COAZ-08	75.87 bc	69.10 cd	123.94 bcd	76.04 cd
COAZ-11	87.87 b	79.62 c	87.93 de	50.35 fg
COAZ-21	60.43 bcd	91.35 b	108.52 bcde	66.96 def
COAZ-18	123.90 a	124.28 a	174.55 a	142.00 a
COAZ-01	52.63 cdef	65.24 c	121.59 bcd	49.15 fg
COAZ-22	55.00 cde	97.82 ab	136.20 b	87.58 c
COAZ-02	72.20 bc	78.77 c	129.79 bc	76.37 cd
LSD	27.93	31.41	36.40	18.34

[z] Means in the same column followed by the same letter are not significantly different at P = 0.05.
[y] The 2 mmho/cm control was used as the covariate in statistical analysis, so it is not shown in the table.

Table 4. Effect of different salinity levels on root viability (ml absorption per gram of root dry mass) of saltgrass selections

high salinity level observed that resulted in 25% clipping yield reduction for saltgrass would rank it as one of the most salt-tolerant turfgrass species.

Based on the number of times in the best statistical category for turf quality, leaf firing, root growth, and root viability in this experiment, selections COAZ-18 and COAZ-01, and COAZ-22 have superior salt tolerance. These selections exhibited high turf quality, low leaf firing, and greater root growth under high salinity conditions. In addition, selection CO-01 also had high turf quality and low leaf firing under high salinity conditions.

Our experiment agrees with previous studies indicating saltgrass possesses excellent salt tolerance. Dahlgren et al. (4) found that mature inland saltgrass stands survived soil salinity at approximately 36,000-43,000 mg/L (i.e. 56 - 67 mmho/cm) under dry salt playa conditions. Alshammary et al. (2) found that saltgrass shoot growth was not reduced as salinity increased from control to 23 mmho/cm and root growth was stimulated at salinity levels ranging from 5 to 23 mmho/cm. In laboratory experiments, Hansen et al. (8) also found that maximum growth of saltgrass was obtained at 15,000 ppm (about 23 mmho/cm) soluble salts in nutrient solution cultures. Nearly equal concentrations of sodium and potassium were found in the plant tissue when the growth of the plants was optimal. Although no growth occurred, Ungar (20) found that saltgrass survived 3.93 % NaCl (61 mmho/cm).

Our results also agree with those of Marcum et al. (11) who found that a substantial range of salinity tolerance exists among saltgrass germplasms. However, despite its halophytic

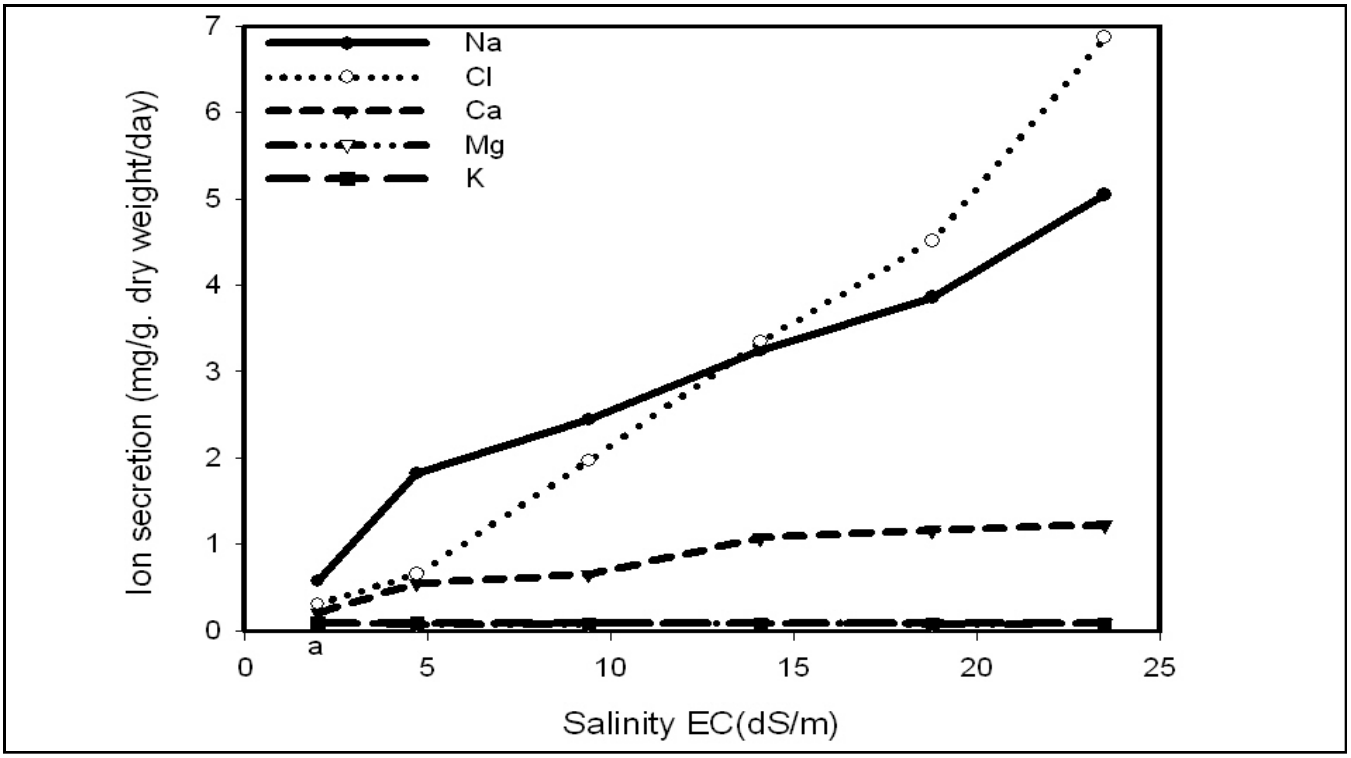


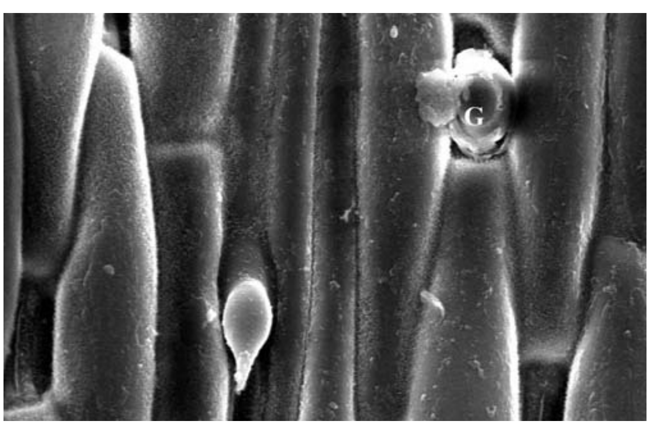
Figure 1. Ion secretion (mg/g dry weight/day) of saltgrass leaf salt glands when exposed to salinity stress in the hydroponic study.

behavior, saltgrass is not immune to salinity stress. It may suffer salinity stress as the level of salinity increases in the growth medium, as indicated by increased leaf firing, reduced turf quality and shoot growth.

Salt Tolerance Mechanisms

Saltgrass has several adaptation features that result in tolerance to salinity. Salt glands (extruding salts from leaves), root growth stimulation under saline conditions, maintenance of high root to shoot ratio, regulation of ion concentrations, and maintenance of higher potassium to sodium ratio in shoots are important salinity tolerance mechanisms in saltgrass that allow saltgrass to utilize salty water (1). Salt glands have been discovered in many plants in the Chlorideae subfamily. However, in salt sensitive species, such as buffalograss, salt glands are less active and effective.

To determine the effectiveness of salt glands in saltgrass, saltgrass were grown in a hydroponic system at 2 to 23 mmho/cm salinity



Salt glands (G) were located on leaf surfaces in longitudinal rows parallel to rows of stomata.

Salt glands of saltgrass are so efficient that secreted salt crystals are visible on leaves of saltgrass growing in salty solution.

levels. After culture for two months, salt glands on the leaves were examined by scanning electron microscopy. These salt glands were located on leaf surfaces in longitudinal rows parallel to rows of stomata. Salt glands were bi-cellular with a basal and a cap cell.

To determine the effectiveness of the salt glands, intact leaves were thoroughly rinsed to remove all external salts. Plants were allowed to grow under different salinity conditions for an additional 24 hours. Then, mature leaves were cut, immediately placed in distilled water within a scintillation vial, sealed, and shaken for ten seconds to dissolve all external secreted salt crystals. Leaves were then removed and dried 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 milligram ion per gram leaf dry weight per day.

Our results found that the ion secretion rate of salt glands increased as salinity level increased, reaching 5.05, 6.86, and 1.22 milligram ion per gram leaf dry weight per day for Na^+, Cl^- and Ca^{++}, respectively, at 23.5 mmho/cm (Figure 1). Interestingly, almost no Mg^{++} and K^+ were secreted (please note that Mg^{++} and K^+ overlap each other on the figure). In fact, the salt glands of saltgrass are so efficient that secreted salt crystals are visible on leaves of saltgrass growing in salty solution. This observation suggest that salt glands of saltgrass are very active and effective.

It is interesting that despite the excellent salt tolerance, saltgrass had low concentrations of sodium and chloride in shoot tissues when compared to Kentucky bluegrass and tall fescue (1). The low sodium and chloride concentrations of saltgrass were likely associated with the efficient sodium and chloride secretions as salinity levels increased. Along with Na^+ and Cl^- exclusion from shoots, root selectivity of K^+ over Na^+ is critical in turfgrass salinity tolerance.

In summary, the genetic variability of saltgrass in this experiment offers great promise for continued improvement of saltgrass. The salinity screening procedures used in these experiments will be both valuable and efficient for screening parental germplasm in our breeding program. Further, turf-type lines developed by breeders can be tested for salinity tolerance prior to release or further development.

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