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Research Article

Growth Responses and Nitrogen Uptake by Saltgrass (*Distichlis spicata* L.), a Halophytic Plant Species, under Salt Stress, Using the ^{15}N Technique

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Various saltgrass clones were studied hydroponically, using Hoagland solution, in a greenhouse to evaluate their DM weights and nitrogen uptake under control and salt stress conditions. Treatments included control (no added salt) and plants grown under NaCl salinity. Twelve clones were grown with 4 replications of each treatment in a RCB design trial. Ammonium sulfate, 5.3%^{15}N was used to enrich the plants by adding 5 mg ^{15}N as 22.931 mg $(^{15}NH_4)_2SO_4$, per liter of the culture solution per day. Plant shoots were harvested weekly, oven-dried at 65°C, and DM weights were recorded. At the last harvest, plant roots were also harvested, oven-dried at 65°C, and DM weights were determined. Harvested plant materials were analyzed for total-N and ^{15}N contents. The results showed non-significant differences in shoot DM weights and total-N and ^{15}N concentrations and contents in salinized plants compared with the controls. Total-N and ^{15}N concentrations of the roots were higher than that of the shoots under either control or saline condition. Overall, due to the high degree of salt tolerance of saltgrass, the results showed generally no difference in nitrogen uptake by most of the clones under salt stress compared with the control plants.

1. Introduction

Saltgrass [(*Distichlis spicata* L.) Greene var. *stricta* (Gray) Beetle] [1] is a warm-season potential turfgrass species that has the ability to grow under highly saline (salt stress) conditions and with limited available water sources [2–9]. This characteristic could prove to be beneficial in certain turfgrass areas requiring low maintenance such as arid regions with saline soils and limited water and nutrients (i.e., nitrogen) availability.

Except for one publication of these authors on only one accession of this grass [8], to our knowledge, there is not any other work reported in the literature regarding the nitrogen (particularly, ^{15}N) nutrition of saltgrass. The previous reports and those of Sigua and Hudnall [10], Sowa and Towill [11], Enberg and Wu [12], Miyamoto et al. [13], Rossi et al. [14], and Miller et al. [15] are concerned only with the growth of this species either under normal or stressful conditions apart from nitrogen absorption. Since saltgrass is a potential turfgrass and landscaping species with a low maintenance/cultural practices, it would be a substantial savings in using this grass as a turf species. However, due to the lack of the adequate information on its water and nutrient requirements, more research should be conducted on this plant before being used as a turfgrass species and a landscaping plant in large scale. Therefore, the objectives of this study were to gather more information on this grass and to compare growth responses in terms of dry-matter (DM) yields and nitrogen requirements (i.e., total-N and ^{15}N absorptions) of various clones of saltgrass grown under control (nonsaline) and salt stress conditions.

2. Materials and Methods

Various saltgrass (*Distichlis spicata*) clones (Table 1) collected from several southwestern states of the United States [2] were studied in a greenhouse to evaluate their nitrogen uptake

Table 1: Various saltgrass clones and their locations, where they have been collected.

Clone	Description	Location collected
A37	Vegetative Clone	Front Range, 35 miles East of Denver, Colorado
A49	Vegetative Clone	Front Range, 35 miles East of Denver, Colorado
A50	Vegetative Clone	Front Range, 35 miles East of Denver, Colorado
A60	Vegetative Clone	Front Range, 35 miles East of Denver, Colorado
72	Vegetative Clone	Front Range, 35 miles East of Denver, Colorado
A86	Vegetative Clone	Front Range, 35 miles East of Denver, Colorado
A107	Vegetative Clone	Front Range 20 miles SE Ft. Collins, Colorado
A126	Vegetative Clone	Front Range 20 miles SE Ft. Collins, Colorado
A136	Vegetative Clone	Front Range 20 miles SE Ft. Collins, Colorado
A138	Vegetative Clone	Front Range 20 miles SE Ft. Collins, Colorado
239	Vegetative Clone	Fresno, California
240	Vegetative Clone	Fresno, California

under normal (control) [no added salt, but, EC 0.95 dSm^{-1} (deci Siemens per meter) equal to 608 $mg\,L^{-1}$ TDS (total dissolved solids) due to the half strength Hoagland solution reagents] and salt stress condition [NaCl at EC 20 dSm^{-1} equal to 12,800 $mg\,L^{-1}$ TDS (total dissolved solids)] using ^{15}N in a hydroponics system. Salt stress is the stress caused by increasing the osmotic pressure (decreasing osmotic potential) of the growth medium (i.e., the culture solution) by adding any salt or solids (i.e., sodium chloride, NaCl) to the growth medium (i.e., culture solution). Saltgrass, a true halophytic plant species, has a very high level of salt tolerance. Several studies by the authors of this paper have shown that, at EC 6 dSm^{-1} (salt concentrations of 3,840 $mg\,L^{-1}$ TDS), this grass performed better than the control plants. The EC 6, 20, 34, and 48 dSm^{-1} have been considered, normal, low, medium, and high, respectively, for the degree of salt tolerance of saltgrass. These EC values are equal to 3,840, 12,800, 21,760, and 30,720 $mg\,L^{-1}$ TDS, respectively. Nitrogen-15 was used as a marker to find exactly how much nitrogen was uptaken and partitioned between the roots and the shoots of the grasses under stress and control conditions.

The plants were grown vegetatively in cups, 9 cm diameter and 7 cm height, following the procedures of Marcum et al. [3], Pessarakli et al. [8], Pessarakli [5], and Pessarakli and Kopec [6]. Briefly, silica sand was used as the plant anchor medium. Each cup was fitted into one of the 9 cm diameter holes cut in a rectangular plywood sheet measuring 46 cm × 37 cm × 2 cm. The plywood sheets served as the lids for the hydroponics tubs and supported the cups above the solution to allow for root growth. The sheets were placed on 42 cm × 34 cm × 12 cm Carb-X polyethylene tubs (total of 8 tubs, 4 replications of 2 treatments) containing half strength Hoagland solution number 1 ([16], Table 2), modified with Fe-sodium ferric diethylenetriamine pentaacetate (DTPA) chelate to provide 3 $mg\,L^{-1}$ elemental Fe. Plants were grown in a randomized complete block (RCB) design with four replications of each treatment. The experiments were conducted in two different seasons (started on March 15, 2008 and repeated on March 15, 2009) for more validity of the data and to take the possible seasonal variations on the studied parameter into consideration. The averages of the data for the two experiments (two growing seasons) are reported here. The day/night temperatures of the greenhouse were 30°C/20°C, with maximum photosynthetically active radiation (PAR) of 1200 $\mu mol\,m^{-2}\,s^{-1}$. Light levels were supplemented for 2 h during early morning (after sunrise) and late afternoon (before sunset) with high-pressure sodium lamps (1000 W; Energy Technics, York, Pa). In each experiment, the plants were allowed to grow in this nutrient solution for 6 months. During this period, the plant shoots (clippings, all above ground biomass) were harvested weekly in order to allow the grass to reach full maturity and develop uniform and equal plant size. The harvested plant materials were discarded. At the last harvest, all roots and shoots were cut to have uniform roots and shoots prior to the initiation of the salt stress phase of the study.

The salt treatments were initiated by adding sodium chloride (NaCl) to the culture solutions to raise the electrical conductivity (EC) of the solutions 5 dSm^{-1} (3,200 $mg\,L^{-1}$ TDS) every other day until the final EC 20 dSm^{-1} (12,800 $mg\,L^{-1}$ TDS) was reached. Two treatments were used, including control (no salt addition) and salinized (EC = 20 dSm^{-1} (12,800 $mg\,L^{-1}$ TDS). The culture solution levels in the tubs were maintained at 10 liter volume, and solution conductivity was monitored and adjusted to maintain prescribed treatment salinity levels. After the final salinity level was reached, the shoots were harvested and the harvested plant materials were discarded prior to beginning the ^{15}N treatment.

The ^{15}N treatment was started by adding 5 mg ^{15}N as 22.931 mg ammonium sulfate ($(^{15}NH_4)_2SO_4$), 5.3%^{15}N (atom percent ^{15}N) per liter of the culture solution per day (following procedures used by Pessarakli and Tucker [17, 18], Al-Rawahy et al. [19], Pessarakli [20], and Pessarakli et al. [8]). Briefly, 22.931 mg ammonium sulfate ($(^{15}NH_4)_2SO_4$) with 5.3%^{15}N (Atom percent ^{15}N) enrichment to provide exactly 5 mg ^{15}N was added per liter of the culture solution per day. After the ^{15}N addition, plant shoots were harvested weekly for the determination of the ^{15}N absorption. The harvested plant materials were oven dried at 65°C and dry weights (DM) were measured and recorded. Six harvests were made in each of these experiments. Plant samples were analyzed for total N and ^{15}N concentrations using Automated ^{15}N Analysis by the Rittenberg Technique (the complete system is referred to as an ANCA-MS for automated N/C analyzer-mass spectrometer and CF-IRMS for continuous flow-isotope ratio mass spectrometer (Spectrumedix Corporation, State College, PA)), following procedures reported

Table 2: Nutrient elements content of Hoagland solution.

Macronutrients	g L^{-1}	m LL^{-1} nutrient solution
1 M KH_2PO_4	136.09	1
1 M KNO_3	101.10	5
1 M $Ca(NO_3)_2$	164.09	5
1 M $MgSO_4$	120.37	2
Micronutrients		
H_3BO_3	2.86	1
$MnCl_2 \cdot 4H_2O$	1.81	1
$ZnSO_4 \cdot 7H_2O$	0.22	1
$CuSO_4 \cdot 5H_2O$	0.08	1
$H_2MoO_4 \cdot H_2O$	0.02	1
$Na_2MoO_4 \cdot 2H_2O$	0.12	1
Iron (Sprint 330, iron chelate)	25.00	1

by Pessarakli and Tucker [17, 18], Al-Rawahy et al. [19], Pessarakli [20], and Pessarakli et al. [8]. Briefly, 0.5 g ground dry plant samples were digested using concentrated sulfuric acid (H_2SO_4) on heated block under the hood, followed by distillation and titration, and then were analyzed for total-N and ^{15}N content using the aforementioned apparatus. Nitrogen isotope (^{15}N) analyses were performed by the Rittenberg technique, in which alkaline hypobromite was used to oxidize NH_4^+-N to N_2 in the absence of air. For the ^{15}N analysis, conversion of sample N to NH_4^+ was done by the Kjeldahl method, which involves digestion with concentrated sulfuric acid (H_2SO_4) to convert plant organic forms of N to NH_4^+-N, followed by steam distillation of the digest with alkali substance. Then, the following steps were performed.

(1) The distillate was placed on microplate that is moved with an *x-y* plotter to position a well-containing the NH_4^+-N sample beneath a pneumatically actuated reaction head.

(2) The head drops, and air is purged from the well with nitrous oxide (N_2O).

(3) A small amount of lithium bromide (LiOBr) is introduced by a peristaltic pump to oxidize NH_4^+-N to N_2.

(4) A valve opens briefly to admit a small amount of the gaseous headspace to a vacuum manifold. Nitrous oxide is frozen out in a cold trap immersed in liquid N_2.

(5) The pressure of residual gas (N_2) is measured with a pressure transducer and is regulated as required.

(6) The N_2 is admitted to the mass spectrometer for isotope-ratio analysis.

(7) When data collection is complete, the N_2 is evacuated, and the cold trap is heated to remove N_2O.

(8) Reference N_2 (air without O_2) is analyzed for calibration of the mass spectrometer.

The combined data of the two experiments were subjected to analysis of variance (ANOVA), using SAS statistical package [21]. The combined means of the two experiments were separated, using Duncan Multiple Range test at the 0.05 probability level.

3. Results and Discussion

The results for the average weekly shoot and final root dry matter (DM) weights and nitrogen (total-N and ^{15}N) contents and concentrations of various saltgrass clones are presented in Tables 3–7.

3.1. Shoot Dry Matter (DM) Weight. Except for the A136 and A138 clones, there was statistically no difference between dry matter (DM) weights of the other clones under salt stress compared with the control plants (Table 3). Three entries, clones 72, A136, and A138, produced the highest shoot DM weights and were statistically the same under the control (nonsaline) condition. Only clone A86 produced significantly lower DM weight than the previous group under the control condition. The rest of the clones produced statistically the same DM weights under the control condition, and the values were statistically the same as those of A86 clone but significantly lower than those of A136 and A138 clones. While there were statistically some differences in shoot DM weights of some of the clones under the control condition, their DM weights were statistically the same under salt stress condition (Table 3). Therefore, there was a wider range among the clones in regards to shoot DM weights under the control compared to that under salt stress condition.

3.2. Root Dry Matter (DM) Weight. As was reported for the shoot DM weights, there was a wider range in root DM weights of the various clones under the control compared to that under salt stress condition. Clone 72 produced the highest root DM weight under either the control or salt stress condition, and the root DM weights of this clone were statistically the same under salt stress compared with that under the control condition (Table 3). Three entries, clones A49, A126, and 239, produced numerically the lowest root DM weights under the control condition which were statistically the same as root DM weights of the clones A50 and A107. Under the control condition, the root DM weights of the rest of the clones were statistically the same and were between the highest and the lowest groups. Under salt stress condition, clone 72 produced numerically the highest root DM weight which was statistically the same as that of clone 240. Two entries, clones A126 and 239, produced the lowest root DM weights, but statistically the same as the root DM weights of the rest of the clones, except clone 72 (Table 3).

Salt stress had more severe effects on shoots DM weights than that of the roots. Sagi et al. [22], Pessarakli [5, 20], Marcum et al. [3], Pessarakli et al. [4, 8, 9], and Pessarakli and Kopec [6, 7] also found that the adverse effect of salinity stress was more pronounced on the shoot than the root growth and DM weight, which is in agreement with the results of the present study. The major differences of the

Table 3: Shoot and root dry matter (DM) weights of various saltgrass clones under the control and salt (NaCl) stress conditions.

Grass clones ID	Shoot DM wt. Treatment		Root DM wt. Treatment	
	Control, 0.95 dSm^{-1} (608 $mg\,L^{-1}$)	EC 20 dSm^{-1} (12,800 $mg\,L^{-1}$)	Control, 0.95 dSm^{-1} (608 $mg\,L^{-1}$)	EC 20 dSm^{-1} (12,800 $mg\,L^{-1}$)
	(g)			
A138	0.96a	0.59bc	0.18b	0.20b
A136	0.83a	0.47bc	0.14b	0.18b
72	0.72ab	0.51bc	0.45a	0.57a
240	0.59bc	0.47bc	0.18b	0.32ab
A50	0.56bc	0.44bc	0.12bc	0.17b
A126	0.55bc	0.45bc	0.06c	0.11bc
A37	0.47bc	0.34bc	0.17b	0.19b
A60	0.46bc	0.42bc	0.14b	0.18b
239	0.45bc	0.39bc	0.05c	0.09bc
A49	0.39bc	0.41bc	0.07c	0.16b
A107	0.39bc	0.36bc	0.11bc	0.18b
A86	0.31c	0.47bc	0.16b	0.18b

Shoot DM values are averages of 4 replications and 6 harvests for both experiments. Root DM values are averages of 4 replications at the final harvest for both experiments.
Values for shoot or root under control and salt stress treatment columns compared together followed by the same letters are not statistically different at the 0.05 probability level.

Table 4: Total nitrogen concentration ($mg\,g^{-1}$) and atom percent ^{15}N of various clones of saltgrass shoot tissues under the control and salt (NaCl) stress conditions.

Grass clones ID	Shoot total-N concentration Treatment		Shoot atom percent ^{15}N Treatment	
	Control, 0.95 dSm^{-1} (608 $mg\,L^{-1}$)	EC 20 dSm^{-1} (12,800 $mg\,L^{-1}$)	Control, 0.95 dSm^{-1} (608 $mg\,L^{-1}$)	EC 20 dSm^{-1} (12,800 $mg\,L^{-1}$)
	($mg\,g^{-1}$)		(%)	
240	37.72a	38.34a	2.58ab	2.52ab
239	33.88ab	33.50ab	2.33bc	1.91c
A37	33.80ab	27.90c	2.94a	2.61ab
A86	33.00ab	31.10bc	2.65ab	2.33bc
A138	32.90b	30.30bc	2.65ab	2.68ab
A136	31.76bc	29.40bc	2.90a	2.40b
A60	31.60bc	29.60bc	2.77a	2.55ab
A50	31.13bc	31.50bc	2.60ab	2.54ab
A49	30.74bc	29.16bc	2.27bc	2.25bc
A126	28.30c	27.70c	2.21c	1.95c
A107	27.90c	27.65c	2.16c	2.12c
72	27.30c	31.62bc	2.78a	2.24bc

Values are total N concentrations and atom %^{15}N of the shoots, averages of 4 replications and 6 harvests for both experiments.
Values for Total-N concentration or %^{15}N for control and salt stress treatment columns compared together followed by the same letters are not statistically different at the 0.05 probability level.

present study with the ones conducted with these authors and listed previously are as follows. First, saltgrass, a true halophytic plant species, with a very high degree of salt tolerance was much less affected by salt stress compared with the other plants used in the aforementioned studies. Second, to the best of our knowledge the nitrogen nutrition, particularly using ^{15}N, has not been conducted on this plant and not been reported in the literature, except for the only one study carried out by the senior author of the present study that was done only on one accession of this plant species.

3.3. Total Nitrogen Concentration and ^{15}N Percentage of Saltgrass Shoots. The average values of the total-N concentration

Table 5: Total nitrogen and ^{15}N contents (mg) of various clones of saltgrass shoot tissues under the control and salt (NaCl) stress conditions.

Grass clones ID	Shoot total N content Treatment		Shoot ^{15}N content Treatment	
	Control, 0.95 dSm^{-1} (608 mg L^{-1})	EC 20 dSm^{-1} (12,800 mg L^{-1})	Control, 0.95 dSm^{-1} (608 mg L^{-1})	EC 20 dSm^{-1} (12,800 mg L^{-1})
	(mg)			
A138	31.58a	17.88cde	0.84a	0.48bcd
A136	26.36b	13.82efgh	0.76a	0.33fgh
240	22.26bc	18.02cde	0.57b	0.45cde
72	19.66cd	16.13def	0.55bc	0.36efg
A50	17.43cde	13.86efgh	0.45cde	0.35efg
A37	15.89def	9.49h	0.47bcd	0.25h
A126	15.57defg	12.47fgh	0.34fgh	0.24h
239	15.25defg	13.07fgh	0.36efg	0.25h
A60	14.54efgh	12.43fgh	0.40def	0.32fgh
A49	12.00fgh	11.96fgh	0.27gh	0.27gh
A107	10.88gh	9.95h	0.24h	0.21h
A86	10.23h	14.62efgh	0.27gh	0.34fgh

Values are total N and ^{15}N contents of shoots, averages of 4 replications and 6 harvests for both experiments.
Values for Total-N or ^{15}N content for control and salt stress treatment columns compared together followed by the same letters are not statistically different at the 0.05 probability level.

Table 6: Total nitrogen concentration (mg g^{-1}) and atom percent ^{15}N of various clones of saltgrass root tissues under the control and salt (NaCl) stress conditions.

Grass clones ID	Root total N concentration Treatment		Root atom percent ^{15}N Treatment	
	Control, 0.95 dSm^{-1} (608 mg L^{-1})	EC 20 dSm^{-1} (12,800 mg L^{-1})	Control, 0.95 dSm^{-1} (608 mg L^{-1})	EC 20 dSm^{-1} (12,800 mg L^{-1})
	(mg g^{-1})		(%)	
240	43.02a	42.28a	2.67ab	2.64ab
A37	38.78ab	33.94c	3.01a	2.73ab
239	38.68ab	39.52ab	2.46bc	2.03c
A86	38.22ab	37.23bc	2.74ab	2.44bc
A138	37.85b	36.40bc	2.78ab	2.80ab
A60	36.96bc	36.76bc	2.89a	2.66ab
A50	36.83bc	37.45bc	2.68ab	2.67ab
A136	36.77bc	35.47bc	2.99a	2.52b
A49	36.37bc	35.19bc	2.37bc	2.36bc
A126	33.83c	33.69c	2.35c	2.07c
A107	33.00c	33.56c	2.27c	2.23c
72	32.73c	37.46bc	2.97a	2.35bc

Values are total N concentrations and atom %^{15}N of the roots, averages of 4 replications at the final harvest for both experiments.
Values for Total-N concentration or Atom %^{15}N for control and salt stress treatment columns compared together followed by the same letters are not statistically different at the 0.05 probability level.

and ^{15}N percentage of the shoots are presented in Table 4. At this relatively high level of salt stress (EC 20 dSm^{-1} (12,800 mg L^{-1} TDS)), there was not a statistically significant difference found between the shoot total-N concentration (except, for A37 clone) and ^{15}N percentage (except, for clones 72 and A136) of the salinized plants compared with the controls. Among all the clones, 239 and 240 had numerically the highest total-N concentrations under both the control and salt stress conditions. Under the control condition, the total-N concentrations of clones A37 and A86 were statistically the same as the above group (clones 239 and 240). Except for clones 72, A107, and A126 which had numerically the lowest total-N concentrations under control condition, the rest of the clones had statistically similar total-N concentrations as clones A37, A86, and 239. Under salt stress condition, clones A37, A107, and A126

Table 7: Total nitrogen and ^{15}N contents (mg) of various clones of saltgrass root tissues under the control and salt (NaCl) stress conditions.

Grass clones ID	Root total N content Treatment		Root ^{15}N content Treatment	
	Control, 0.95 dSm^{-1} (608 mg L^{-1})	EC 20 dSm^{-1} (12,800 mg L^{-1})	Control, 0.95 dSm^{-1} (608 mg L^{-1})	EC 20 dSm^{-1} (12,800 mg L^{-1})
	(mg)			
72	14.73b	21.35a	0.44ab	0.50a
240	7.74c	13.53b	0.21c	0.36b
A138	6.81c	7.28c	0.19c	0.20c
A37	6.59c	6.45c	0.11cdefg	0.18cd
A86	6.12c	6.70c	0.17cde	0.16cdef
A60	5.17cd	6.62c	0.15cdefg	0.18cd
A136	5.15cd	6.39c	0.15cdefg	0.16cdef
A50	4.42de	6.37c	0.12cdefg	0.17cde
A107	3.63def	6.04c	0.08defg	0.14cdefg
A49	2.55ef	5.63cd	0.06fg	0.13cdefg
A126	2.03f	3.71def	0.05g	0.08defg
239	1.93f	3.56def	0.05g	0.07efg

Values are total N and ^{15}N contents of the roots, averages of 4 replications at the final harvest for both experiments.
Values for Total-N or ^{15}N content for control and salt stress treatment columns compared together followed by the same letters are not statistically different at the 0.05 probability level.

had numerically the lowest total-N concentrations. However, there was statistically no difference between the total-N concentrations of these clones with the rest of the clones, except 239 and 240.

Clones A107 and A126 had numerically the lowest ^{15}N percent under both control and salt stress conditions and their values were statistically the same for the control and the salinized plants. Under salt stress condition, the ^{15}N percent of clone 239 was among the lowest group. Under the control condition, clones A49 and 239 which had statistically the same ^{15}N percent as the lowest group, but numerically higher values, had statistically the same ^{15}N percent as the rest of the clones, except A37, A60, 72, and A136. Under salt stress condition, the range in the ^{15}N percent of the various clones was narrower. Except for the three entries (clones A107, A126, and 239) which were numerically in the lowest group of the ^{15}N percent and statistically the same as A49, 72, and A86, the rest of the clones (including the latter 3; A49, 72, and A86) had statistically the same ^{15}N percent.

For both the total-N concentration and the ^{15}N percent of the shoots, there were statistically significant differences found among the various clones under the control and salt stress conditions. Clones A107 and A126 had numerically the lowest shoot total-N concentration and ^{15}N percent under the control and salt stress and their values were statistically the same under both conditions (Table 4).

Some clones (i.e., 240) that were more drought and salt stress tolerant in our previous studies [7, 9] had higher shoot total-N concentrations in the present study. This is supported by Khalil et al. [23], Pessarakli and Tucker [17, 18], Al-Rawahy et al. [19], Pessarakli and Fardad [24], Pessarakli [20], and Pessarakli et al. [8] that showed various salt and drought tolerant plants had a higher total-N concentrations in shoots and roots under salt stress conditions compared with the control plants. The difference of the present study with the ones conducted with these authors on saltgrass and listed previously is that in the previous studies on saltgrass, except for only one study that was done on only one accession of this plant, nitrogen nutrition, particularly using ^{15}N, has not been conducted on this plant species, rather only growth responses of saltgrass under salt or drought stress conditions were evaluated.

Since saltgrass is a true halophyte, it is expected to perform better or as good as other salt tolerant plants, thereby, accumulate nitrogen under salt stress conditions, and use the accumulated nitrogen for its continuous growth and development under harsh environmental stress (i.e., salt and drought) conditions.

3.4. Total Nitrogen and ^{15}N Contents of Saltgrass Shoots. The average values of the total-N and ^{15}N contents of the shoots are presented in Table 5. Except for 3 entries (clones A37, A136, and A138), the rest of the clones showed statistically no difference in their total-N contents under salt stress condition as compared with the control plants. Nevertheless, all the clones, except A86, had numerically lower total-N contents under salt stress condition as compared with the control (nonsalinized) plants. Under the control condition, clone A138 statistically had the highest and clone A86 numerically the lowest total-N content. However, under salt stress condition, clone 240 had numerically the highest and clone A37 the lowest total-N content.

The ^{15}N content of the shoots did not follow the same pattern as the total-N content. For clones A37, 72, A136, A138, 239, and 240, there were statistically significant differences found in the ^{15}N contents of their shoots under

salt stress condition as compared with the control plants. The differences in the ^{15}N contents of the shoots of the rest of the clones were not significant between the salinized plants as compared with their corresponding controls. Under control condition, clones A136 and A138 had statistically the highest and clone A107 numerically the lowest ^{15}N content. However, under salt stress condition, clones A138 and 240 had statistically the highest and clone A107 numerically the lowest ^{15}N content. As was observed for the total-N content of the shoots, all the clones, except A86, had numerically lower ^{15}N content under salt stress condition compared with the control plants.

For both total-N and ^{15}N contents of the shoots, there were statistically significant differences found among the various clones under either the control or salt stress condition. Some clones (particularly, A138 and 240) that were more drought and salt stress tolerant in our previous studies [7, 9] had higher shoot total-N and ^{15}N contents in the present study. As previously mentioned, the difference of the present study with the ones conducted with these authors on saltgrass and listed previously is that in the previous studies on saltgrass, except for only one study that was done on only one accession of this plant, nitrogen nutrition, particularly using ^{15}N, has not been conducted on this plant species, rather only growth responses of saltgrass under salt or drought stress conditions were evaluated. Again, this is probably because the more tolerant plants perform better and accumulate nitrogen under stress conditions and use it for their continuous growth and development under harsh environmental stress (i.e., salt and/or drought) conditions.

3.5. Total Nitrogen Concentration and ^{15}N Percentage of Saltgrass Roots. Total-N and ^{15}N concentrations of the roots are presented in Table 6. Total-N and ^{15}N concentration of the roots essentially followed the same pattern as these values for the shoots, but with substantially higher magnitudes. This is because saltgrass is a true halophytic plant species, continued to absorb nitrogen (both total-N and ^{15}N) under salt stress condition, and accumulated the excess absorbed nitrogen in its roots before being transported to its shoots for utilization and metabolism. Also, probably some nitrogen in the form of ammonium (NH_4^+ cation) was absorbed to the root surfaces of the plants as was suggested by Pessarakli and Tucker [17, 18] and Pessarakli et al. [8]. There was not statistically any significant difference found between total-N concentrations (except, for A37 clone) and ^{15}N percentage (except, for clones 72 and A136) of the roots of the salinized or the control plants. Among all the clones, clone 240 had significantly higher root total-N concentrations under both control and salt stress conditions. This phenomenon is probably due to the higher drought and salt stress tolerance of this clone compared to the other clones observed in our previous studies [7, 9] as mentioned previously. Again, as previously mentioned, the difference of the present study with the ones conducted with these authors on saltgrass and listed previously is that in the previous studies on saltgrass, except for only one study that was done on only one accession of this plant, nitrogen nutrition, particularly using ^{15}N, has not been conducted on this plant species, rather only growth responses of saltgrass under salt or drought stress conditions were evaluated. Clone 239 had statistically the same root total-N concentration as that of clone 240 under both the control and salt stress conditions. These two clones have similar growth characters, collected from the same location (Fresno, California), and have been reported as turf-type saltgrass clones [2]. Under the control (non-saline) condition, clones A37 and A86 also had statistically the same root total-N concentrations as the previous two clones (239 and 240) but significantly lower total-N concentrations under salt stress condition compared to these two clones. Numerically, the lowest root total-N concentrations and ^{15}N percents were found in clones A107 and A126 under either control (nonsaline) or salt stress condition. The values of the roots total-N concentrations and ^{15}N percents of all the other clones were between the aforementioned two groups (Table 6).

Compared to the ^{15}N percent of the roots of the plants under the control with that under salt stress condition, the differences between the values for the clones under salt stress were narrower. Clones A37, A50, A60, A138, and 240, and 3 clones (72, A86, and A136, only under control) had the highest and statistically the same root ^{15}N percents under the control and salt stress conditions (except for noted previously). Clones A107 and A126 had numerically the lowest root total-N concentrations and ^{15}N percents under the control and salt stress conditions, and their values were statistically the same under both conditions (Table 6). The values of the root total-N concentrations and ^{15}N percents of the rest of the clones were between the highest and the lowest groups.

3.6. Total Nitrogen and ^{15}N Contents of Saltgrass Roots. Table 7 presents total-N and ^{15}N contents of the roots. As shown in this table (Table 7), total-N and ^{15}N contents of the roots essentially followed the same pattern as these values for the shoots, but with substantially lower magnitudes. This is mainly due to the substantially lower root DM weights of the plants compared to their corresponding values for the shoots. Among all the clones, clone 72 had significantly higher total-N and ^{15}N contents under both control and salt stress conditions. As was mentioned before, this phenomenon is probably due to the higher drought and salt stress tolerance of this clone compared to the other clones observed in our previous studies [7, 9]. Again, as previously mentioned, the difference of the present study with the ones conducted with these authors on saltgrass and listed previously is that in the previous studies on saltgrass, except for only one study that was done on only one accession of this plant, nitrogen nutrition, particularly using ^{15}N, has not been conducted on this plant species, rather only growth responses of saltgrass under salt or drought stress conditions were evaluated. Clone 240 had the second highest total-N and ^{15}N contents under both the control and salt stress conditions. As mentioned for clone 72, clone 240 was always more tolerant to stress than the other studied clones in our previous salt and drought stress studies. Therefore, the same reasoning applies for the

higher total-N and ^{15}N contents of this clone. The values of the total-N and ^{15}N contents of the roots of these clones (72 and 240) and most of the other clones (except for clone A37 for total-N and clone A86 for ^{15}N content) were markedly higher under salt stress condition compared with those of the control plants.

4. Conclusions

The results showed that while all the 12 clones produced numerically different amounts of shoot DM weights under the control, they were statistically the same under salt stress condition. Therefore, there was a wider range among the clones in regards to shoot DM weights under the control as compared with those under salt stress condition. Shoots DM weights and nitrogen (total-N and ^{15}N) concentrations were more severely affected than those of the roots under salt stress condition.

Most of the clones showed statistically no difference in their total-N or ^{15}N contents or concentrations under salt stress condition as compared with the control plants. Except for clone A86, the rest of the clones had only numerically lower total-N and ^{15}N contents under salt stress condition as compared with the control (nonsalinized) plants. These findings are significant, indicating that at this relatively high level of salt stress, EC 20 dS m^{-1} (salt concentration of 12,800 mg L^{-1} TDS), there was not a statistically significant difference between total-N or ^{15}N contents of the shoots or roots of the salinized and the control plants. For both total-N and ^{15}N contents of the shoots and the roots, there were statistically significant differences found among the various clones under either the control or salt stress condition. Some clones (particularly, clones A138 and 240) that were more tolerant to drought and salt stress in our previous studies had higher shoot total-N and ^{15}N contents and concentrations in the present study.

Overall, although the results showed that due to the high degree of salt tolerance of saltgrass, generally no significant adverse effects of salt stress were found on growth, DM production, and nitrogen (total-N and ^{15}N) uptake by the various saltgrass clones under this relatively high level of salt stress (EC 20 dS m^{-1}, salt concentration of 12,800 mg L^{-1} TDS), there were some differences found among the various clones under both control and salt stress conditions.

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