

# TURFGRASS SCIENCE

## Salinity Effects on Zoysiagrass Cultivars and Experimental Lines

Y. L. Qian,\* M. C. Engelke, and M. J. V. Foster

### ABSTRACT

Salt tolerant turfgrasses are highly desirable in areas associated with saline soils and/or saline irrigation waters. To determine the salt tolerance of 29 zoysiagrass (*Zoysia* spp.) experimental lines and cultivars, two greenhouse studies were conducted by means of a hydroponic culture system. Sodium chloride was gradually added to a basic nutrient solution to obtain a final salinity level of 42.5 dS m<sup>-1</sup>. Relative salt injury, indicated by leaf firing percentage, was associated with zoysiagrass leaf length and texture. Short, fine leaf-textured zoysiagrass ('Diamond', DALZ8501, and their hybrids) were most salt tolerant, coarse leaf-textured entries (*Z. japonica* Steud. and their hybrids) intermediate, whereas long, fine leaf-textured entries ('Cavalier', 'Emerald', and 'Zeon') exhibited the least salt tolerance. Salinity resulted in decreased K<sup>+</sup> and increased Na<sup>+</sup> in shoots of all cultivars. Sodium content was significantly correlated with percentage of leaf firing, with Zeon, Emerald, 'DeAnza', Cavalier, and TAES4377 having the highest concentration of Na<sup>+</sup>, and TAES4357, TAES4361, and TAES4373 the lowest concentration. Most hybrids with Diamond and DALZ8501 as parents exhibited good to intermediate salinity tolerance. The broad sense heritability was 0.67, 0.50, 0.41, 0.54, and 0.40 for relative leaf firing, shoot and root growth, and Na<sup>+</sup> and K<sup>+</sup> content, respectively. Results suggest that genetic progress may be made to develop salt tolerant zoysiagrass with conventional breeding techniques.

LIMITED POTABLE WATER AVAILABILITY has resulted in increased use of effluent and other low quality water sources to irrigate golf courses and other large turf facilities (Gill and Rainville, 1994). Although these waters may provide nutrients for turfgrass growth, they often contain significant concentrations of dissolved salts (Harivandi, 1994). Problems associated with saline soils and saline irrigation water may increase in the future as more effluent or poor quality water is applied to turf sites. In addition, seawater intrusion in coastal states and the use of salt for deicing along roadways of the northern USA have increased the need for salt tolerant turfgrasses (Harivandi et al., 1992).

Zoysiagrass, indigenous to the Orient, is an important turfgrass in the USA. Although zoysiagrass is in general ranked as salt tolerant turfgrass (Harivandi et al., 1992), Marcum et al. (1998) reported that considerable variability in salt tolerance exists among zoysiagrass species and genotypes. This may be due to the fact that zoy-

siagrass genotypes come from diverse origins and are classified as different species. At least five species of zoysiagrass [*Zoysia japonica*, *Z. matrella* (L.) Merr., *Z. tenuifolia* Trin., *Z. sinica* Hamce, and *Z. macrostachya* Franch. & Sav.] and their hybrids have been introduced for turfgrass breeding in the USA. Since all these species appear to be sexually compatible and may hybridize, some hybrids are not clearly classified. White et al. (1993) divided 21 zoysiagrass cultivars into four groups (short-narrow, short-wide, long-narrow, and long-wide) on the basis of differences in leaf length and texture. To develop highly salt-tolerant zoysiagrass with good turf characteristics, more information is needed on relative salinity tolerance among different leaf-textured zoysiagrasses and their hybrids.

The objectives of this study were to: (i) determine relative salt tolerance for newly developed zoysiagrasses and experimental lines; (ii) determine if it is possible to rank relative salt tolerance on the basis of leaf texture characteristics; and (iii) explore the opportunity of developing salt tolerant zoysiagrass through hybridization and selection.

### METHODS AND MATERIALS

#### Plant Materials

A previous study (Marcum et al., 1998) indicated that Diamond and DALZ8501 had good salinity tolerance, whereas 'Crowne' exhibited relatively poor salt tolerance. These three cultivars were selected as parents for crosses, and included as checks. At least 30 crosses were made in all possible parents combinations during summer and fall, 1995. However, only 11 crosses (DALZ8501 × Diamond, Diamond × DALZ8501, DALZ8501 × Crowne, Crowne × DALZ8501, and Crowne × Diamond) produced seed (Table 1). After harvest, seed from these crosses were germinated individually in plastic pots containing a sand:peat:fertilizer mix in the greenhouse in May 1996. In August 1996, plants were transplanted to 25- by 53-by 10-cm plastic bedding flats.

Fifteen additional cultivars and experimental lines were also planted in bedding flats: (i) six hybrids with Diamond as maternal parent; (ii) 'Meyer', 'El Toro', and Emerald (standard cultivars); (iii) Cavalier and 'Palisades' (Texas Agricultural Experimental Station newly released cultivars); (iv) 'Jamur' and Zeon (experimental proprietary lines); and (v) DeAnza and 'Victoria' (hybrid cultivars between Emerald × El Toro: California Experimental Station) (Table 1).

#### Plant Culture and Treatment Procedures

From 1 March to 26 July 1997 (Study I) and from 1 Aug. to 18 Dec. 1997 (Study II), 29 zoysiagrass cultivars and experimental lines were screened for salinity tolerance in a green-

Y.L. Qian, Dep. of Horticulture and Landscape Architecture, Colorado State Univ., Fort Collins, CO 80523-1173; M.C. Engelke and M.J.V. Foster, Texas A&M Res. and Ext. Ctr., Texas A&M Univ. System, 17360 Coit Road, Dallas, TX 75252-6599. Received 26 May 1999. \*Corresponding author (yaqian@lamar.colostate.edu).

**Table 1. Relative salinity tolerance (indicated as percentage leaf firing relative to control) of 29 zoysiagrasses exposed to NaCl in hydroponic solution (EC = 42.5 dS m<sup>-1</sup>) for 3 wk.**

Cultivar	Species or origin	Percentage leaf firing		Leaf length and texture
		Study 1	Study 2	
Diamond	<i>matrella</i>	2.2	2.5	short and fine
DALZ8501	<i>matrella</i>	7.3	2.0	short and fine
TAES4377	DALZ8501 ( <i>matrella</i> ) × Diamond	2.8	3.2	short and fine
TAES4375	DALZ8501 ( <i>matrella</i> ) × Diamond	4.9	3.5	short and fine
TAES4373	DALZ8501 ( <i>matrella</i> ) × Diamond	8.5	2.8	short and fine
TAES4376	DALZ8501 ( <i>matrella</i> ) × Diamond	8.8	2.2	short and fine
TAES4361	DALZ8501 ( <i>matrella</i> ) × Crowne	23.5	4.5	short and fine
TAES4357	DALZ8501 ( <i>matrella</i> ) × Crowne	30.1	7.3	short and fine
TAES4355	DALZ8501 ( <i>matrella</i> ) × Crowne	34.0	11.8	short and fine
TAES4370	Crowne × Diamond	15.7	14.5	coarse
TAES4364	Crowne × DALZ8501	50.1	16.2	coarse
TAES4366	Crowne × DALZ8501	38.9	5.5	coarse
TAES4365	Crowne × DALZ8501	42.6	4.0	coarse
F1-26	hybrids of Diamond	3.3	7.3	coarse
F1-23	hybrids of Diamond	7.8	9.2	coarse
F1-15	hybrids of Diamond	17.4	5.8	coarse
F1-18	hybrids of Diamond	18.6	4.5	coarse
F1-96-1	hybrids of Diamond	22.6	6.5	coarse
F1-24	hybrids of Diamond	34.0	9.8	coarse
Victoria	Emerald × El-Toro	27.3	20.4	coarse
DeAnza	Emerald × El-Toro	30.0	38.0	coarse
El Toro	<i>japonica</i>	19.8	14.5	coarse
Crowne	<i>japonica</i>	22.0	12.0	coarse
Jamur	<i>japonica</i>	30.3	16.0	coarse
Palisades	<i>japonica</i>	39.3	9.00	coarse
Meyer	<i>japonica</i>	45.1	22.5	coarse
Emerald	<i>japonica</i> × <i>tenuifolia</i>	33.6	40.1	long and fine
Zeon	<i>matrella</i>	45.4	38.0	long and fine
Cavalier	<i>matrella</i>	48.3	40.7	long and fine
LSD (0.05)		8.5	9.9	

house solution culture system (Marcum and Murdoch, 1994). Uniform sprigs of each grass were planted into 9-cm-diam. by 6-cm-deep plastic cups filled with a 1-cm layer of coarse, sterilized silica sand. The cup bottom was removed and covered with nylon screen to hold sand and allow roots to grow through. Twenty-nine cups consisting of 29 entries were placed into holes of a 1.25-cm-thick plywood lid, with the lid suspended over a 50-L tank. A total of six tanks were used with each accommodating 29 cups, representing each of the 29 cultivars. The tanks contained 48 L of constantly aerified full strength Hoagland solution, which was replaced weekly. This volume allowed the bottom of each cup to be submersed 3.0 cm into the solution. Approximately 0.75 mL of Subdue [methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-DL-alanine] was added to each tank to prevent Pythium root rot in both experiments.

About 3 mo after planting, shoots and roots were clipped and discarded prior to initiation of salinity treatments. Roots were clipped at the base of the cups; shoots were clipped to a 2.5-cm height. Three tanks were then subjected to salinity treatment while the other three tanks were used as the control. Experimental design was a split plot with three replications, salt treatment (tank) being the main plot effect, and cultivars within each tank being subplot effects. For salinity treatment, a total of 1250 g of NaCl was gradually added during a 25-d period, with daily application ranging from 50 to 100 g. During this period, solution electrical conductivity was measured daily using an electrical conductivity bridge (Model RC-16C, Beckman Industrial, Cedar Grove, NJ) and adjusted to the same reading for all three salinity-treated tanks. Upon reaching a final EC of 42.5 dS m<sup>-1</sup>, no additional salt was added, and grasses were exposed to the final salt concentration for 3 wk. Electrical conductivity in the control tank was maintained at 2.5 dS m<sup>-1</sup> throughout the study. During establishment and salinity increasing periods, grasses were clipped weekly at a

2.5-cm height. Upon reaching a final EC of 42.5 dS m<sup>-1</sup>, no clipping was made until the termination of studies.

### Data Collection

Data were collected on leaf firing percentage (as an indication of salt injury), root and shoot growth, and shoot Na<sup>+</sup> and K<sup>+</sup> content. Leaf firing percentage was determined weekly by visually estimating the total percentage of chlorotic leaf area. After 3-wk exposure to the final salinity level, shoots (clipping + verdure) and roots were harvested and dried for 48 h at 60°C, then weighed for dry mass. Shoot and root response to salinity was also presented as a percentage ratio of growth under salt treatment relative to control. Dried shoots were ground in a Wiley mill to pass through a screen with 425- $\mu$ m openings, and then ashed at 450°C for 7 h. Ash was dissolved in 1 M HNO<sub>3</sub> and diluted in deionized water. Solution aliquots were analyzed for K<sup>+</sup> and Na<sup>+</sup> content by atomic absorption spectrometry (SpectrAA 40, Varian Analytical Instruments, Palo Alto, CA).

Two identical studies were conducted: analysis of variance indicated significant difference in relative percentage leaf firing and insignificant differences in Na<sup>+</sup> and K<sup>+</sup> content and shoot and root growth between the two experiments. Therefore, data on leaf firing were presented separately, whereas data on shoot and root growth and Na<sup>+</sup> and K<sup>+</sup> content from the two experiments were combined for analysis. All data were subjected to analysis of variance tests, and cultivar means were separated by Fisher's LSD (SAS Institute, 1989). Pearson's correlation analysis was performed to determine the relationship between response variables. Broad-sense heritability estimates were calculated as genotype variance/total variance with each parameter collected under salt treatment (Burton and Monson, 1972). Parents and F1 progeny relations for percentage leaf firing were estimated for crosses in which both parents were known.

**Table 2. Analysis of variances with mean square and treatment significance.**

Parameters	Salinity treatment	Cultivar	Treatment × cultivar
Leaf firing	9974****	2248****	290ns
Shoot wt.	4.13****	0.39****	0.04ns
Root wt.	0.86ns	18.4****	6.9ns
Na <sup>+</sup> content	68.7****	0.128**	0.127***
K <sup>+</sup> content	14.4****	0.014ns	0.01ns

ns, \*\*, \*\*\*, \*\*\*\* not significant or significant at 0.01, 0.001, and 0.0001 levels, respectively.

**RESULTS AND DISCUSSION**

Salinity was incrementally increased to a point in which leaf firing occurred. When salinity reached ≈20 dS m<sup>-1</sup>, shoot growth inhibition was apparent compared with the control. Leaf blade growth inhibition has been reported as a sensitive response to salinity (Munns and Termaat, 1986). As salinity increased, all cultivars exhibited responses similar to that of turf undergoing drought stress. When the final concentration of NaCl was reached (>42.5 dS m<sup>-1</sup>), genetic differences in leaf chlorosis (leaf firing) were clearly evident.

Analysis of variance indicated significant salinity treatment and cultivar effects for leaf firing, shoot growth, and shoot Na<sup>+</sup> content (Table 2). Differences in root mass were only significant among cultivars, whereas salinity treatment had a greater effect on shoot K<sup>+</sup> content than did cultivar.

Considering the fact that turf quality (color and canopy cover) is of paramount importance to turfgrass growers, leaf firing was used as a primary indicator of

salt injury in this study. Leaf firing increased over time, and mean leaf firing percentage (relative to the control) are presented (Table 1). Relative leaf firing ranged from 2 to 50% in Study I and 2 to 40.7% in Study II among entries, indicating a large range in salinity tolerance within the *Zoysia* genus. Lowest overall leaf firing ratings were obtained from Diamond, DALZ8501, and hybrids of Diamond and DALZ8501 in both studies. Highest percentage leaf firing occurred in TAES4364, Cavalier, Zeon, Meyer, and TAES4365 in Study I, and Cavalier, Emerald, Zeon, and DeAnza in Study II. Relative salinity tolerance among nine hybrids was not consistent between Study I and II (Table 1).

Clipping yields of all entries were significantly reduced by salinity (data not shown). However, only seven coarse leaf textured entries (Victoria, Palisades, Crowne, DeAnza, F1-96-1, TAES4370, and TAES4365) and two fine and long leaf textured entries (Cavalier and Zeon) exhibited significant reduction in total shoot dry weight (clipping + verdure) (Table 3). Six short and fine leaf-textured entries (TAES4376, Diamond, TAES4377, TAES4375, TAES4373, and DALZ8501) showed an increasing (though not statistically significant) trend in verdure in the salinity treatment. Relative shoot growth has been used to assess salinity tolerance in many studies (Francois, 1988; Greub et al., 1983; Marcar, 1987; Weimberg and Shannon, 1988), and the U.S. Salinity Laboratory characterizes salt tolerance on the basis of 50% yield reduction compared with plants receiving no salinity treatments (U.S. Salinity Lab. Staff, 1954). Results of this study indicate that fine and short

**Table 3. Effect of NaCl salinity on the shoot and root growth of zoysiagrasses.**

Cultivars	Shoot (verdure† + clipping)		Percentage of control	Root mass		Percentage of control
	Control (2.5 dS m <sup>-1</sup> )	Salt (42.5 dS m <sup>-1</sup> )		Control (2.5 dS m <sup>-1</sup> )	Salt (42.5 dS m <sup>-1</sup> )	
	g			mg		
TAES4376	1.32	1.8	136.4	91	127	139.6
Diamond	1.36	1.75	128.7	126	147	116.7
TAES4377	1.54	1.82	118.2	75	105	140.0
TAES4375	1.54	1.73	112.3	46	40	87.0
TAES4373	1.52	1.63	107.2	114	120	105.3
DALZ8501	1.88	1.97	104.8	35	54	154.3
F1-26	1.45	1.35	93.1	58	95	163.8
TAES4357	0.9	0.78	86.7	41	60	146.3
TAES4355	0.96	0.82	85.4	18	28	155.6
F1-24	1.72	1.21	70.3	120	136	113.3
TAES4361	1.79	1.24	69.3	57	53	93.0
Emerald	0.87	0.55	63.2	35	52	148.6
F1-23	2.31	1.39	60.2	152	96	63.2
TAES4370	2.88	1.68	58.3	131	131	100.0
Zeon	1.3	0.75	57.7	19	16	84.2
F1-18	1.85	1.06	57.3	95	78	82.1
TAES4366	2.42	1.32	54.5	143	96	67.1
Palisades	2.42	1.31	54.1	103	132	128.2
TAES4364	2.41	1.29	53.5	77	67	87.0
Victoria	1.35	0.71	52.6	54	41	75.9
TAES4365	3.12	1.59	51.0	92	96	104.3
Meyer	2.48	1.22	49.2	102	116	113.7
Crowne	2.52	1.23	48.8	98	83	84.7
Jamur	2.43	1.16	47.7	71	88	123.9
F1-96-1	2.61	1.19	45.6	69	41	59.4
El Toro	2.29	1.3	44.5	136	154	113.2
F1-15	2.34	1.04	44.4	158	113	71.5
DeAnza	1.71	0.7	40.9	63	45	71.4
Cavalier	1.8	0.61	33.9	23	13	56.5
LSD (0.05)	0.90	0.49	-	62	52	-

† Verdure is defined as the quantity of shoot tissue remaining after clipping.

leaf-textured zoysiagrass, having the least clipping and verdure reduction, is more salt tolerant than coarse leaf-textured and fine and long leaf-textured zoysiagrasses. Percentage leaf firing due to salinity stress was highly and negatively correlated to shoot growth (Table 5).

As daily salt concentration increased, root growth of all entries increased relative to control. However, when salt concentration increased beyond 25 to 30 ds/m, root growth declined. Since only the final root mass of each cultivar was measured, no differences in root growth were detected between treatments (Table 3). Dudeck et al. (1983) also found that, as the salt concentration increased, root growth of bermudagrass increased to a maximum point and then declined.

Shoot K<sup>+</sup> decreased, while Na<sup>+</sup> increased due to salinity for all cultivars (Table 4). However, cultivars differed in their shoot Na<sup>+</sup> content. Zeon, Emerald, DeAnza, Cavalier, and TAES4377 had the highest concentration of Na<sup>+</sup>, whereas TAES4357, TAES4365, TAES4361, and TAES4373 had the lowest concentration. This result indicated that some cultivars had a higher capacity to exclude Na<sup>+</sup> from shoots. Sodium content was significantly correlated with percentage leaf firing ( $r = 0.43$ ) (Table 5).

Mean shoot K<sup>+</sup> ranged from 21.9 g to 31.0 g kg<sup>-1</sup> for controls, whereas the salt treatment ranged from 5.4 to 11.7 g kg<sup>-1</sup>. Overall, zoysiagrasses under salinity contained only one-third the shoot K<sup>+</sup> compared with control. The negative correlation between Na<sup>+</sup> and K<sup>+</sup> concentrations suggest Na<sup>+</sup> was competing with K<sup>+</sup> for absorption (Table 5). However, there were no varietal differences in K<sup>+</sup> concentration for either salinity treat-

**Table 4. Effect of NaCl salinity on the concentration (g/kg dry weight) of K<sup>+</sup> and Na<sup>+</sup> in shoot tissue of 29 zoysiagrasses.**

Cultivars	Na <sup>+</sup>		K <sup>+</sup>	
	Control (2.5 dS m <sup>-1</sup> )	Salt (42.5 dS m <sup>-1</sup> )	Control (2.5 dS m <sup>-1</sup> )	Salt (42.5 dS m <sup>-1</sup> )
TAES4370	1.01	48.5	28.3	8.4
DALZ8501	0.88	34.3	26.4	7.3
F1-23	1.32	41.0	26.5	10.9
Diamond	1.24	45.7	27.0	7.9
F1-26	1.59	43.2	28.6	8.8
TAES4376	1.09	47.6	25.0	7.3
TAES4361	1.27	21.7	27.8	7.4
El Toro	1.37	42.2	27.6	10.0
TAES4377	1.22	50.7	31.0	8.2
TAES4373	0.98	30.8	25.3	8.0
F1-24	1.10	35.1	25.0	6.4
TAES4375	1.30	36.0	27.2	8.6
F1-96-1	0.95	40.6	24.4	9.0
Meyer	1.30	31.6	23.4	9.4
Jamur	1.19	43.4	26.4	9.1
TAES4366	0.86	41.9	28.6	8.2
F1-15	1.31	32.5	25.4	8.2
F1-18	1.29	39.6	28.0	5.4
Crowne	1.38	43.7	28.7	8.8
TAES4364	2.62	34.4	24.4	10.6
TAES4355	1.51	34.8	28.2	7.8
TAES4365	0.88	26.5	26.1	9.3
Palisades	1.14	42.4	28.6	9.6
Emerald	1.07	53.4	24.8	8.4
DeAnza	1.82	53.6	29.7	11.7
TAES4357	1.25	18.4	21.9	9.1
Cavalier	1.60	50.4	22.8	10.0
Victoria	1.37	49.2	27.0	9.3
Zeon	1.25	55.5	28.7	10.7
LSD (0.05)	0.65	16.8	NS	NS

**Table 5. Simple correlation coefficients for relative salinity tolerance (indicated by percentage injury), shoot growth, root growth, shoot Na<sup>+</sup> content, and shoot K<sup>+</sup> content of salt treated and control plants among 29 zoysiagrasses grown in the greenhouse hydroponic solution.**

Parameter	Shoot growth	Root growth	Shoot Na <sup>+</sup> content	Shoot K <sup>+</sup> content
% leaf firing	-0.72****	-0.56***	0.43**	0.26ns
Shoot growth		0.68****	-0.49**	0.24ns
Root growth			-0.49**	-0.15ns
Shoot Na <sup>+</sup> content				-0.87****

\*, \*\*, \*\*\*, \*\*\*\* correlation coefficients significantly different from 0 at the 0.05, 0.01, 0.001, and 0.0001 probability levels, respectively.

ment or the control plants. Also, shoot K<sup>+</sup> content was not correlated with percentage leaf firing. This contrasts with buffalograss and bentgrass studies, reporting negative relationships between shoot K<sup>+</sup> concentration and salinity tolerance (Ahmad et al., 1981; Wu and Lin, 1994).

Broad sense heritability for the respective response variables were as follows: percentage leaf firing = 0.67, shoot growth = 0.50, root growth = 0.41, Na<sup>+</sup> accumulation = 0.54, and K<sup>+</sup> content = 0.40. The moderate to high heritabilities suggested that reasonable progress could be made in a breeding program to introgress salinity tolerance. Parent-F1 progeny relationships of salinity tolerance could become very important in zoysiagrass breeding programs. In all cases, percentage leaf firing of F1 hybrids was intermediate relative to the two parents (Table 6). Hybrids between two salinity tolerant parents exhibited equally excellent salinity tolerance.

Substantial cultivar differences in salinity tolerance among zoysiagrasses were demonstrated by this study, in agreement with Marcum et al. (1998). On the basis of the classification of White et al. (1993), cultivars in this study fell into three groups: (i) short and fine leaf textured zoysiagrass, including Diamond, DALZ8501, and some hybrids with Diamond and DALZ8501 as parents; (ii) fine and long leaf textured, including Emerald, Cavalier, and Zeon; and (iii) coarse leaf textured, including *Z. japonica* and some hybrids with *Z. japonica* as parent plant. Analysis of variance indicated that short and fine leaf-textured zoysiagrass had the most salt tolerance, whereas the fine and long leaf-textured zoysiagrass had the least salt tolerance. The coarse leaf-textured zoysia had intermediate salt tolerance. An ongoing phenology study at Texas A&M University suggested that the short and fine leaf-textured zoysiagrass included in this study are *Z. matrella*, whereas long and

**Table 6. Leaf firing of zoysiagrass parental clones and F1 progeny exposed to NaCl hydroponic solution (42.5 ds/m).**

Crosses	Parents		F1 progeny	
	Maternal	Paternal	Mean	Range
	Leaf firing (%)			
DALZ8501 × Diamond	4.7	2.4	3.9	2.2–8.8
Crowne × Diamond	17.0	2.4	15.2	14.7–15.7
DALZ8501 × Crowne	4.7	17.0	8.0	4.5–11.8
Crowne × DALZ8501	17.0	4.7	12.9	4.0–16.2
Emerald × El Toro	40.1	14.5	29.2	20.4–38

fine leaf-textured entries may be a result of hybridization between *Z. tenuifolia* and other species (Sharon Anderson, 1997, personal communication). Our results agreed with Marcum et al. (1998), showing *Zoysia matrella* (short and fine leaf textured zoysia) to be more salt tolerant than *Z. japonica*. The high salt tolerance may be associated with the origin of *Z. matrellas*. *Zoysia matrella* was first introduced into the USA from Manila by botanist C.V. Piper, who described the grass as abundant on or near the seashore in the Philippine Islands, where salinity pressure was great (Cunningham, 1984). In this study, we further observed that many hybrids with Diamond and DALZ8501 (fine and short leaf-textured *Z. matrella*) as parents also exhibited good salinity tolerance. Results of this study suggests that salinity tolerance is heritable, and that development of salt tolerant zoysiagrass with good turf characteristics using conventional breeding techniques is possible.

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