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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 400 projects at a cost of $31 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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Salinity Tolerance Screening and Breeding in Annual and Perennial Ryegrass

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SUMMARY

Researchers at Texas A&M University initiated efforts to develop a salinity-tolerance screening procedure and select and increase salt-tolerant annual ryegrass (*Loluim multiflorum* L.) genotypes. Preliminary results of this study include:

- Although researchers were able to measure significant differences over different experiments and years, results from the greenhouse immersion experiments were not highly correlated to field trial.
- Germplasm which was selected over three cycles of recurrent selection at Pecos, Texas for salinity tolerance has been increased in Oregon and will be tested in 2011-12 under golf course conditions in Texas to determine if it may be useful as a salt-tolerant variety in the future.

Annual (*Lolium multiflorum* L.) and perennial ryegrass (*L. perenne* L.) are considered to be susceptible to high salinity. Research by Aydemir et al. (1) indicated that many municipal water wells in southeast Texas have relatively high levels of sodium (Na) and low totals of dissolved solids. This often results in accumulation of Na and increased salinity on highly irrigated turfgrass areas.

Marcum (7) rated most cool-season grasses suitable for overseeding as very susceptible to high salinity with perennial ryegrass having better tolerance than both rought bluegrass (*Poa trivialis*) and annual ryegrass. With the decline in water availability during the drought of 2011 in Texas and the increased use of irrigation water with higher levels of salt and Na, there is a need for a cool-season salt-tolerant turf for golf courses, sports fields, and home lawns.

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Lee et al. (5) indicated the necessity to develop different criteria for salt screening of halophytic turfgrass compared to glycophytes (salt-sensitive plants). Mass and Hoffman (9) classified crops with a threshold EC greater than 10 dS m L^{-1} (6,400 mg L^{-1}) as very salt tolerant. Brilman (2) screened perennial ryegrass for germination in 10,000 mg salt L^{-1} and seedlings at a concentration of 12,000 mg L^{-1} and reported differences among genotypes for rate of germination and for seedling growth.

Numerous techniques for screening turf grass for salinity tolerance have been developed to identify and select genotypes with increased tolerance. Techniques include hydroponic saline solution (3, 6, 8, 11, 12). Nelson and Foster (10) reported on an immersion technique where plants grown in flats were submerged in salt water which resulted in differentiation between genotypes for salt tolerance.

Techniques for screening genotypes for salinity tolerance under greenhouse or laboratory conditions should be correlated to field trials where high salt is known to be a problem. Koch and Bonos (4) reported on a study where they were able to correlate results from a hydroponic

Numerous techniques for screening turfgrass for salinity tolerance have been developed to identify and select genotypes with increased tolerance. Techniques include the use of hydroponic saline solution shown above.

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technique, an overhead-irrigation method, and a field screening study. The objectives of this research were to screen for salinity tolerance between ryegrass genotypes, select improved genotypes under field conditions, and correlate greenhouse screening techniques and field screening techniques.

Materials and Methods

Field Study

In the field study at Pecos, Texas, 13 ryegrass genotypes were seeded into 3 x 3 ft plots on October 27, 2010. The soil analysis for Pecos is shown in Table l. Seeding rate was 33 g seed in a 3 x 3 ft plot. The experiment had three replications. The experiment was watered daily by sprinkler irrigation until a stand was obtained.

The water analysis at Pecos had a pH of 7.8, and the SAR was 4.7. Elemental analyses were 446 ppm Na, 16 ppm K, 455 ppm Ca, 136 ppm Mg, 350 ppm sulfate, 906 ppm Cl, and 172 ppm bicarbonate. Thereafter the experiment was watered as needed; however, the trial was watered about every 7 days as little rainfall occurred during the growing season. Plots were mowed at a mowing height of 1.5 inches approximately every 14 days.

Greenhouse Studies

Soil was collected from Pecos and transported to College Station and was used in the greenhouse studies. In experiment A, 100% Pecos soil was placed in plastic flats (9 X 20 inches) at a depth of 2 inches. Entries were seeded into rows 2 inches apart. Each row was seeded with about 30 seeds, and seedlings were thinned to 12 plants per row. Plants were watered once or twice per day until emergence. Pecos soil dried out in the greenhouse after 3 to 4 hours, so consistent stands were difficult to obtain and some entries had to be replanted. Pecos soil is a silty clay soil which becomes brick-like when dry.

In experiment B, sand was mixed with Pecos soil in a 60% sand and 40% Pecos soil ratio. This resulted in a soil mixture which adsorbed moisture and did not dry out as rapidly as the 100% Pecos soil. Experiment B was seeded on February 5, 2011. Obtaining good stands were much improved with this soil mixture. The same planting procedure was used in experiment B as with experiment A.

In both experiments, plants were irrigated with a sprinkler from a hose using city water every other day. When plants were in the 3-tiller stage, flats were subjected to the salt treatment. Fourteen genotypes were tested in these experiments. There were 5 replications in both Experiment A & B. A sixth replication was immersed in city well water and was used as a

The experiment was watered as needed; however, the trial was watered about every 7 days as little rainfall occurred.

Data suggested that the immersion technique used in these experiments was not reliable or useful to differentiate genotypes for salinity tolerance.

control to determine effects of environmental conditions in the greenhouse. Fertilization was accomplished by adding plant nutrients to the water. Salt concentration was regulated by mixing OCEANIC Natural Sea Salt Mix to the salt tank in which flats were immersed. The entire plants including leaves were immersed in the salt solution. An Oakton Salt 6 salinity meter was used to measure the salt concentration for each day prior to the immersion process.

In experiment A, flats were immersed in salt water beginning February 9 and the trial was terminated on March 28. At first immersion, salt concentration was 3,800 ppm. Flats were immersed for 2 minutes before being returned to greenhouse bench. Plants in flats were immersed every 2 to 3 days and salt concentration was gradually increased by 300 to 400 ppm between each immersion. The salt concentration was 11,000 ppm by March 23, 2011, and severe salt damage had occurred and the experiment was terminated on March 28. Salinity tolerance ratings were recorded on a 1 to 9 scale where 1 = 10% green leaves, and 9 = 90% green leaves.

Result

In the field study at Pecos adequate stands were obtained on all genotypes. The trial was evaluated or rated for salinity tolerance at two dates (November 28, 2010 and April 19, 2011). Elemental soil analysis at Pecos for sodium, potassium, calcium, magnesium, as well as SAR and SSP are shown in Table 1.

Salinity ratings are shown on Table 2. All ryegrass plots looked good in this study. No significant differences were determined between entries. Mean ratings on the November rating date were between 4.3 and 6.0. Most salt-tolerant entries were STPRG-1 and STPRG-2; however, they were not significantly different from other

	Pecos Soil			60% Sand: 40% Pecos Soil	
Parameter[1]	from field	Salt-treated end of exp	Non-salt treated end of exp	Salt treated end of exp.	Non-salt treated end of exp.
Sodium (ppm)	847	6,867	2,331	3,560	1,555
Potassium (ppm)	65	177	60	168	58
Calcium (ppm)	660	846	401	351	141
Magnesium (ppm)	119	437	101	185	48
SAR	7.9	47.8	26.9	38.3	28.9
SSP	45.4	78.3	77.3	80.7	84.5

[1] Analyses were conducted by the Soil, Water and Forage Testing Laboratory at Texas AgriLife Extension at College Station.

Table 1. Soil analysis of Pecos, Texas soil and greenhouse soil from Pecos for pre- and post-salt treatments in Experiment A and Experiment B.

Entry	Nov 28[1]	April 19
STPRG-1	4.7	5.7
STPRG-2	4.3	4.3
TXR2010-SS3G	6.0	5.3
TXR2011-S-10	5.0	5.3
Pecos Bulk 2010	5.3	5.3
T-10 Bulk	5.7	5.7
Panterra	5.7	6.0
Axcella 2	5.3	5.6
ProSaline	5.0	4.6
Intercross	5.0	5.0
Gulf	5.3	5.3
B-9. 1578	5.0	4.7
A-9. 1580	4.7	4.7
Mean	5.2	5.2
CV	18.0	29.6
Significance	NS	NS

Test was planted on Oct 27, 2010. Trial was watered as needed, on a weekly basis. All genotypes looked relatively good throughout growing season. Seed was overseeded onto bare soil and cultipacted in. Good stands were obtained.

[1]Date all plants in one row were rated

Table 2. Salinity ratings on ryegrass genotypes grown in field at Pecos, TX in 2010-11. Ratings were on a 1 to 9 scale where 1 = best and 9 = dead plants.

entries. On the April 19 rating date, mean ratings were between 4.3 and 6.0; however, again there were no significant differences. Entry TXR2011-S-10 did have a more tolerant salinity rating in both the field trial and the greenhouse trials.

There were three snow events at Pecos during the winter of 2010-11 and perhaps the moisture from these events reduced salt levels in the soil and helped mask genetic differences for salt tolerance between entries in this trial. Perhaps rating genotypes is not a reliable technique and a more definitive technique such as weighing plant tissue would improve the technique. In previous years, we have measured significant differences in the field rating a Pecos.

In greenhouse experiment A, ratings were recorded on three dates which were March 15, March 22, and March 28 (Table 3). The increase in element concentration in the soil is shown in Table 1. For experiment A, the Pecos soil had 847 ppm sodium at the beginning of the experiment and 6,867 ppm at the termination of the trial. However, using just the city water, sodium had increased to 2,331 ppm. Significant differences were obtained between genotypes. Two genotypes (TXR2011-S-10 and Pecos Bulk 2010) had higher tolerance ratings (March 15); however, they were not significantly better than several other entries.

The above genotypes make up the majority of a bulk population which was increased in Oregon in 2011 as a salt-tolerant population. On the March 22, TXR2011-S-10, A-9.1580 and A-9.1578 had slightly better salinity tolerance ratings; however, they were not significantly better than several other entries. On March 28, A-9.1580 and B-9.1578 had slightly high green leaf ratings; however, on this date very little green leaf tissue remained on any genotype. The *Poa trivialis* line TMI-S had significantly less salt tolerance compared to all ryegrass entries. This is in agreement with research reported by Marcum (7).

In experiment B, immersion began on March 14 at the 3-tiller stage at a salt concentration of 4,600 ppm. Experiment B was terminated on April 8, 2011, when the salt concentration level was 10,000 ppm. Most plants were either dead, or nearly dead. Salinity ratings are shown in Table 4. Greenhouse temperatures were much higher during March and April for both experiments, and likely affected experiment B, more than A. On the April 5 rating date, there were not much differences between genotypes except for the TMI-S (*Poa trivialis*) which had a lower rating for green leaf tissue. On the April 7 date, more tolerant entries were B-9.1578, TXR2010-SS3G and 'Intercross'. However, they were not significantly better that several other entries. On the April 11 rating, there were no significant differences.

In comparing results from the field trial and experiments A & B, results are not encouraging or consistent. The two checks are 'Panterra' and 'Axcella 2', which should have little salt tol-

Genotype	Date		
	March 15	March 22	March 28
TXR2011-S-10	6.8	4.4	1.8
Pecos Bulk 2010	6.8	3.6	1.8
A-9.1580	6.4	4.0	2.0
B-9.1578	6.2	4.2	2.0
TXR2010-SS3G	6.0	3.4	1.0
Panterra (ck)	5.8	4.0	2.0
TXR2009-SSBlk	5.6	3.8	1.6
Intercross	5.4	3.2	1.4
Axcella 2 (ck)	5.2	3.0	1.6
TXR	5.0	2.8	1.6
STPRG1	4.6	1.8	1.0
ProSaline	4.4	2.4	1.4
STPR G2	4.4	2.4	1.2
TMI-S (*Poa trivialis*)	1.8	1.0	1.0
Mean	5.3	3.1	1.5
CV	22.9	32.9	40.3
LSD (0.05)	3.8	2.9	1.1

Plants were grown in rows in Pecos soil in flats and were immersed in salt water every 3 days. Salt concentration was increased gradually until all plants died due to high salt concentration. There were 5 replications.

Table 3. Salinity ratings on 13 ryegrass genotypes and one *Poa trivialis* genotype grown in Pecos soil in flats in greenhouse in 2011. Ratings were from 1 to 9 where 1 = 10% green and 9 = 90% green leaves.

erance. In the field trial, they are slightly more susceptible, however not significantly more susceptible that other entries. In the greenhouse trials some genotypes which have been "selected for salt tolerance" are slightly more salt tolerant than 'Panterra' and 'Axcella 2'. However, these differences are small and often not significant.

These data suggest that the immersion technique used in these experiments was not reliable or useful to differentiate genotypes for salinity tolerance. A problem we had with the immersion technique was that soil in which the plants were growing silted out or washed out through holes in the bottom of the flats. Since the soil was 100% saturated with water and with movement of the flats into the immersion tank or when replacing flats on the bench in the greenhouse, perhaps the soil was washed out (not uniformly) with each immersion and over time this may have resulted in a significant loss of soil. This could have contributed to a high variability or error factor with the process. If the immersion technique is used in the future, we recommend that a cloth or paper towel be used to cover all holes in the bottom of the each flat to eliminate or reduce soil being washed out of the flats.

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Genotype	Date		
	April 5	April 7	April 11
TXR2011-S-10	5.6	4.6	2.8
Pecos Bulk 2010	4.8	3.2	2.0
A-9.1580	5.4	4.8	1.6
B-9.1578	5.6	5.0	2.4
TXR2010-SS3G	6.4	5.0	2.2
Panterra (ck)	5.2	3.8	1.6
TXR2009-SS Blk	4.8	3.8	2.2
Intercross	6.2	5.6	2.4
Axcella 2 (ck)	5.4	4.0	2.2
TXR	6.2	5.6	2.4
STPRG1	4.8	3.6	2.0
ProSaline	5.2	4.6	1.6
STPRG2	5.2	3.8	2.0
TMI-S (*Poa trivialis*)	3.0	2.0	1.2
Mean	5.3	4.3	2.0
CV	23.3	34.1	46.2
LSD (0.05)	2.5	2.9	NS

Plants were grown in rows in soil mix (40% Pecos soil and 60% sand) in flats and were immersed in salt water every 3 days. Salt concentration was increased gradually until all plants died due to high salt concentration. There 5 replications.

Table 4. Salinity ratings on 13 ryegrass genotypes and one *Poa trivialis* genotype grown in 60% sand and 40% Pecos soil in flats in greenhouse in 2011. Ratings were from 1 to 9, where 1 = 10% green and 9 = 90% green leaves.

Literature Cited

1. Aydemir, S., N. F. Najjar, and C. T. Hallmark. 2005. Exchangeable sodium accumulation with irrigation in soils under turfgrass. *Comm. Soil Sci. and Plant Anal.* 36:1611-1624. (TGIF Record 112687)

2. Brilman, Leah. 2006. Salt tolerance of perennial ryegrass varieties. Seed Research of Oregon (Online, http://www.sroseed.com). Verified 29 June 2006. (TGIF Record 189434)

3. Dai, J., D. R. Huff, and M. J. Schlossberg. 2009. Salinity effects on seed germination and vegetative growth of greens-type *Poa annua* relative to other cool-season turfgrass species. *Crop Sci.* 49:696-703. (TGIF Record 148866)

4. Koch, M. J., and S. A. Bonos. 2011. Correlation of three salinity tolerance screening methods for cool-season turfgrasses. *HortSci.* 46:1198-1201. (TGIF Record 187911)

5. Lee, G., R. N. Carrow, and R. R. Duncan. 2004a. Photosynthetic responses to salinity stress of halophytic seashore paspalum ecotypes. *Plant Sci.* 166:1417-1425. (TGIF Record 116836)

6. Lee, G., R. R. Duncan, and R. N. Carrow. 2004b. Salinity tolerance of seashore paspalum ecotypes: shoot growth responses and criteria. *HortSci.* 39:1138-1142. (TGIF Record 97229)

7. Marcum, K. B. 2004. Use of saline and nonpotable water in the turfgrass industry: constraints and developments. 4th International *Crop Sci.*

Congress, Brisbane Australia. (TGIF Record 129141)

8. Marcum, K. B. 2000. Salt tolerance varies in modern creeping bentgrass varieties. *Golf Course Management* 68(10):54-58. (TGIF Record 68874)

9. Mass, E. V., and G. J. Hoffmann. 1977. Crop salt tolerance-current assessment. *ASCE J.* Irr. and Drainage Div. 103(IR2):115-134.

10. Nelson, L. R., and M. A. Foster. 2009. Breeding annual ryegrass (*Lolium multiflorum*) and perennial ryegrass (*Lolium perenne*) for salinity tolerance. Pages 21-22. *In* Proceeding of 2009 International Turfgrass Society Annex – Technical Papers – Chile. (TGIF Record 188212)

11. Pessarakli, M., and D. M. Kopec. 2009. Screening various ryegrass cultivars for salt stress tolerance. *Journal of Food, Agriculture and Environment.* 7:739-743. (TGIF Record 189433)

12. Rose-Fricker, C., and J. K. Wipff. 2001. Breeding for salt tolerance in cool-season turfgrasses. *ITS Research Journal* 9:206-212. (TGIF Record 74292)