USGA EXECUTIVE SUMMARY

Determining the Heritability of Salt Gland Density: a Salinity Tolerance Mechanism of Chloridoid Warm Season Turfgrasses

November 10, 1999

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Water shortages are resulting in a major shift to use of secondary, saline water sources for turf irrigation, particularly in the western U.S., and in coastal areas. Though there is increasing need for improved salt tolerant turfgrass cultivars, breeding progress has been limited. Understanding of basic salt tolerance mechanisms and their genetic control may greatly expedite turf breeding programs.

Salt tolerance in the Chloridoid grasses, including bermudagrass, buffalograss, zoysiagrass, and saltgrass (Distichlis spp.) is stongly associated with shoot salt exclusion, which seems to be associated with leaf salt gland density. We are examining the relationship between salt gland density and salt tolerance in the zoysiagrasses. Fifteen zoysiagrass varieties are being examined for salinity tolerance and salt gland density. These fifteen are being crossed to produce offspring to examine salt gland heritability, or genetic control (i.e. if it is passed on from parent to offspring). Research to date supports initial observations that salt gland density plays a premier role in salt tolerance of Chloridoid turfgrasses.

Currently, development of salt tolerant turfgrass cultivars has been very limited, due to the difficulty of screening thousands of breeding accessions for salt tolerance. If gland density is highly heritable, it should be possible to select new salt tolerant individuals in breeding programs simply by looking at their leaves under a microscope to determine their salt gland density. This procedure should be much easier, and more accurate than screening large numbers of individuals under salt stress, and so may greatly expedite progress in developing new salt tolerant turfgrass cultivars.

USGA ANNUAL REPORT

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The main purpose of this study is to determine broad and narrow sense heritabilities of salt gland density, an important salinity tolerance mechanism of Chloridoid warm season turfgrasses. A second goal is to determine the range of salinity tolerance existing within the *Zoysia japonica* species.

A total of fifteen *Zoysia* genotypes are utilized in this study. They have been assigned the following numeral designations:

Expt. #	I.D.
I	Belair
II	Crowne
III	El Toro
IV	J-21
V	J3-2
VI	J94-5
VII	JS-23
VIII	K-12
IX	K157
X	K-162
XI	Korean Common
XII	Meyer
XIII	P-58
XIV	Palisades
XV	Sunrise

Genotypes were collected and increased during the summer of 1998, and then used to make two plantings - a crossing block bench and a hydroponics bench. The crossing block, consisting of 10 pots of each genotype, was planted Nov. 17-19, 1998, and the hydroponics bench Feb. 23-25, 1999. A rapid, inexpensive method for counting salt gland densities has been developed for this project, in which leaf peels (nail polish) are observed under a stereo light microscope.

OBJECTIVE 1: Determine narrow sense heritability of salt gland density.

To determine narrow sense heritability, progeny of crosses must be evaluated. The crossing bench was designed as a polycross nursery, but incompatible flowering timing among genotypes has forced us to pursue individual crosses. Due to the smallness of the flowering culms, and the lack of sufficient numbers of pots to pair up for individual crosses, a detached culm technique has been used. Individual culms from the male and female parent are cut from the base of the crown, and immediately suspended in "Floralife" solution in a microcentrifuge. Floralife, designed to prolong flower life, contains sugars and an antibiotic. Culm stems are fit through small holes in the microcentrifuge tube lid, and sealed to minimize evaporative losses. Inflorescences are then covered with a small glacine bag, and sealed around the microcentrifuge tube base with a small dental brace rubber band. Crosses are kept in a controlled environment chamber having a long day/night cycle. Bags are agitated daily, and Floralife fluid levels maintained, and changed periodically.

In addition to the above detached culm method, crosses were performed where the female culm remained attached to the crown in the growing pot. The male culm was detached and immediately suspended in the Floralife solution in a microcentrifuge tube as described above.

Crossing began in April, 1999. As of November 1, 1999 a total of 127 individual crosses have been performed. Out of the 105 possible crosses between the 15 genotypes (i.e., 15 x 14 ÷ 2 = 105), 43 or 41.0% have been performed. Fourteen of the 15 genotypes have flowered to date.

Below is a listing by genotype of the 127 individual crosses performed (as of November 1, 1999):

I--Belair is included in 23 crosses. 2 with II--Crowne, 1 with VI--J94-5, 4 with VIII--K-12, 3 with XI--Korean Common, 7 with XII--Meyer and 6 with XIII--P-58, for a total of 23.

II--Crowne is included in 10 crosses. 2 with I--Belair, 1 with III--El Toro, 1 with VI--J94-5, 1 with VIII--K-12, 1 with XI--Korean Common, 1 with XII--Meyer and 3 with XIII--P-58, for a total of 10.

III--El Toro is included in 27 crosses. 1 with II--Crowne, 1 with itself (a selfing test), 2 with VI--J94-5, 1 with XI--Korean Common, 1 with XII--Meyer, 17 with XIII--P-58 and 4 with XIV--Palisade, for a total of 27.

IV--J-21 is included in 3 crosses. 1 with VII--JS-23, 1 with XII--Meyer and 1 with XIII--P-58, for a total of 3.

V--J3-2 is included in no crosses at this time, as flowers have not developed.

VI--J94-5 is included in 12 crosses. 1 with I--Belair, 1 with II--Crowne, 2 with III--El Toro, 1 with VIII--K-12, 4 with XII--Meyer, 2 with XIII--P-58 and 1 with XV--Sunrise for a total of 12.

VII--JS-23 is included in 11 crosses. 1 with IV--J-21, 1 with VIII--K-12, 2 with X--K-162, 3 with XI--Korean Common, 2 with XII--Meyer and 2 with XIII--P-58, for a total of 11.

VIII--K-12 is included in 18 crosses. 4 with I--Belair, 1 with II--Crowne, 1 with VI--J94-5, 1 with VII--JS-23, 7 with XII--Meyer, 3 with XIII--P-58 and 1 with XIV--Palisades, for a total of 18.

IX--K157 is included in 5 crosses. 1 with XI--Korean Common, 3 with XII--Meyer and 1 with XIII--P-58, for a total of 5.

X--K-162 is included in 4 crosses. 2 with VII--JS-23 and 2 with XII--Meyer, for a total of 4.

XI--Korean Common is included in 17 crosses. 3 with I--Belair, 1 with II--Crowne, 1 with III--El Toro, 3 with VII--JS-23, 1 with IX--K157, 7 with XII--Meyer and 1 with XIII--P-58, for a total of 17.

XII--Meyer is included in 47 crosses. 7 with I--Belair, 1 with II-Crowne, 1 with III--El Toro, 1 with IV--J-21, 4 with VI--J94-5, 2 with VII--JS-23, 7 with VIII--K-12, 3 with IX--K157, 2 with X--K-162, 7 with XI--Korean Common, 9 with XIII--P-58, 1 with XIV--Palisades and 2 with XV--Sunrise, for a total of 47.

XIII--P-58 is included in 56 crosses. 6 with I--Belair, 3 with II-Crowne, 17 with III--El Toro, 1 with IV--J-21, 2 with VI--J94-5, 2 with VII--JS-23, 3 with VIII--K-12, 1 with IX--K157, 1 with XI--Korean Common, 9 with XII--Meyer, 10 with XIV--Palisades and 1 with XV--Sunrise, for a total of 56.

XIV--Palisades is included in 16 crosses. 4 with III--El Toro, 1 with VIII--K-12, 1 with XII--Meyer and 10 with XIII--P-58, for a total of 16.

XV--Sunrise is included in 4 crosses. 1 with VI--J94-5, 2 with XII--Meyer and 1 with XIII--P-58, for a total of 4 crosses.

Crosses are summarized in a crossing table on the next page.

Two months into the crossing, it appeared that the detached culm technique has not been as effective as hoped, though some seed was still obtained. We have switched to another crossing technique, in which the female culm is not detached from the mother plant. The male is detached and suspended in a microcentrifuge tube filled with Floralife, and placed next to the female flower in the turf canopy. The two Inflorescences are then bagged, as before. The male is put into solution, because zoysia flowers (both male and female) open and are receptive over a period of several days to one week. Fourty-two crosses have been made with the new technique.

OBJECTIVES 2 AND 3: Determining broad sense heritability of salt gland density, and the range of salinity tolerance present within the Z. japonica species.

To accomplish these objectives, the 15 genotypes are being evaluated in hydroponics. A split plot, randomized complete block (4) experimental design is being used, with each block suspended over tubs containing 31 L of constantly-aerified Hoaglands #2 nutrient solution, modified with EDDHA chelate. Broad sense heritability will be determined by comparing salt gland densities of moderately salinized plants vs. nonsalinized plants within each genotype. Following heritability determination, relative salinity tolerances will be determined under highly salinized conditions.

Grasses were cut at constant height (1.5"), then treatment tubs were initially raised to 75 mM $NaCl/CaCl_2$ and held for 31/2 weeks to allow new growth to occur. During this time, control plants were clipped and leaf peels were made using clear finger nail polish. Salinized leaves were then clipped and leaf peels made. Forty peels were made per grass entry, 5 per replication. The 600 peels have been mounted on glass slides. Salt gland density of control and salinized grasses will be quantified from the leaf peels via light microscope, and broad sense heritability calculated from this data. A high degree of conservation of salt gland density within a genotype under both control and salinized conditions will indicate a large degree of genetic control for the trait, i.e. a high broad sense heritability.

Plants are now being tested for relative salinity tolerance in hydroponics. Plants are being trimmed at 1.5 inches, 1x per week. Salinity has been gradually raised by 40mM daily, to achieve 160mM per week. Measurements of clipping dry weights, percent leaf firing, and rooting depth are taken at each 160 mM NaCl treatment level. Root weights will be determined at the end of the experiment. Greg is currently taking final shoot and root data at the 960mM level. Plants will then be allowed to sit at the final level to monitor survival.

Zoycros.xls
28-Oct-99 University of Arizona -- Ken Marcum and Greg Weiss
10:15 AM Number of Zoysia crosses performed to date

	I--Belair	II--Crowne	III--El Toro	IV--J-21	V--J3-2	VI--J84-5	VII--J8-23	VIII--K-12	IX--K157	X--K-152	Korean Com	XII--Meyer	XIII--P-56	IV--Palisade	XV--Sunrise	Total
I--Belair ->		3				1		4			3	7	6			23
II--Crowne ->			1			1		1			1	1	3			8
III--El Toro ->			1			2					1	1	17	4		26
IV--J-21 ->							1					1	1			3
V--J3-2 ->																0
VI--J84-5 ->								1				4	2		1	8
VII--J8-23 ->								1		2	3	2	2			10
VIII--K-12 ->												7	3	1		11
IX--K157 ->											1	3	1			5
X--K-152 ->												2				2
XI--Korean Common												7	1			8
XII--Meyer ->													9	1	2	12
XIII--P-56 ->														10	1	11
XIV--Palisades																0
XV--Sunrise																0
Grand Total																127

As of Nov. 1, 1999 a total of 127 individual crosses have been performed. 43 out of the 105 possible combinations or 41.0% of the total possible number of crosses.

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<u>PLANS FOR THE UPCOMING YEAR</u>

OBJECTIVE 1

Seed collected from all crosses will be germinated in greenhouse pots for narrow sense heritability estimates. We will attempt to force another flowering cycle by lengthening daylength via artificial lighting, coupled with moderate drought stress. Further crosses will be made if flowering is successful, otherwise crosses will be made next spring/summer.

OBJECTIVE 2

Salt gland density of hydroponically grown zoysiagrasses will be determined by quantifying gland density of the 600 leaf peels. Broad sense heritability estimates of salt gland density will be obtained, along with relative salinity tolerances of the 15 entries. Results will be published in a refereed journal.