USGA ANNUAL REPORT

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

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EXECUTIVE SUMMARY

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. Salt tolerance in the Chloridoid warm season grasses, including bermudagrass, buffalograss, zoysiagrass, and saltgrass (Distichlis spp.) is strongly 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 *Zoysia japonica*. Fifteen entries 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).

Results show a broad range of salinity tolerance. The most tolerant entries were: Palisades, El Toro, J3-2, P58, Belair, Meyer, and Crowne. In addition, salt gland density was highly correlated with salt tolerance, indicating that salt gland density is an important salt tolerance mechanism in zoysiagrass. Also, broad sense heritability estimates of salt gland density were high, indicating that genetics, not environment, controls salt gland density. This would support the use of salt gland density as a selection tool in turfgrass breeding programs for development of salt tolerant cultivars. The use of gland density as a selection tool would greatly expedite the breeding process, as there would no longer be a need to screen large numbers of accessions under controlled environmental stress (e.g. salinity) conditions. F1 progeny are currently being produced, and sampled for salt gland density, which will allow an estimation of narrow sense heritability of salt gland density.

RESEARCH PROGRESS

There are three main objectives of these studies:

I. Determine the range of salinity tolerance existing within the *Zoysia japonica* species.

II. Determine the broad sense heritability of salt gland density of the zoysiagrasses, and if gland density is an important salinity tolerance mechanism within *Z. japonica*.

III. Determine the narrow sense heritability of salt gland density in zoysiagrass.

These objectives will be discussed separately in the sections that follow.

A total of fifteen *Zoysia* genotypes are included in these studies. They have been assigned the following numeral designations:

#	Name
I	Belair
II	Crowne
III	El Toro
IV	J21
V	J3-2
VI	J94-5
VII	JS-23
VIII	K12
IX	K157
X	K162
XI	Korean Common
XII	Meyer
XIII	P58
XIV	Palisades
XV	Sunrise

I. Determining the range of salinity tolerance existing within the Zoysia japonica species.

Genotypes were collected and increased during the summer of 1998. Rhizomes of the 15 genotypes were planted into 3.5 inch wide X 3 inch deep plastic pots. Pots were filled with acid-washed coarse silica sand, and had coarse plastic screen bottoms to allow roots to grow unimpeded into hydroponics nutrient solutions. A split plot, randomized complete block (4) experimental design was used, each block containing all 15 zoysiagrass entries. Each block was suspended over a tub containing 31 L of constantly-aerified Hoaglands #2 nutrient solution, modified with EDDHA chelate. Grasses were allowed to become established over a period of 3 months before salinity treatments, and the experiment began.

Grasses were clipped weekly at 1.5 inches height. Roots extending into nutrient solutions were clipped 1 week prior to initiation of treatments to allow monitoring of rooting depth during salinity stress. Salinity was gradually raised by 40 mM (2340 ppm) NaCl daily, to achieve 160 mM (9360 ppm) per week. Final salinity treatment levels were: 0, 160, 320, 480, 640, 800, and 960 mM NaCl (9360, 18720, 28080, 37400, 46800, and 56160 ppm). For comparison, full strength sea water is approximately 35000 ppm total salinity.

Data was taken at each salinity level. Measurements included:

Leaf clipping dry weights
Percent green leaf canopy area
Rooting depth (length)
Root dry weight

II. Determining the broad sense heritability of salt gland density of the zoysiagrasses, and if gland density is an important salinity tolerance mechanism within Z. japonica.

All potted entries within the salinity experiment (both control and salinity treatments) were sampled for salt gland density. Grasses were sampled under both control and salinized conditions to determine if gland density is affected by salinity stress (gland density of salinized plants ≠ gland density of control plants), or if gland density is an innate genetic trait, not affected by the environment. If gland density is innate, and not affected by the environment, then gland density has a high broad sense heritability.

Zoysia entry gland densities were analyzed to determine if they were correlated with salinity tolerance indicators growth rate and percent green leaf area. A significant positive correlation would indicate that salt gland density is involved in salinity tolerance in *Z. japonica* species.

Each experimental unit (zoysia entry and replication) for both control and salinized plants were sampled: 15 entries X 4 replications X 2 treatments = 180 pots sampled. From each pot sampled, 5 leaves were taken for analysis. To count salt glands, leaf peels were made by painting the leaves with an acrylic, then carefully peeling off the impression, and mounting it onto a microscope slide. A total of 900 leaf peels were made. The number of salt glands present within a light microscope observation field were counted, giving a density of glands per unit area. Twelve fields were counted per leaf impression, making a total of 10,800 fields counted. Data was obtained for salt gland density for each zoysiagrass entry, under both control and salinized conditions.

III. Determining the narrow sense heritability of salt gland density in zoysiagrass.

Narrow sense heritability of salt gland density indicates whether salt gland density is passed from parent to progeny. To evaluate narrow sense heritability, mixed progeny from the 15 zoysiagrass entries must be generated, and evaluated for salt gland density. To make the crosses, crossing blocks were planted in controlled environment greenhouses at U.A. Crossing blocks, planted during late 1998 into early 1999, consisted of multiple planting flats of each zoysiagrass entry.

The crossing bench was designed as a polycross nursery, but flowering timing (nicking) was largely incompatible among genotypes during the first flowering season (early summer, 1998). Since the zoysiagrasses revealed a long day flowering habit, supplemental lighting was added during the spring of 1999 to try and force uniform flowering. The summer flowering of 1999 was not much better, and so a complete polycross (every combination of every entry) could not be obtained. However, over the 2 year period, 269 individual crossed were made (Fig. 1).

Due to the smallness of the flowering culms, a detached culm technique has been developed. Individual culms from the male and female, or male only parent are cut from the base of the crown, and immediately suspended in "Floralife" solution in a sealed microcentrifuge tube. Floralife, designed to prolong flower life, contains nutrients and an antibiotic, preventing bacterial clogging of phloem as seeds mature on the detatched culm. 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.

For initial crosses, culms of both male and female parents were detatched, and placed adjacent to each other in the same microcentrifuge tube, constituting a single cross. Crosses of this type wee kept in a controlled environment chamber having a long day/night cycle. In later crosses, only the culm from the male parent was detatched, and placed in a microcentrifuge tube. The detatched male culm was then placed into a zoysiagrass canopy adjacent to the receptor female culm, and the two culms then bagged with a glacine bag *in situ*. All glacine bags containing crossed were agitated daily, and Floralife fluid levels maintained, and changed periodically.

RESULTS

I. Determining the range of salinity tolerance existing within the Zoysia japonica species.

Leaf clipping dry weight declined with increasing salinity in all entries (Fig. 2). Linear regressions were fit to the leaf clipping dry weight data of each zoysiagrass entry, and the predicted 50% yield reduction value determined as a relative salinity tolerance criteria (Fig. 2). Other growth parameters measured were % green leaf canopy area, root depth, and root dry weight (Table 1). These parameters were tested for correlation (Table 2). The shoot (leaf) parameters Y50 and % green leaf area were highly correlated (0.84), indicating that they were both in agreement in predicting salinity tolerance. However, rooting parameters depth and dry weight were not correlated with each other, or with shoot parameters. It is not known why, except that it was observed that root growth in this experiment was almost nil, even under control conditions. Some other factor must have inhibited rooting, possibly a root borne fungus. Roots were not tested for disease, however. Shoot parameters indicate that the most salt tolerant zoysiagrass entries are: Palisades, El Toro, J3-2, P58, Belair, Meyer, and Crowne.

II *Determining the broad sense heritability of salt gland density of the zoysiagrasses, and if gland density is an important salinity tolerance mechanism within Z. japonica.*

Salt gland densities under control and salinized conditions are shown in Table 3. Gland densities under salinized conditions ranged from 14.0 in P58 to 7.1 in JS23. Salt gland density was highly correlated with shoot salinity tolerance indicators Y50 and % Green leaf area (up to 0.70 and 0.83 respectively, Table 2). This indicates that salt gland density is an important salt tolerance mechanism for *Z. japonica* species.

Differences between salt gland densities of salinized vs. control plants were low. The overall average (including all entries together) was only 0.8%, indicating that salt stress (environment) played no role in the expression of salt gland density. This indicates that the broad sense heritability of salt gland density is high, and that it could be used as a selection tool in turfgrass salt tolerance breeding programs.

III. *Determining the narrow sense heritability of salt gland density in zoysiagrass.*

A total of 269 crosses have been made: 127 in 1999 and 142 in 2000. Of the 1999 crosses, only 45 were successful in producing seed, or 35%. Due to relatively low percentage of successful crosses, we have modified the crossing technique, detatching only the male culm (leaving the female on the plant). We are currently processing the 2000 crosses, and it appears that we have a much better crossing success rate. Figure 3 shows the number of successful 1999 crosses, followed by the number of emerged seedlings from each successful cross, and finally the number of new crosses made in 2000.

All seed produced by crosses went through a thorough processing to prepare them for germination, including processing them in NaOH to improve germination. Seeds were then germinated on blotting paper primed with CaCl2, and protected from damping off with Benlate, in Petri dishes. Dishes were placed in a controlled environment chamber (32C day/22C night), with a 12 hour lighted day. Seedlings were transferred to greenhouse pots for growing out.

The 1999 crosses are now grown out (mature). We are now in the process of sampling these for salt gland density, using the leaf peel technique previously described. A total of 225 peels are being made, with 12 observations taken per peel. The 2000 crosses are currently being planted (as they are processed). When they mature (spring, 2001) we will sample these also for salt gland density. Leaf peels from this group will be substantially more than the 1999 crosses. Analysis of salt gland density of these F1 progeny will hopefully allow us to estimate narrow sense heritability of *Z. japonica* salt gland density, giving us a good indication of the possibility of breeding high gland density, salt tolerant Chloridoid warm season turfgrasses.

Table 1. Relative growth parameters indicating salinity tolerance of 15 Zoysia japonica entries. Y50 - predicted 50% leaf clipping dry weight reduction, % Green - % green leaf canopy area, Root Dep. - relative (to control) root depth, Root D.W. - relative (to control) root dry weight. All Growth Parameters except Y50 were taken at high salinity (960 mM).

Grass	Y50	% Green	Root Dep.	Root D.W.
Palisades	177	45.3	113.3	93.5
Crowne	157	23.7	76.0	134.5
El Toro	136	51.9	74.6	104.5
J3-2	131	47.9	92.9	118.0
P58	117	47.4	88.2	108.6
Belair	109	53.8	79.7	114.8
Meyer	71	9.7	73.6	100.6
K12	63	9.0	98.9	133.3
J21	62	5.6	122.4	131.3
K162	61	0.0	86.2	123.6
J94-5	53	3.3	109.2	117.3
Sunrise	48	0.0	98.9	101.4
Korean Common	44	2.3	89.6	157.5
JS-23	42	0.0	90.0	94.2
K157	23	0.0	100.0	226.1
$LSD_{0.05}$	-	17.1	15.5	78.1

Table 2. Correlation (r^2) matrix of growth parameters. Y50 - predicted 50% leaf clipping dry weight reduction, % Green - % green leaf canopy area, Root Dep. - relative (to control) root depth, Root D.W. - relative (to control) root dry weight. All Growth Parameters except Y50 were taken at high salinity (960 mM).

	% Green	Root Dep.	Root D.W.	SGCont.	SCSalt
Y50	.84****	-.21NS	-.43NS	.50*	.70**
% Green		-.28NS	-.35NS	.68**	.83****
Root Dep.			.14NS	.14NS	-.15NS
Root D.W.				-.25NS	-.20NS
SCCont					.79***

Table 3. Salt gland densities of Z. japonica entries under control (SG Cont.) and salinized (SG Salt) conditions. Broad sense heritability is estimated in this table as the % change between gland densities under control vs. salinized conditions. % Change = (SC Cont. - SC Salt) / SC Cont.

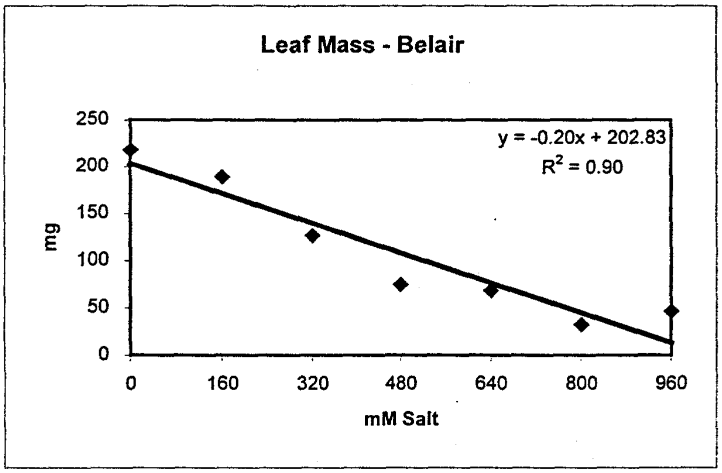
Grass	SG Salt	SG Cont.	% Change
P58	14.00	12.17	-15.0%
J3-2	13.79	14.12	2.4%
K12	12.20	11.11	-9.8%
Belair	12.18	11.68	-4.4%
El Toro	11.50	12.45	7.7%
Palisades	11.38	10.05	-13.3%
Crowne	11.28	9.91	-13.7%
J94-5	9.90	11.23	11.8%
Meyer	9.14	8.48	-7.8%
Sunrise	9.00	10.18	11.6%
K157	8.80	8.38	-5.0%
J21	8.58	12.04	28.7%
Korean Common	7.97	8.09	1.4%
K162	7.67	8.23	6.8%
JS23	7.06	7.59	7.0%
Overall Means	10.30	10.38	0.8%

Zoycros.xls
28-Nov-00
11:33 AM

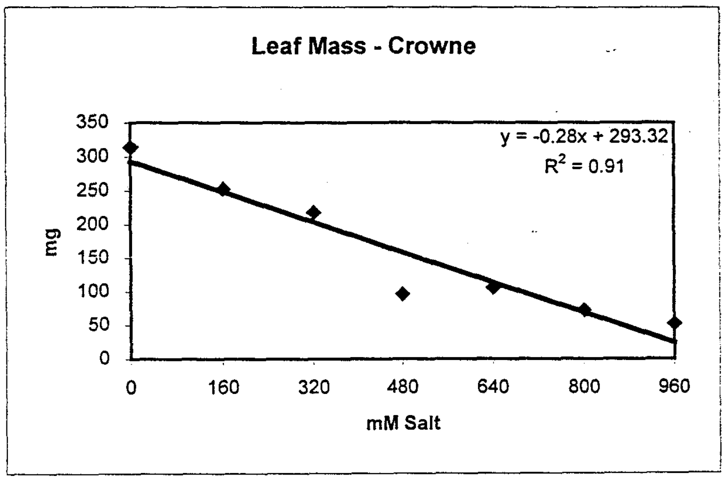
University of Arizona -- Ken Marcum and Greg Wess
Number of Zoysia crosses performed to date

	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	Korean Comn	XII--Meyer	XIII--P-58	XIV--Palisades	XV--Sunrise	Total
I--Belair ->		2				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--J94-5 ->								1				4	2		1	8
VII--JS-23 ->								1		2	3	2	2			10
VIII--K-12 ->												7	3	1		11
IX--K157 ->											1	3	1			5
X--K-162 ->												2				2
XI--Korean Common												7	1			8
XII--Meyer ->													9	1	2	12
XIII--P-58 ->														10	1	11
XIV--Palisades																0
XV--Sunrise																0
Grand Total																127

Fig. 1. Zoysiagrass crosses made to date.
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.



Y-50 = 109



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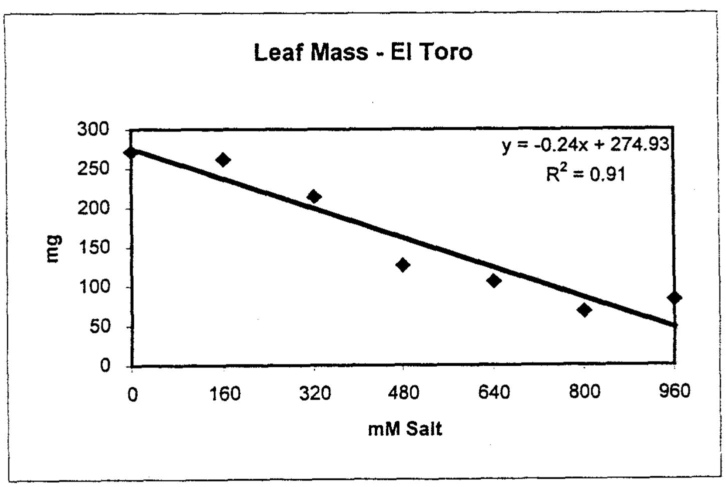
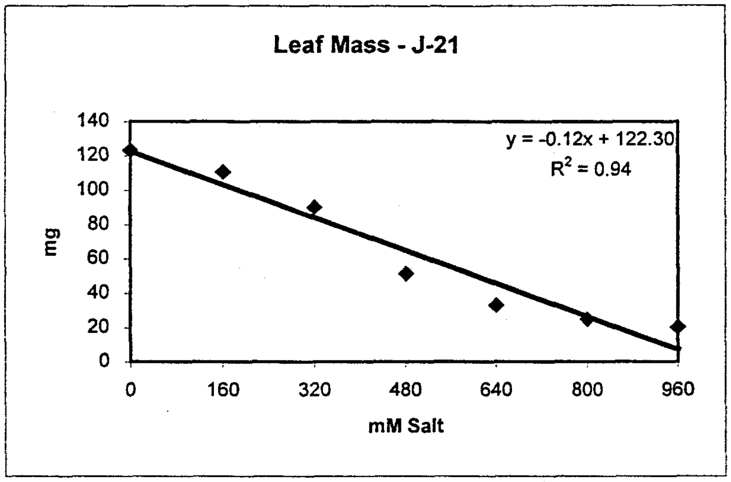


Y-50 = 136

Figure 2 (5 pages total). Decline in leaf clipping dry weight with increasing salinity. A Linear regression was fit to data of each entry, and predicted salinity value resulting in 50% clipping dry weight yield reduction was obtained.

'2000



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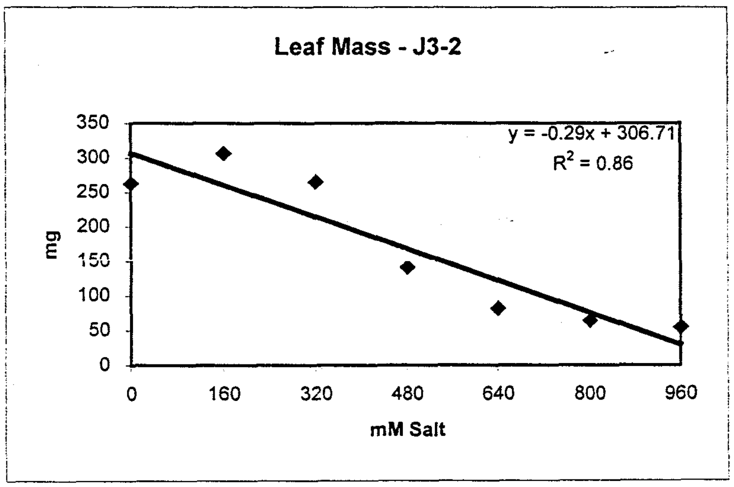

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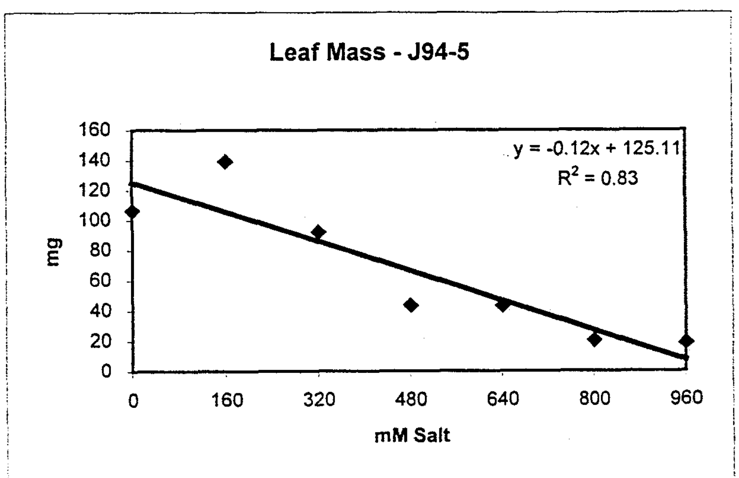

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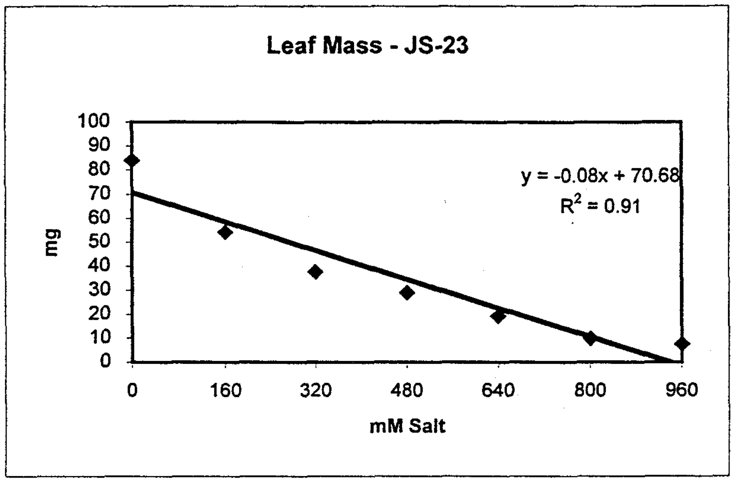

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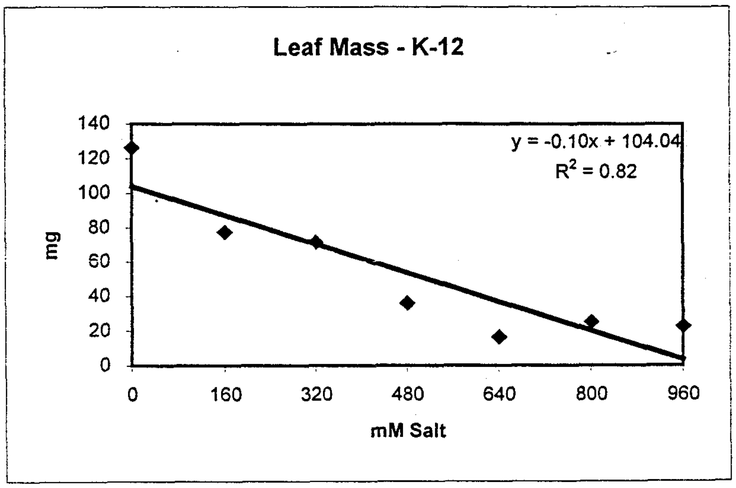

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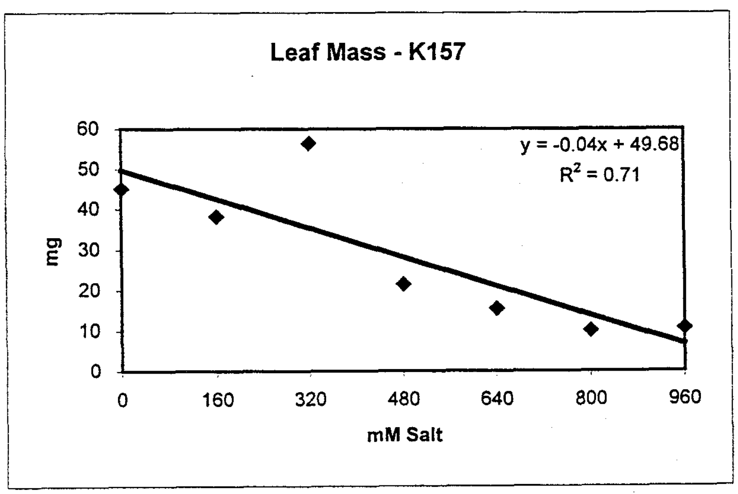

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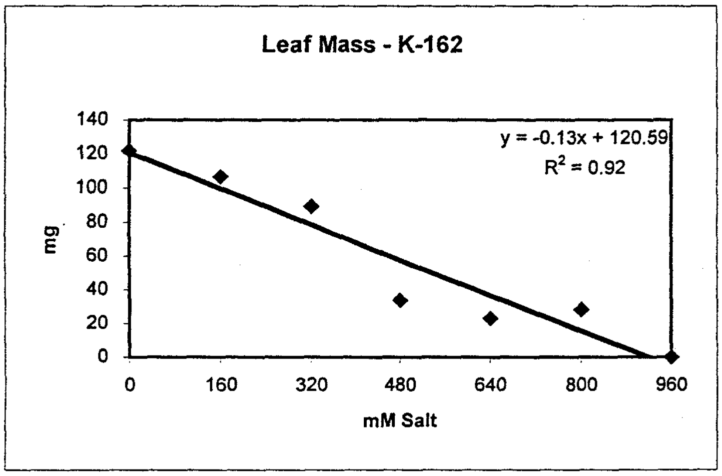

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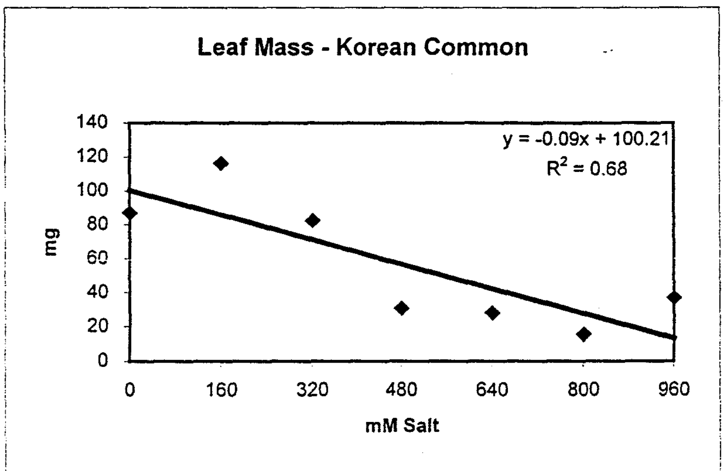

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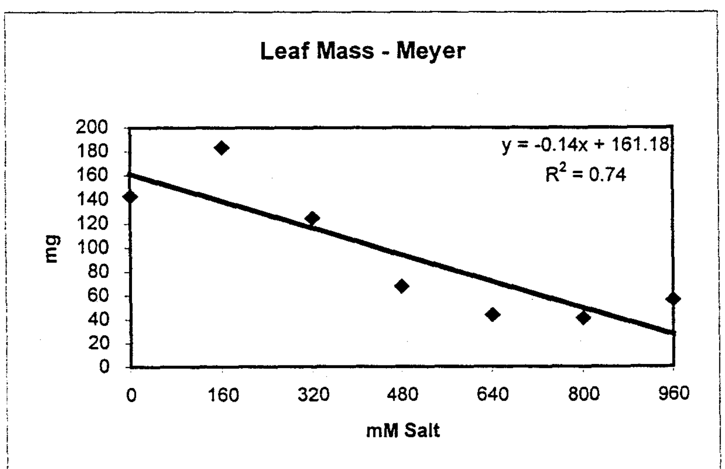

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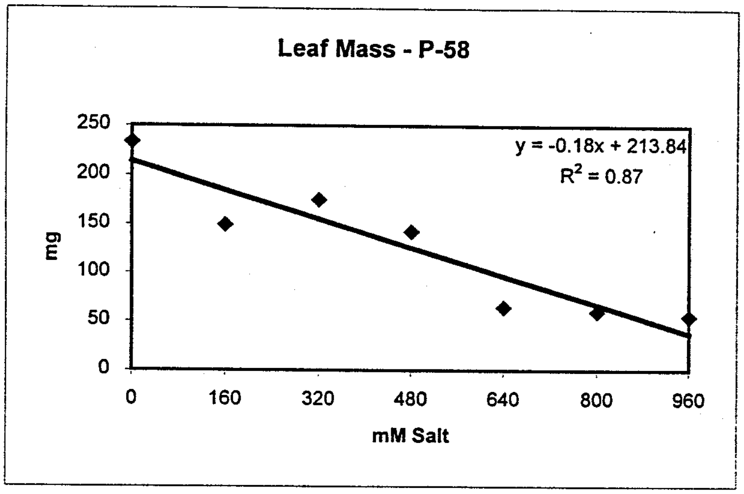

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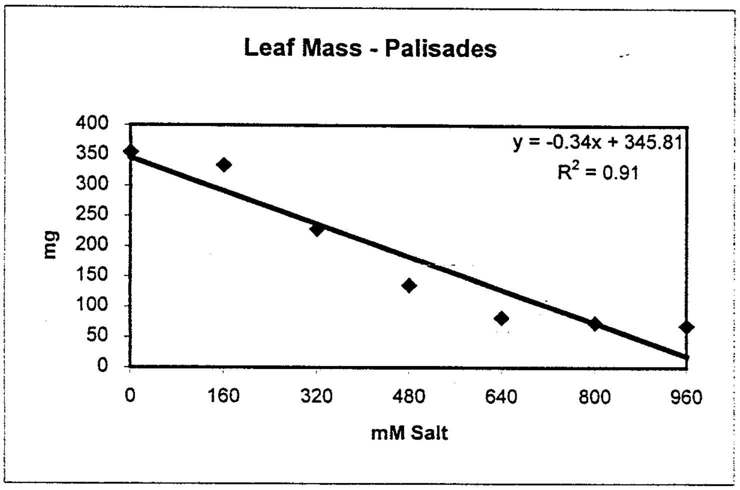

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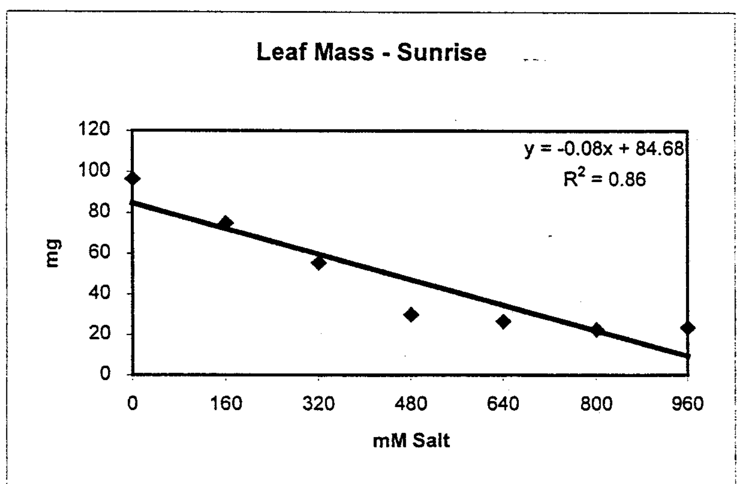

Y-50 = 48

Table Code -- Number of Crosses Successful: Number of Emerged Seeds//New 2000 Crosses
Updated as of 26-July-2000

SGD -->	14.12	12.45	12.17	12.04	11.68	11.23	11.11	10.05	9.91	8.48	8.38	8.23	8.09	7.59	
Female -->	V: J3-2	Iii: El Toro	XIII: P-58	IV: J-21	I: Belair	VI: J94-5	VIII: K-12	XIV: Palisa	II: Crowne	XII: Meyer	IX: K157	X: K-162	XI: Korean	VII: JS-23	Total
V: J3-2		//2						//1	//2						0:0//5
Iii: El Toro	//3		5:8		//4		//3	//7	//11	1:21			1:4		3:33//28
XIII: P-58	//1	8:59			1:2		2:2	1:6//16	1:4//17	3:9					6:82//34
IV: J-21										1:11					1:11//0
I: Belair		//2				1:4	3:8	//9	//3	4:19					3:31//14
VI: J94-5		2:10			1:1		1:15		1:12						4:38//0
VIII: K-12		//4	2:3		1:6//1				1:1//2	2:7					4:17//7
XIV: Palisa	//3	2:7//3	0:0		//3				//11	1:2					2:9//20
II: Crowne	//3	//12	1:2		1:3//3	1:13		//11		1:4//2			1:13		5:35//31
XII: Meyer	//2	1:6//1	1:1	1:6	2:3	2:20	1:2	1:23	1:11				4:17		9:89//3
IX: K157													1:1		1:1//0
X: K-162										1:9					1:9//0
XI: Kor Com		1:8								2:14	1:7				3:29//0
VII: JS-23				1:9						2:7		1:3			3:19//0
Totals	0:0//12	5:90//24	4:14//0	2:15//0	5:15//11	3:37//0	4:27//3	2:29//44	4:28//46	11:106//2	1:7//0	1:3//0	4:35//0	0:0//0	45:403//142

Figure 3. Number of successfull 1999 zoysiagrass crosses, seedlings emerged from crosses, and number of crosses made in 2000.