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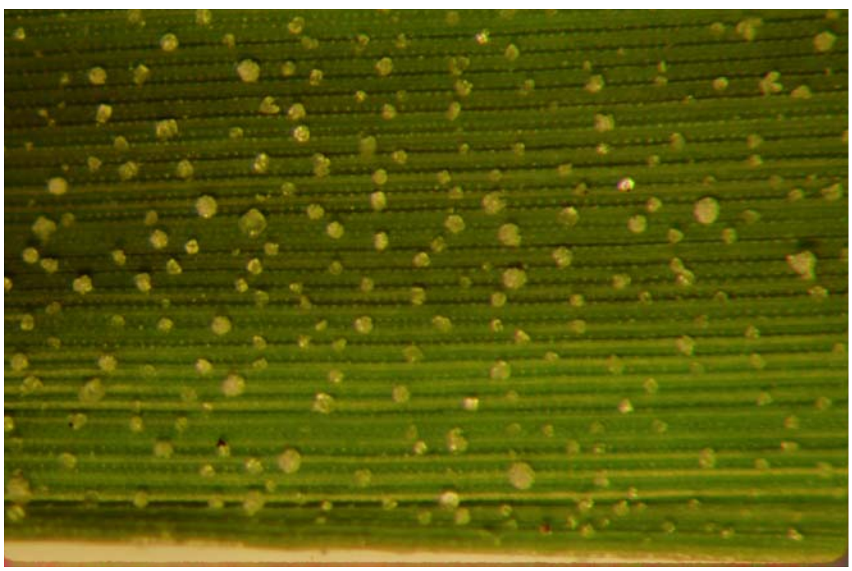

Salt glands secrete salt from leaves and can be a major means of excluding saline ions in salt-tolerant grasses. Secreted salt crystals can be seen on leaves of plants growing in salty soils (above). Researchers at Arizona State University, University of Arizona, and Texas A&M are testing whether salt gland density, as a genetic trait, can be used to screen for salt-tolerant germplasm.

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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 225 projects at a cost of $21 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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Zoysiagrasses, Salt Glands, and Salt Tolerance

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SUMMARY

Researchers at Arizona State University, University of Arizona, and Texas A&M are testing whether salt gland density, as a genetic trait, can be used to screen for salt-tolerant germplasm.

- Fifteen zoysiagrasses (Japanese lawngrass - *Zoysia japonica*) were tested for salinity tolerance using a solution culture-hydroponics growing system. The most salt-tolerant varieties were El Toro and Palisades, and the least tolerant were Sunrise, K162, JS-23, and K157.
- Salt gland densities were determined for all entries, growing under both salt-free (control) and saline conditions. Results of this study show that salt tolerance and salt gland activity is related to the actual density of salt glands on the leaves, or the number of glands per unit leaf area.
- Salt gland density on grasses grown in salt-free conditions predicted salinity tolerance, as well as plants grown under saline conditions. This is the first report of a morphological (visual) trait which can be used to predict salt tolerance of grasses. Salt gland density is an innate, genetically-controlled, heritable trait which does not require environmental stress conditions to express itself.
- Salt gland density is a much easier screening procedure which could expedite selection of salt-tolerant grasses, in that the breeder need only count salt gland density on leaves of plants growing under regular (non-salt) conditions.

Water shortages throughout the U.S. are resulting from rapid urbanization and drought. In the western U.S., limited water supplies have caused municipalities to implement xeriscape programs (16). With 50% or more of total urban water consumption being utilized for landscape irrigation in western states (1, 10), many municipalities are requiring use of recycled, or other saline secondary water sources for turf landscapes (3, 4, 5).

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Though there is increasing need for improved salt-tolerant turfgrass cultivars, breeding progress has been limited. Turf breeders typically need to select among hundreds or thousands of progeny to come up with an improved cultivar. Selection for salt tolerance among so many progeny is difficult, time-consuming, and expensive. Therefore, improved accurate and efficient salt tolerance screening tools are needed to expedite turf cultivar development. These tools may be morphological or physiological markers which can be used to predict salt tolerance.

Salt Glands - A Salt Tolerance Mechanism in Turf

Most plants, including grasses, exclude saline ions (sodium, chloride, etc.) from shoots and leaves to minimize their toxic effects (7, 18). Saline ion exclusion from shoots has been associated with salt tolerance among grasses in a number of studies and is a major physiological process associated with salt tolerance (8, 9, 11, 17).

Salt glands are found in a number of warm-season (C_4) grasses, including the turfgrasses bermudagrass, zoysiagrass, and buffalograss, and potential turfgrasses saltgrass (*Distichlis spicata* var. *stricta*), dropseeds

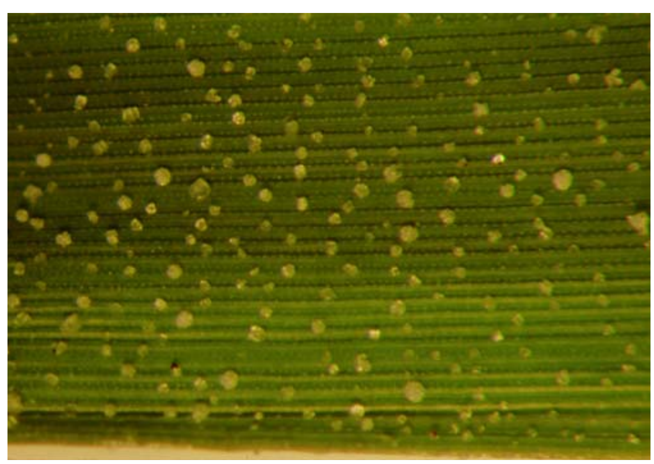

Figure 1. Salt crystals on a zoysiagrass (*Z. japonica*) leaf growing on saline soil.

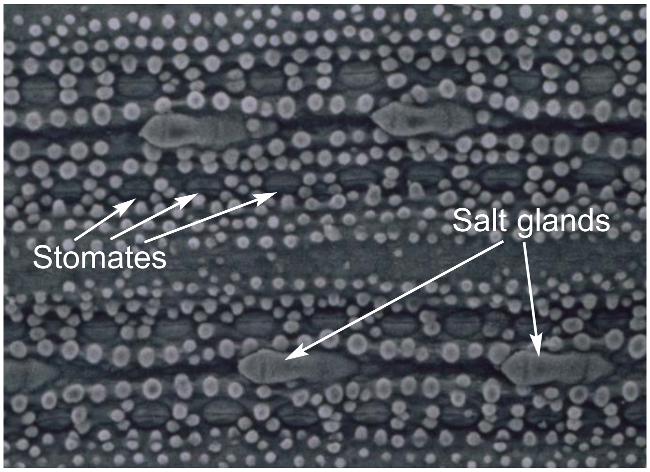

Figure 2. Electron micrograph (550X) of salt glands on the leaf surface of zoysiagrass. Glands are in rows, parallel to stomates.

(*Sporobolus spp*.), gramagrasses (*Bouteloua spp.*), and curly mesquite (*Hilaria belangeri*) (2, 6, 13). Salt glands, which are actually miniature ion pumps, secrete salt from leaves, and can be a major means of excluding saline ions in salt-tolerant grasses (12, 15). In fact, in salt-tolerant grasses having active glands, secreted salt crystals can be seen on leaves of plants growing in salty soils (Fig. 1).

Salt glands, which are modified leaf microhairs (trichomes), are microscopic two-celled structures which lie flat on the leaf surface, in rows parallel to stomates (Fig. 2). Unlike internal physiological plant markers, salt glands are externally visual and morphological. They can be easily observed on grass leaves. The goal of this research was to determine if salt gland density can be used to predict turfgrass salt tolerance, and if they can be used as an effective salt-tolerance selection tool by turfgrass breeders.

The Experiment - Salt Tolerance

Fifteen zoysiagrasses (Japanese lawngrass - *Zoysia japonica*) were tested for salinity tolerance using a solution culture-hydroponics growing system (Fig. 3). This system allows precise control of salinity levels and can accurately determine differences in salt tolerance among turfgrass varieties. It also allows monitoring of both root and shoot responses to salinity. To compare salt tolerance among entries, changes in shoot growth (clipping weight), root growth, and visual quality (percent green leaf area) were observed at six different salinity levels over a growth period of several months.

Throughout the experiment, grasses were clipped twice per week at one inch. As salinity increased, leaf clipping weight decreased linearly (Fig. 4). The relative shoot growth and visual quality (percent green leaf area) at high salinity were used to indicate the relative salinity tolerance of varieties (Table 1). The most salt tolerant varieties were El Toro and Palisades, and the least tolerant were Sunrise, K162, JS-23, and K157.

Salt Glands - A Potential Tool for Turfgrass Breeding

Salt gland densities were determined for all entries, growing under both salt-free (control) and saline conditions. Densities were determined using a light microscope, with 120 observations

Figure 3. Hydroponics system used in turfgrass salinity tolerance research. Both shoot and root responses to salinity can be monitored.

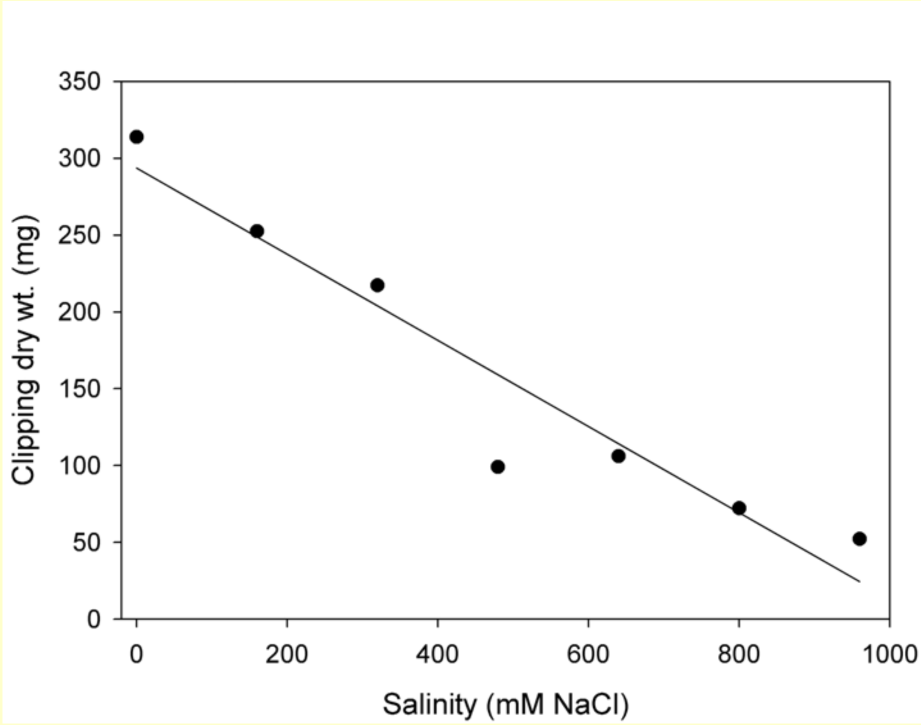

Figure 4. Decrease in shoot growth of 'Crowne' zoysiagrass with increasing salinity.

Grass	Relative Clipping Wt.	%Green Leaf Area
El Toro	83	52
Palisades	68	45
Meyer	56	20
J3-2	55	48
P58	55	47
Belair	54	54
Crowne	52	24
K12	23	9
J21	20	6
J94-5	19	3
Korean Common	12	2
K16	20	0
Sunrise	0	0
JS-23	0	0
K157	0	0

Table 1. Predicting salt tolerance of zoysiagrasses, based on the relative clipping weight and on visual quality (percent green leaf area) at high salinity. Grasses are listed from highest to lowest salt tolerance. Shoot growth and green leaf area variables were highly correlated (R^2 = 0.73) and are equally effective in predicting salinity tolerance.

taken on each zoysiagrass variety (each observation was a gland count).

It had been previously found that salt tolerance in zoysiagrasses, and in general among Chloridoid warm-season grasses (bermudagrass, buffalograss, zoysiagrass, gramagrasses, *Sporobolus*, and saltgrass) was related to the amount of salt which the salt glands were able to secrete (the amount pumped out of the leaves) (12, 14).

In addition, results of this study show that salt tolerance and salt gland activity is related to the actual density of salt glands on the leaves, or the number of glands per unit leaf area. Figures 5 and 6 show the relationships between leaf salt gland density and salt tolerance, indicated by visual quality (% green leaf area) and relative clipping yield at high salinity. There is a positive correlation with both salt tolerance parameters. In other words, as leaf salt gland density increases, so does salt tolerance.

If salt gland density is to be a valuable screening tool for turfgrass breeders, it must be genetically, not environmentally, controlled. In

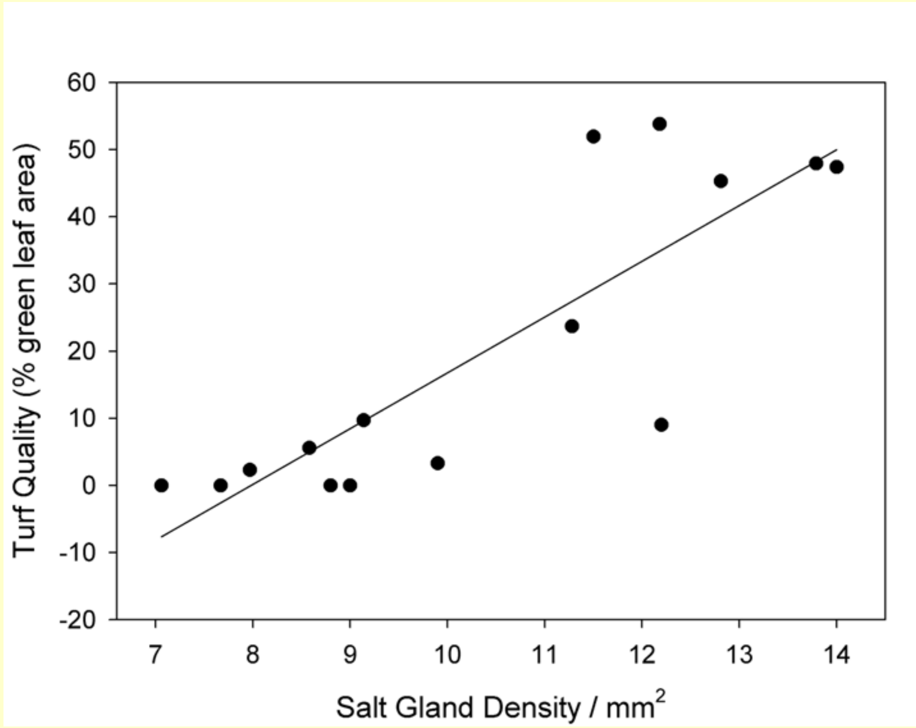

Figure 5. Turf visual quality (% green) vs. salt gland density (of salinized plants) of zoysiagrasses growing under high salinity.

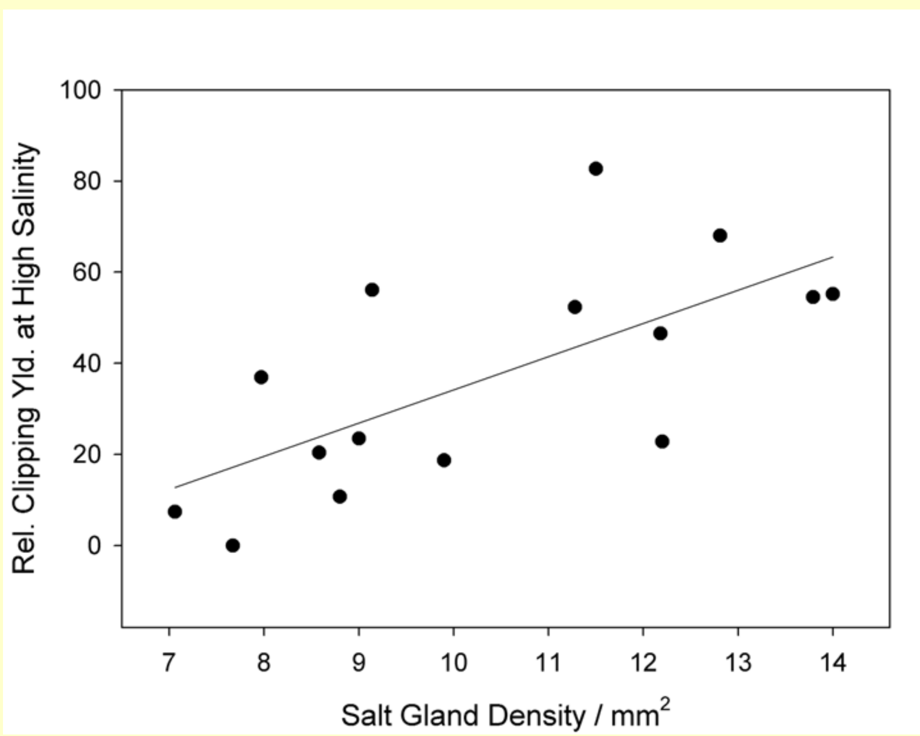

Figure 6. Relative clipping yield vs. salt gland density (of salinized plants) of zoysiagrasses growing under high salinity.

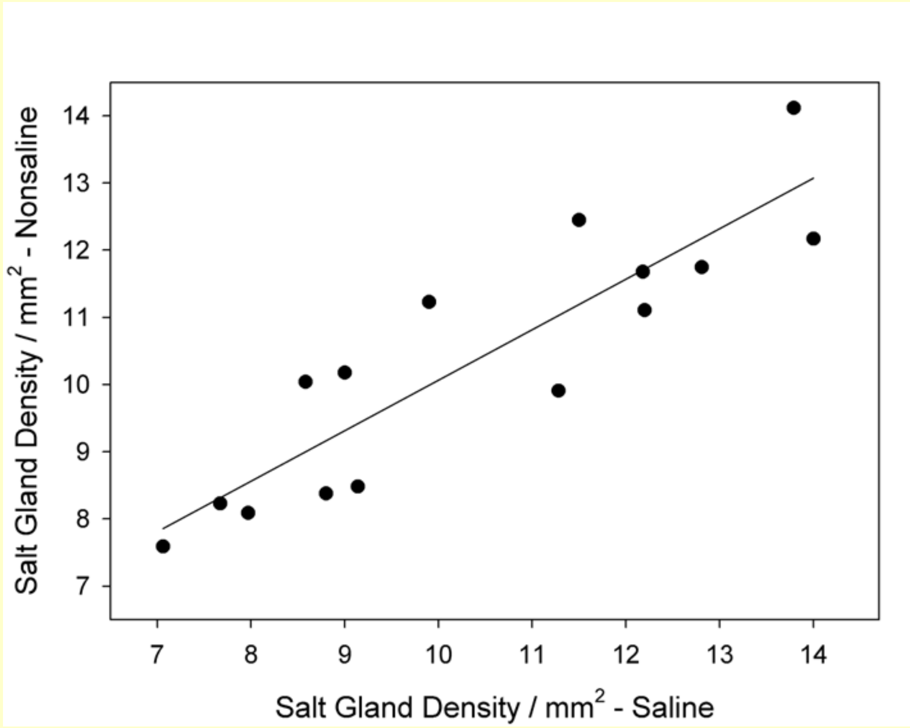

Figure 7. Salt gland densities of zoysiagrasses growing in saline, vs. nonsaline conditions.

other words, salt gland density must be highly heritable (passed from parent to offspring). To determine this, we measured salt gland density of varieties growing both in saline environment and in a salt-free environment.

It was found that salt glands are not induced by salt stress, i.e. within a variety, there was no difference in gland density between a plant grown in either saline conditions or salt-free conditions (Fig. 7). In other words, the grasses are "born" with a certain density, depending on their genetics. Averaged across all genotypes and within a genotype, there was less than a 1% difference between control (non-stressed) and salt stressed grasses, indicating a very high heritability for this trait.

Salt gland density on grasses grown in salt-free conditions predicted salinity tolerance as well as plants grown under saline conditions. This is the first report of a morphological (visual) trait which can be used to predict salt tolerance of grasses. Salt gland density is an innate, genetically-controlled, heritable trait, which does not require environmental stress conditions to express itself.

Accurately screening hundreds or thousands of breeding selections for salt tolerance is difficult and expensive. Salt gland density is a much easier screening procedure which could expedite selection of salt tolerant grasses in that the breeder need only measure salt gland density on leaves of plants growing under regular (non-salt) conditions.

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