Improved understanding and testing for salinity tolerance in cool-season turfgrasses

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Objectives:

1. *Explore which physiological measurements are most efficient for the evaluation of salt stress and selecting for salt tolerance.*
2. *Use a field study to explore salt tolerance in lines of perennial ryegrass, Kentucky bluegrass, and alkaligrass.*
3. *Evaluate alkaligrass for variation in salt tolerance, establishment, and turfgrass quality characteristics.*
4. *Test tissue expression of the three calreticulin genes in tolerant and susceptible germplasm (including cultivars) from the three species, sequence the calreticulin alleles, and test association* with salt tolerance.

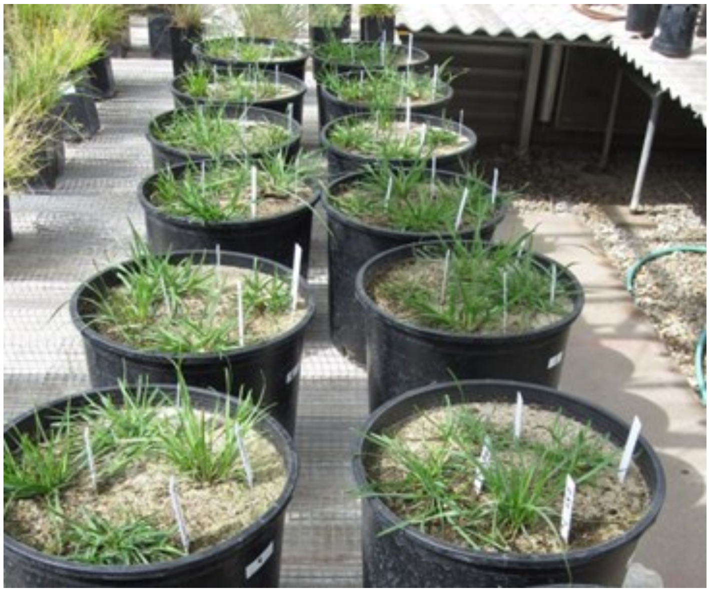
Water is a central issue for agriculture and urbanization in the North American West and likewise is the focus of work at Utah State University (USU) and the USDA-ARS Forage & Range Laboratory (FRRL). As a result, our efforts touch the lives of the most citizens possible. Golf course superintendents are definitely part of our stakeholder group as they are frequently asked or forced to use less irrigation water and/or from lower quality sources. Yet at the same time, these turfgrass areas are asked to do more and be used by more people due to the rapid population growth in the SW United States. Turfgrass with high quality, greater salt tolerance, and greater drought tolerance is essential.

Beginning in 2005, the FRRL and USU, both in Logan, Utah, started work in the area of salt and drought tolerance evaluation in Poa. Through a previous grant from the USGA, a large number of Kentucky bluegrass collections from the National Plant Germplasm System and other sources were evaluated for salt tolerance by submersing samples into solutions of increasing salt concentrations. Significant variation in salt tolerance was identified and those with potential were carried forward into further turf quality evaluations. Separate germplasm was evaluated under summer drought conditions common throughout the West. Results from these studies have been have been published in Robins et al., 2009 and Bushman et al., 2012. Additional genetic and physiological investigations on selected germplasm has been carried out to explore physiological and genetic mechanisms of drought and salt tolerance in Poa.

However it has historically been difficult to characterize and find consistent tolerance in germplasm due to interactions of climatic factors and variability in soil salinity. Therefore, this USGA funded project is focused on evaluating materials under increasingly representative conditions and to gain a better understanding of the mechanisms of salt tolerance.

In a two-pronged approach, our first effort was to determine how to best quantify tolerance to salinity. For

Figure 1. Pots in greenhouse to test for response to salt treatments.



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this work, larger containers of sand/peat growing media were used to mimic field rootzone conditions (Fig. 1). Lijun Wang, a M.S. student, evaluated four Kentucky bluegrass lines representing the range from salt tolerant to salt susceptible, under control and three levels of salinity. This work confirmed our salt tolerant lines (´768´ and ´603´) were the most tolerant as indicated by higher visual quality, higher levels of stomatal conductance, maintenance of higher plant water potential, lower electrolyte leakage, and lower leaf temperatures. The most consistent and indicative measures of tolerance were visual quality and electrolyte leakage. Soil salinity and soil moisture was also measured using buried sensors, but limitations of the sensors prevented measurements at high salt levels. At the end of the experiment, the plants were harvested for ion quantification in shoots and roots. Additionally, a separate but similar experiment was conducted and tissue collected for quantification of RNA–expression of genes thought to be involved in salt tolerance (currently being analyzed). Important outcomes thus far have included: (1) the identification of electrolyte leakage as the most effective measure of salt stress irrespective of immediate environmental conditions, and (2) that tolerant plants accumulate less sodium in leaves than susceptible plants.

Figure 3. Electrolyte leakage (EL) measurements on July 9, 2013 of bluegrass, ryegrass, and alkaligrass entries in salt treatment and control plots. A larger EL value means more plant stress.

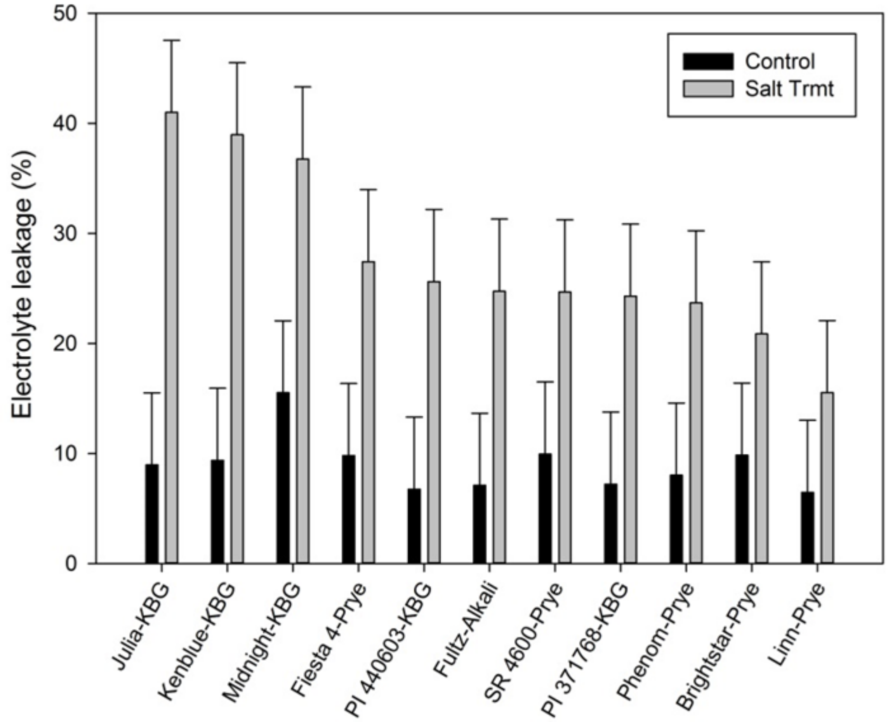
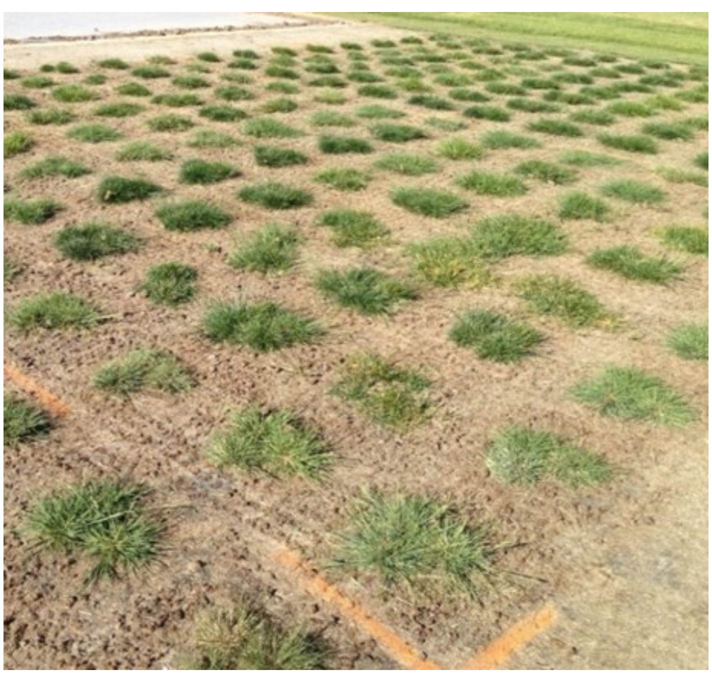
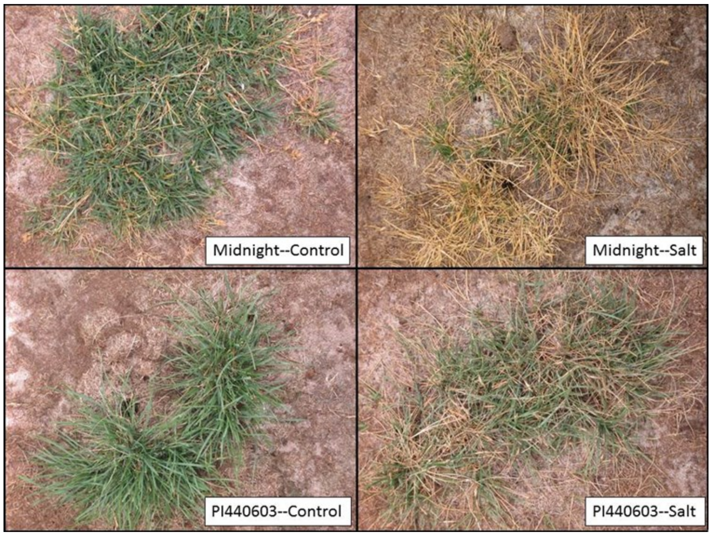


Figure 2. Field salt tolerance testing in June 2013.



Our second effort comprises a field study that was started in 2012 with the establishment of plants in a sand/peat rootzone; including five accessions and cultivars each of salt–tolerant Kentucky bluegrass, salt–sensitive Kentucky bluegrass, salt–tolerant perennial ryegrass, and salt sensitive perennial ryegrass, and two alkaligrass (*Puccinellia distans*) cultivars. In early June, 2013 salt treatments were applied using overhead watering to each plant. Control plots received water only. The salt treatments began at 3 dS/m and increased to 9 dS/m by the end of June. Concentrations were lowered to 3 dS/m in July because of high temperatures. Salinity treatments were increased in August to 6 dS/m and continued through early October. From June through early October, visual quality was recorded along with regular measurements of volumetric water content of the soil, soil salinity, soil temperature, percent green cover and electrolyte leakage. Shoot and root tissue was harvested three times from a subset of entries for confirmation of gene expression changes initiated in the greenhouse study described previously.

Figure 4. Visual comparison of Midnight and ´603´ under control and salt-treated conditions.



RNA analysis is currently underway. Figure 3 shows electrolyte leakage data from July 9th indicating a range of stress levels on the entries in the study. As expected, perennial ryegrasses showed generally less stress and Kentucky bluegrass entries more stress, however PI 440603 (´603´) and PI 371768 (´768´) showed high tolerance (Fig. 4) compared to other bluegrass entries including cultivars like Kenblue and Midnight. ´603´ and ´768´ were similar to the tolerance observed for most ryegrass entries. Alkaligrass entries showed moderate to relatively low stress although turfgrass quality was poor. We established a second replication of this experiment in August of 2013, which will be treated and analyzed in 2014.

Finally, we began exploring turfgrass quality traits of the salt tolerant species alkaligrass. The two entries included in the salt field trial described above were also transplanted, along with all *Puccinellia distans* and closely related species maintained in the National Plant Germplasm System into a field trial to evaluate turfgrass quality traits. While several entries showed reasonable turf quality in spring, none were acceptable in summer. Heat appears to be the primary limiting factor with this species. Evaluations will continue in 2014 .

Summary Points

- We observed consistent salt tolerance trends in key Kentucky bluegrass lines as we impose salt stress under increasingly more realistic growing conditions.
- The most effective and efficient measurement procedures for detecting fine changes in salt stress included electrolyte leakage along with visual traits.
- Three calreticulin sequences (alleles or paralogs) were identified and sequenced. Primers were designed to test expression of the genes using qPCR.
- Initial evaluation of Puccinellia lines show moderate potential in turfgrass quality in Spring, but high temperatures in summer appear most limiting.

Figure 5. The salt tolerant traits of Alkaligrass (*Puccinellia distans*) are being investigated. Heat appears to be the primary limiting factor

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