Improving our Understanding of Salinity Tolerance in Perennial Ryegrass Through Transcriptome Analysis

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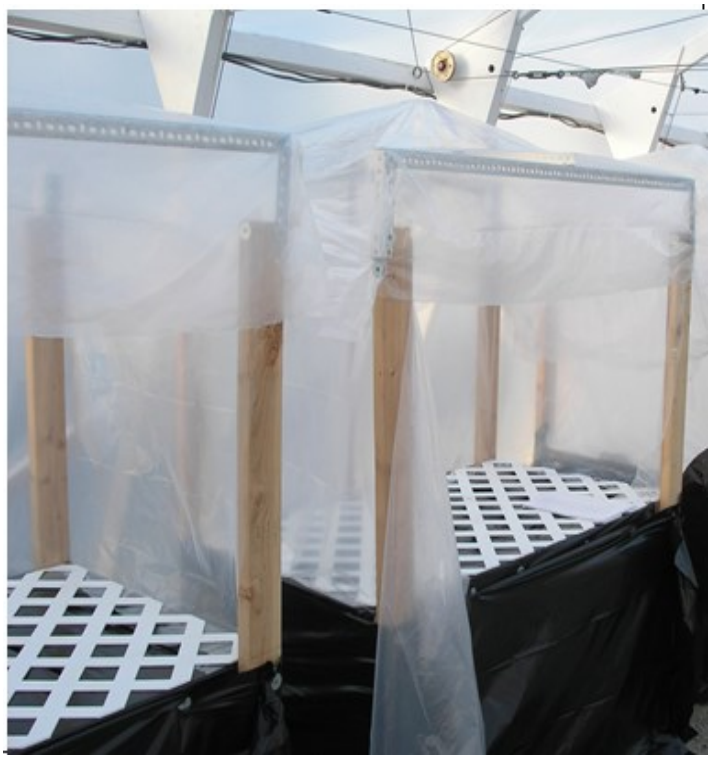
Objectives:

1. *By analyzing the transcriptomes of salinity tolerant and susceptible genotypes, we hope to identify specific transcripts (or genes) responsible for salinity stress tolerance in perennial ryegrass in order to identify the mechanism involved in resistance.*
2. *By comparing transcriptomes of E+ and E− perennial ryegrass plants, we hope to identify whether endophytes play a significant role in salinity tolerance in perennial ryegrass.*
3. *By sequencing the genome and transcriptomes of perennial ryegrass, we hope to identify sequence variation that can be used in the future for genomic selection in perennial ryegrass.*

The use of alternative water sources for irrigation on turfgrass areas would reduce the demand for high quality potable water and promote water conservation on landscapes and golf courses. Alternative sources can contain higher levels of total soluble salts than potable water and can result in salt stress injury and poor turf quality. The development of turfgrass cultivars that can tolerate salinity while maintaining safe, acceptable quality would result in a community and industry more accepting of voluntary utilization of alternative water sources. However, currently breeding for salinity tolerance has been slow and the mechanism is not fully understood. Building on a previously funded USGA project on salinity tolerance in cool−season turfgrass, the goals of this project are to 1) identify specific transcripts (or genes) responsible for salinity stress tolerance in perennial ryegrass in order to identify the mechanism involved in salinity tolerance; 2) identify whether endophytes play a significant role in salinity tolerance in perennial ryegrass; 3) create a sequenced genome of perennial ryegrass and 4) identify sequence variation that can be used in the future for genomic selection in perennial ryegrass.

Four endophyte + perennial ryegrass clones (2 tolerant and 2 susceptible) will be utilized in this study. We plan to 1) remove the endophyte from 6 replicates of each plant; 2) treat the endophyte free and endophyte infected plants with salinity stress; 3) analyze the perennial ryegrass genome for transciptome differences as influenced by salinity stress. An Illumina Genome Analyzer will be used for next generation sequencing to generate and identify transcripts. This project will identify sequence variation between tolerant and susceptible plants that can be used to develop SNP markers which will be useful for future genomic selection in breeding programs. An added benefit to the project is that in addition to the transcriptome sequence analysis we also plan to create

Figure 1. Recirculating salt chambers for salinity tolerance screening of perennial ryegrass.



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a sequence of the perennial ryegrass genome which will provide a resource to many turfgrass scientists throughout the country.

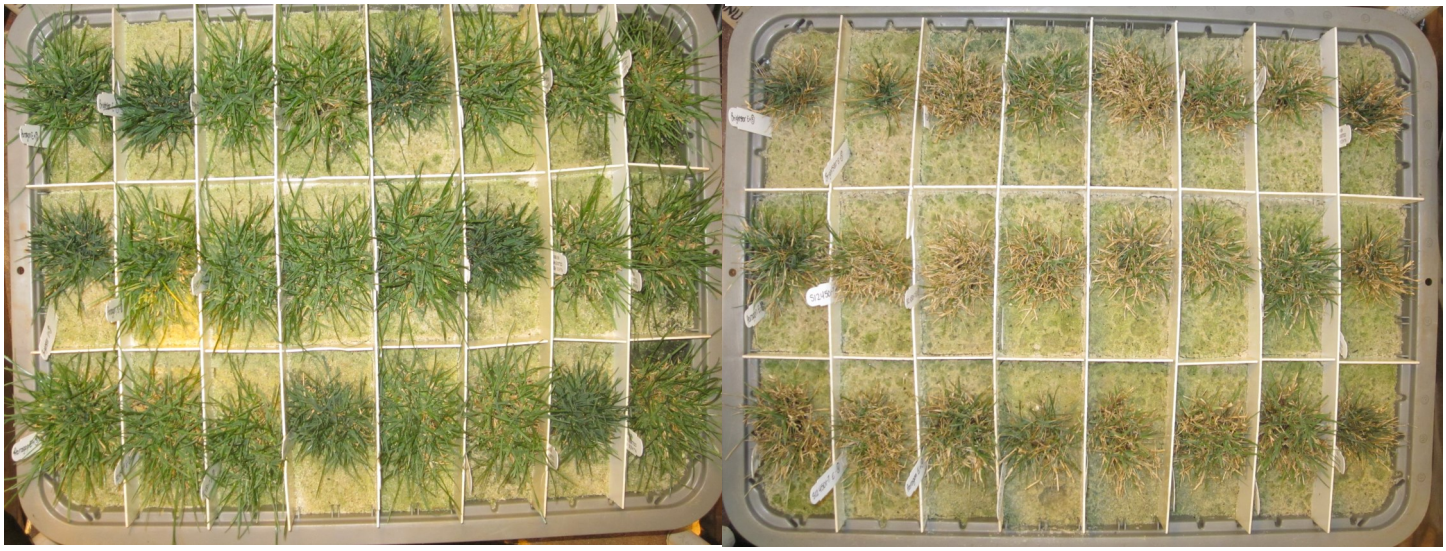
Accomplishments and Progress

Plant Material, Experimental Design and Salinity Treatments. Four perennial ryegrass plants (2 salinity tolerant – [4540–9 and 4501–7] and 2 susceptible – [Paragon GLR clone 4 and Brightstar SLT clone 5]) were screened for salinity tolerance in both the field (Koch and Bonos, 2011) and greenhouse screening (Koch and Bonos, 2010) techniques developed in a previously funded USGA project. All plants tested positive for endophyte infection using an ELISA endophyte test kit. The clones were vegetatively propagated into 288 single tiller replicates and planted into greenhouse flats in potting soil. One half, 144 plants of each clone were treated with weekly applications of propiconazole at 4oz/1000 sq ft for five weeks in order to remove the endophyte. Single tillers were checked weekly for endophyte presence using the ELISA test kit mentioned above. Endophytes are extremely persistent in perennial ryegrass and it was not until the fifth week that tillers were observed to be free of endophyte. At this time phytotoxic effects of the fungicide were also observed on some of the leaf tissue. For each clone, six single tiller replicates without endophyte were obtained. These single tillers were vegetatively repropagated into greenhouse flats along with single tillers of endophyte infected clones so that all clonal replicates were at the same growth stage. They were grown for eight weeks in the greenhouse. Plants were then removed from individual cells and washed free of Pro–mix growing medium. Shoots were cut to 3.8 cm while roots were cut directly below the crown. Three replicates of each clone were randomly planted into a cell of a plastic tray containing Sureplay topdressing sand. Irrigation applications were applied using the overhead irrigation spray chamber system designed at Rutgers University (Koch and Bonos, 2010). Salt solution treatments, made with Instant Ocean, were added to the nutrient solution in the holding tanks at the rate of 2 dS m–1 each irrigation day. The final electrical conductivities (EC) of the two chambers were 1 dS m–1 (This treatment included half–strength Hoagland solution) and 15 dS m–1. Plants were irrigated every other day for 9 weeks after final EC was reached in the salt treatment. Individual plants were rated weekly to quantify leaf senescence. Plants were cut weekly to a height of 3.8 cm and clippings were collected every two weeks. Clippings were dried in an oven at 65°C for 48 hours. Digital images were also taken weekly when stress started to become present to quantify leaf senescence. Roots and shoots were dried in an oven at 65°C for 48 hours after 9 weeks of salt treatments.

Run 1 was completed in the late spring/early summer of 2013. Significant differences in visual percent green between clones were observed starting at week 5 and remained through the end of the study. Endophyte effects were variable. The presence of the endophyte did not influence salinity tolerance in one susceptible and one tolerant clone. For the other two clones where significant contrasts between E+ and E– were significant, the E– clones were significantly more salt tolerant than E+ clones. Clipping yields and digital images are currently being analyzed.

All individuals were retested for endophyte status prior to the initiation of Run 2. All E– were still E–. Run 2 is currently being conducted.

Control (Left) compared to salt treated (Right) perennial ryegrass clones after 9 weeks of salt treatments.



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Next Generation Sequencing and Transcriptome Analysis

The salinity tolerant perennial ryegrass clone 4540–9 (with endophyte) was chosen for genome sequencing. A genomic library was generated and sequenced on an MiSeq benchtop sequencer (Illumina Inc., San Diego, CA). The quality of the genome sequencing runs is currently being analyzed.

For the 16 treatments (2 cultivar, 2 salt tolerances, E+ or E–, and salt application or no salt application), approximately 50 mg of leaf tissue was taken per plant on 6 collection dates: 25 Apr, 29 Apr, 3 May, 9 May, 16 May and 23 May. Leaf tissue was cut and placed in a 1.7 ml tube which was put directly into liquid nitrogen and later stored at –80°C. Scissors and forceps were cleaned with ethanol between each treatment collection. RNA was extracted from 4540–9 and Paragon GLR leaf tissue with and without salt treatment and with and without endophyte from the 3 May collection date. The extraction was done using an RNeasy Plant Mini Kit by Qiagen Inc. (Valencia, CA) according to the manufacturer´s instructions with on–column DNase digestion. The library was made immediately following the RNA extraction using a TruSeq RNA Sample Preparation Kit v2 by Illumina Inc. (SanDiego, CA) according to the manufacturer´s instructions. Samples were run on the MiSeq benchtop sequencer and are currently being analyzed.

Summary Points

- This project will increase our understanding of the genetic mechanisms of salt tolerance in cool–season grasses.
- After Run 1 of the study, endophytes did not seem to influence salinity tolerance.
- Sequence variation will be identified in relation to salinity tolerance. This information will help improve our understanding of salinity tolerance in perennial ryegrass.
- Sequences will also be used to generate markers for mapping and/or marker assisted selection.

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