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

Stacy Bonos, Debashish Bhattachary, Udi ZelZion, Joshua Honig, Jennifer Vaicuinas, and Eric Koch
Rutgers University

Turfgrass and Environmental Research Online
Volume 12, Number 1 | January 2013.

Objectives:

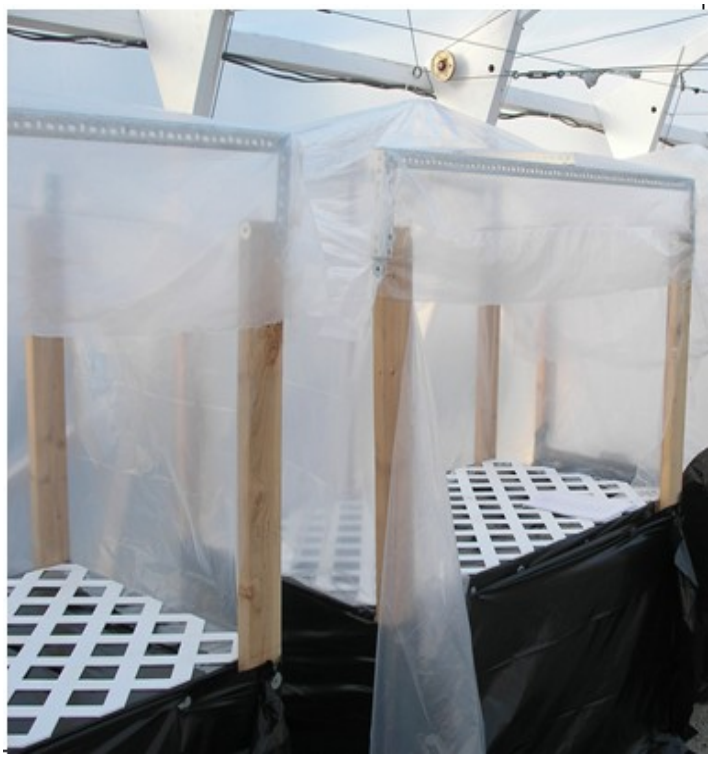
1. *By analyzing the transcriptomes of salinity tolerant and susceptible genotypes, we hope to identify specific transcripts (or genes) responsible for salinity stress tolerance in perennial ryegrass in order to identify the mechanism involved in resistance.*
2. *By comparing transcriptomes of E+ and 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, current breeding for salinity tolerance has been slow and the mechanism is not fully understood. The objectives of this project build upon a previously funded USGA project on salinity tolerance in cool−season turfgrass.

Plant Material, Experimental Design and Salinity Treatments

Four endophyte plus (E+) 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; and 3) analyze the perennial ryegrass genome for transcriptome 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 a sequence of the perennial ryegrass genome which will provide a resource to many turfgrass scientists throughout the country.

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



TERO Vol. 12(1):9−10 | January 2013.
USGA ID#: 2012−01−435
TGIF Number: 217433

Four perennial ryegrass clones (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 fungicide at a rate of 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 re–propagated into greenhouse flats along with single tillers of endophyte infected clones so that all clonal replicates were at the same growth stage. Endophyte status will be rechecked periodically throughout the study as the literature states that the endophyte can grow back.

Since the role of endophytes in salinity tolerance in perennial ryegrass is unknown, three replicates of each plant (with and without endophyte) will be exposed to salinity stress. Salinity stress will be imposed by applying saline water (EC = 15 dS/m) using an overhead irrigated greenhouse screening technique (Figure 1) (Koch and Bonos, 2010). Plants will be exposed to salinity stress for 10 weeks. Percent green ratings (taken weekly), percent green calculated with digital image analysis, and shoot and root weights will be collected at the end of the study, in order to determine the effect of endophyte on growth and quality of perennial ryegrass under salinity stress.

Next Generation Sequencing and Transcriptome Analysis

The salinity tolerant perennial ryegrass clone 4540–9 (with endophyte) was chosen for genome sequencing. A genomic library is currently being generated for this perennial ryegrass clone. In order to get high 8x to 10x genome coverage we will plan to run 4 lanes of an Illumina sequencing platform (GAII) flow cell with paired end reads 150 x 150 bp. This will be used to create the reference genome for mapping and validating cDNA reads. Quality trimming will be run on the ends to estimate the quality of the runs. Smaller reads with higher quality are preferred rather than longer read lengths. The sequences will be assembled into contigs using several assembly programs, CLC genomics, ABySS and VELVET and compared. Scaffolding pipelines will be run on the best assembly to fill the ends between contigs.

In an effort to ensure ample reads and quality transcriptome data, RNA will initially be collected from one tolerant clone and one susceptible clone under control (E+ and E–) and saline–stress (E+ and E–) for a total of 8 samples. Each of these tissues will be run separately in one lane of an Illumina flow cell. mRNA will be isolated with a kit compatible with the Illumina Genome Analyzer IIx such as ScriptSeqTM mRNA–Seq library preparation kit following the manufactures instructions. The samples will be prepared for sequencing using Illumina´s protocol for transcriptome analysis. Each transcriptome will be assembled separately. Blast2GO will be run on the contigs (i.e., putative genes) derived from each transcriptome. The coverage of each contig will be determined to identify copy number. This information will be used to determine which genes are expressed in each transcriptome and their expression level. The EST contigs will then be mapped to the reference genome and differences between transcriptome libraries will be compared (e.g., using CLC Workbench tools or Bowtie [http://bowtie.cbcb.umd.edu]).

Summary Points

- This project will increase our understanding of the genetic mechanisms of salt tolerance in cool–season grasses.
- This project will determine if endophytes influence salinity tolerance.
- This project will create a sequenced genome of perennial ryegrass which will provide a resource to the turfgrass industry.
- This project will identify sequence variation that can be used for potential SNP markers for genomic selection to develop cultivars with improved salinity tolerance. If golf course superintendents are expected to utilize non–potable water for irrigation of golf courses, they need cultivars that can maintain adequate turf quality while being irrigated with water high in total soluble salts. The efficient development of cultivars with improved salinity tolerance will directly benefit golf course superintendents, sod farmers, turfgrass seed companies and turfgrass scientists. The environment and public at large will also benefit from a concerted effort to conserve our natural water resources.

TERO Vol. 12(1):9–10 | January 2013.
USGA ID#: 2012–01–435
TGIF Number: 217433