Materials and Methods

To select the genotypes for use in this study a preliminary dry down study will be conducted to find the genotypes which are most susceptible or most resistant to drought. The 8 genotypes that will be used are: Celebration, Quicksand, Vamont, MS Choice, and 1x9, 2x14, 4x20, and 4x20. Celebration has consistently been found to be a drought resistant standard for C. dactylon (Baldwin 2006, Liu et al., 2013, Steinkie et al. 2011). Quicksand, Vamont and MS choice are commercial C. dactylon cultivars. In previous research 1x9 and 2x14 are genotypes found to be drought susceptible (Liu et al., 2013, Wu et al. 2013). The material will be obtained from the field or greenhouse and then doped for 24 h in an succisside dip, 10 mL 7.5% a.i. bitrin acid. The grass will then be vegetatively propagated into flats. The growth conditions for the preliminary study will be identical to the main study as is described below. Each genotype will have 4 replications and measurements of normalized difference vegetation index (NDVI), visual ratings, evapotranspiration (ET), relative water content (RWC), and electrolyte leakage (EL) will be taken twice per week using the methods described below. Once the genotypes have been selected for the main study two cuttings 4 nodes long will be transplanted into 10 x 16 cm pots. PVC pots 15 cm deep filled with a 1:1 (v:v) screened topsoil and screened sand mix, and then 15 cm pots will be moved to a growth chamber. Each genotype will have 4 treatment levels: no drought, moderate drought stress, severe drought stress and 24h after re-watering. Each treatment level will have 4 replications and the time periods relating to moderate and severe drought stress will be determined based on the preliminary study with a threshold of 60% RWC for moderate drought stress and 30% RWC for severe drought stress (Shi et al., 2012, Zhou et al., 2014). The pots will be arranged in a completely randomized design inside of a growth chamber at 30/25 °C day/night, 14 photoperiod, a photo-synthetically active light density of 480 μmol m⁻² s⁻¹, and 75% relative humidity (Zhou et al. 2014). The plants will be fertilized biweekly using (20-20-20) at a rate of 10 g N m⁻², and watered to field capacity 3 times per week (Zhou et al. 2014) for a total of 6 days. After a 60 days of growth, plant irrigation will be ceased. NDVI will be measured using a FieldScout CM 1000 NDVI Chlorophyll Meter. The pots will be weighed to measure ET. Visual ratings will be taken based on turfgrass visual quality guidelines from NTEP definitions. For RWC measurement 10-15 second and third fully expanded leaves from the apical meristem will be collected from each sample, and then weighed to determine fresh weight (FW), the samples will put in to petri dishes filled with water for 4 h at 4°C, the samples will be blotted dry with paper towels, and then weighed again to determine turgid weight (TW) (Zhou et al. 2014). The samples will be dried to an oven at 90°C for 1 h to determine dry weight (DW). RWC of the leaves will be calculated as: RWC (%)=(FW-DW)/(TW-DW) x 100 (Barr and Wetherby 1982). For electrolyte leakage (EL) 15 second and third fully expanded leaves from the apical meristem will be collected from each sample. Each sample will be rinsed three times in distilled deionized (DD) reagent water in a test tubes with a cap. The samples will be rinsed then added to a tube containing 20 mL of DD water and shaken for 24 h at 20 rpm. Additionally, 2 check samples will be included. The electrical conductivity (EC) will be measured to determine initial conductivity (CI) and then the samples will be autoclaved at 140°C for 20 min and then shaken for an additional 24 h at 20 rpm and then the EC will be measured again to determine maximum conductivity (CMax). Relative electrolyte leakage will be calculated by (C/CMax) x 100 (Su et al. 2013, Zhou et al. 2014). For each treatment level 0.025 g of leaves will be collected and the 4 replications will be pooled together to create a 0.1 g sample. The sample will immediately be frozen in liquid nitrogen and stored at -80°C. After all samples have been collected they will be extracted as per instructions on the extraction kit and the samples will be sent off for sequencing with 4 technical replicates per sample. Once the results are returned from sequencing the unigenes will be matched to sequences in the SwissProt and NCBI databases. Once the unigenes have been assigned names a differential expression analysis will be performed. Genes that are up-regulated in the resistant genotype but not the susceptible genotype will be considered important for drought resistance.

Introduction

Turfgrass is not often thought of as a major agricultural crop, but conservative estimates put the acreage of irrigated turfgrasses at 3x the acreage of irrigated corn (Milesi et al. 2005). According to the EPA the average American family uses 320 gallons of water daily, and between 15-30% of this water is used to irrigate turfgrass lawns (WaterSense 2013). The use of cultivars with superior drought resistance will reduce the amount of water needed for irrigation of turfgrass. Bermudagrass (Cynodon spp.) is a low growing grass from the Poaceae family that is widely used as a forage or turf grass in tropical or warmer temperate regions (Juska and Hanson 1964, Tallaffe & 1995). The main species of bermudagrass that are commonly used in turf are Cynodon dactylon (common bermudagrass), Cynodon transvaalensis (African bermudagrass), and Cynodon dactylon x transvaalensis (hybrid bermudagrass) (Barron 1977, Hanna 1998). Bermudagrass is widely considered to be drought and heat resistant, but the exact methods of drought resistance is not well understood. One method for elucidating the genes and biochemical pathways with drought resistance is by using transcriptomics. A transcriptome is the set of transcripts in a cell and their quantity at a specific condition (Wang et al., 2009). By comparing the transcriptome of a plant between a non drought stressed condition and a drought stressed condition and finding transcripts which are expressed at higher or lower rate it is possible to identify transcripts related to drought stress. Then by comparing the expression level of transcripts in a genotype that has been determined to be resistant to a genotype that has been determined to be susceptible it is possible to identify the biochemistry of drought resistance in the plant. There have been different procedures to evaluate the transcriptome but the most current procedure is RNAseq. RNAseq is a procedure that takes advantage of next generation high throughput sequencing to create transcriptomes more cost effectively and quicker than previous methods (Marguerat and Bähler 2010, Hennett and Wheat 2012). It involves extracting RNA from the sample, converting the RNA to cDNA, sequencing the cDNA, base calling, and then assembling the base called sequences into unigene transcripts more cost effectively and quicker than previous methods (Marguerat and Bähler 2010, Hennett and Wheat 2012). Unigenes will be matched to sequences in the SwissProt and NCBI databases. Once the unigenes have been assigned names a differential expression analysis will be performed. Genes that are up-regulated in the resistant genotype but not the susceptible genotype will be considered important for drought resistance.

Objectives

The objectives of this study are:
1) Identify unigenes involved in response to drought stress.
2) Identify transcripts differentially expressed between a drought resistant variety and a drought susceptible variety of C. dactylon in response to drought stress.
3) Compare to other transcriptomic drought stress studies involving C. dactylon x transvaalensis.

Experimental Design

| Table 2. The design for the experiment. ND is no drought, MD is moderate drought, SD is severe drought, and RW is re-watered. |

<table>
<thead>
<tr>
<th>Time Periods</th>
<th>ND</th>
<th>MD</th>
<th>SD</th>
<th>RW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological Replicates</td>
<td>Leaves</td>
<td>Roots</td>
<td>Leaves</td>
<td>Roots</td>
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<tr>
<td>Peeped Samples</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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<tr>
<td>Leaf Cut</td>
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<td>2</td>
<td>3</td>
<td>4</td>
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<tr>
<td>Leaf Tissue</td>
<td>1</td>
<td>2</td>
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<td>4</td>
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References