Characterization and Validation of Molecular Markers Linked to Heat and Drought Tolerance for Marker Assisted Selection of Stress-tolerant Creeping Bentgrass

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Objectives:

1. *Validate SSR markers linked to six known heat/drought tolerance QTLs and gene-based markers that were developed in previous USGA-funded projects in a bentgrass breeding population with a wide range of variation in drought and heat tolerance in two different environments or locations;*
2. *Determine the stability of known QTLs over a range of test cross parents and environments;*
3. *Assess physiological traits (phenotypes) linked to these molecular markers in drought and heat tolerance;*
4. *Identify and characterize ideal phenotypes of newly developed drought and heat tolerant lines using verified markers to facilitate marker assisted selection in creeping bentgrass breeding programs*

Figure 1. Image from University of Georgia taken on August 6, 2014, showing variations in summer turf performance among the 144 new lines/cultivars.

Drought and heat are two major abiotic stresses which cause declines in the health of many valuable cool-season turfgrass species, including creeping bentgrass (Agrostis stolonifera). Summer declines in turf quality brought about by these two stresses are caused by many complex underlying factors including reduction in photosynthetic capabilities, damage to cellular membranes, or increased reactive oxygen species production. This ultimately results in turf areas experiencing premature senescence and eventual plant death. The development cool-season turfgrass species with improved heat and drought tolerance is indispensible for maintaining high quality turf areas during summer months with elevated temperatures or when irrigation is limited. Our previous projects have identified and developed molecular markers linked to drought or heat tolerance in bentgrass species using both quantitative trait loci (QTLs) and candidate-gene based markers. Candidate gene markers were developed using previous studies which identified important genes for abiotic stress tolerance and confirmed them using real-time PCR (qPCR). These genes include those coding for photosynthetic proteins such as chlorophyll a/b binding protein, protective chaperone proteins such as heat shock protein-70, and antioxidant genes such as glutathione-s-transferase. The current project will further confirm the relationship between these molecular markers and important stress tolerance related traits.

In the current project our aim is to validate previously developed SSR markers associated with drought or heat related QTLs and gene-based markers associated with important tolerance related genes in a genetically diverse population. Screening this population for important

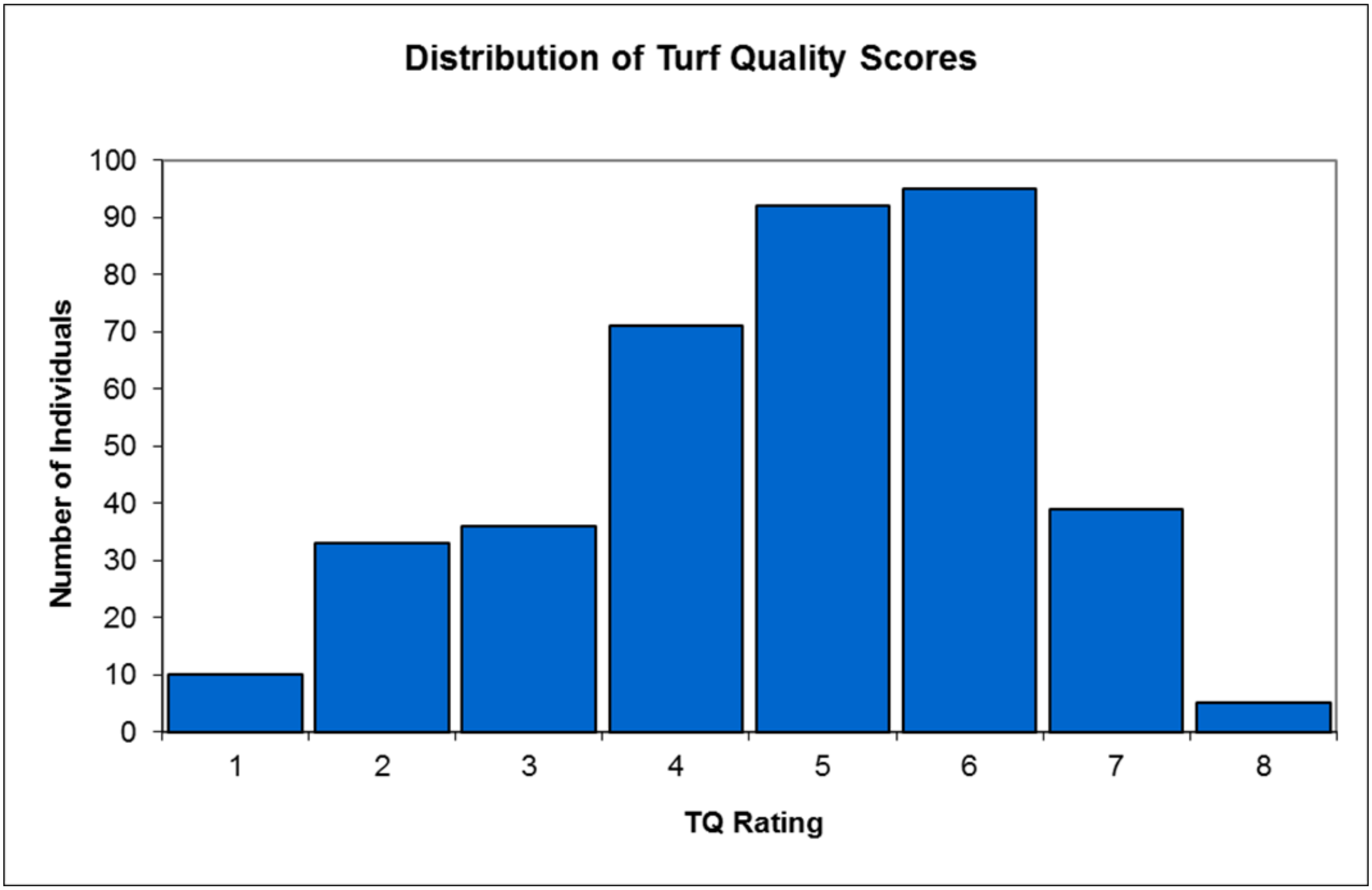

Figure 2. The distribution of turf quality ratings at Rutgers University during the summer of 2014, showing genetic variations in summer turf performance.

physiological characteristics related to abiotic stress tolerance in two locations will allow for the confirmation of markers related to important drought or heat tolerance mechanisms. Once the utility of these markers is confirmed they can be used for marker assisted selection (MAS) for the development of bentgrass lines with improved abiotic stress tolerance.

Two populations of 144 creeping bentgrass germplasm, including several commercial cultivars (Penncross, Crenshaw, Declaration, Penn A-4, Luminary) and new experimental lines from both UGA and Rutgers were planted in two locations, at the University of Georgia in Griffin, GA in fall 2012, and at Rutgers University in New Brunswick, NJ in fall 2013. These populations represent a diverse collection of germplasm from both Rutgers and University of Georgia breeding programs to be used for the confirmation of previously developed markers. Turf plots were rated for heat tolerance during the summer of 2013 at UGA and summer of 2014 at both locations. Genotypic variations in overall visual turf quality rating, membrane stability as measured by electrolyte leakage, chlorophyll content, and NDVI were evaluated at both locations. Additionally images of turf plots were taken using a camera and a light box for digital image analysis to determine turf color and density characteristics.

A large range of genotypic variations in summer performance were observed at both UGA and Rutgers plots (Figure 1). Turf quality rating ranged from 7 to 1 at UGA and 8 to 1 at Rutgers University, with average rating of 5.9 and 5.4, respectively (Figure 2). The NDVI measurements ranged from 0.16 to 0.80. Among the commercial cultivars and new lines from Rutgers or UGA, 40 new lines (27%) performed better than the best-performing commercial cultivar at the UGA site during the summer of 2014, and 46 lines (32%) performed better than the best-performing commercial cultivar at the Rutgers site based on turf quality ratings. These results are consistent to what was found last year at the UGA site.

Genetic variations in drought tolerance are currently being evaluated in turf plots covered with automated rainout shelters at both locations. The above-mentioned parameters will be examined in the 144 lines/commercial cultivars after withholding irrigation (initiated on October 5, 2014) to determine genotypic variations in drought tolerance. In addition, leaf relative water content is being measured to assess hydration status of the plant.

Additionally tissue samples from 144 new lines/ cultivars have been collected and DNA has been extracted from them. These samples are being used to screen 54 SSR markers in the QTL regions associated with drought or heat tolerance. In addition, 13 previously developed candidate gene markers are also examined in the new lines/cultivars. Initial screening results indicate that genetic polymorphism exists for molecular markers from both the QTL regions and candidate genes linked to heat tolerance within the creeping bentgrass population (Figure 3). Ongoing work includes the continued screening of the populations at both locations for drought tolerance, as well as continuing to score previously developed SSR and gene-based markers to characterize the population and confirm the utility of these markers.

Summary

- A total of 144 new lines/cultivars were evaluated for summer heat tolerance in both Georgia and New Jersey, demonstrating large genetic variations, with turf quality ranging from 1.0 to 8.0 during summer 2014. Nearly 30% of new lines outperformed the best-performing cultivar examined in this trial during summer 2014.
- DNA analysis identified genetic polymorphism of some candidate gene markers and SSR markers associated with QTL regions linked to heat tolerance traits within the 144 new lines/cultivars, confirming and validating the usefulness and stability of those molecular markers in two different environments or locations and over a large population of germplasm with different phenotypes.
- Drought tolerance is currently being evaluated using automated rain-out shelters at both locations. DNA has been obtained from those samples and will be screened for genetic polymorphism in drought tolerance.

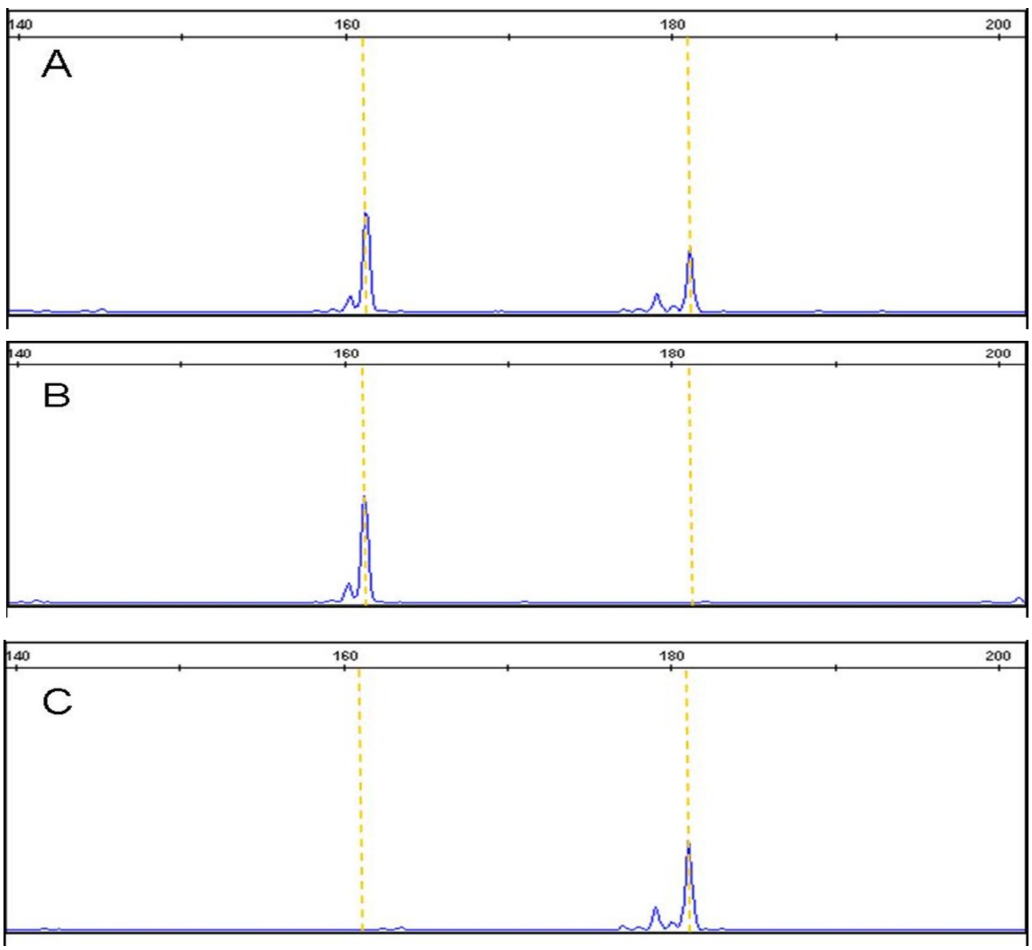

Figure 3: Representative results from SSR marker screening using a capillary electrophoresis system, showing polymorphisms among three genotypes (A, B, C) for a heat-tolerance marker. Peaks are SSR marker products and orange dotted lines represent potential product sizes for the marker.