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 phenotypes of newly developed drought and heat tolerant lines using validated markers to facilitate marker assisted selection in creeping bentgrass breeding programs.

Drought and heat are two major abiotic stresses which cause damage to cool-season turf areas. Creeping bentgrass (*Agrostis stolonifera*) is a high value turfgrass which is particularly susceptible to the stresses of drought and heat. Damages caused by these stresses include reductions in photosynthesis, the production of reactive oxygen species, damage to membranes and degradation of proteins. Ultimately these cellular damages reduce plant growth and canopy density, induce premature senescence and eventual result in 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 include previously identified genes which play important roles in stress tolerance such as anti-oxidant genes, chaperones involved in protein stabilization, and photosynthesis genes. 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 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. Both locations are equipped with rainout shelters which were used in the spring or fall to exclude rainfall and induce drought stress. Turf plots have been assessed for both heat tolerance during the summer months, and drought tolerance at both the University of Georgia and Rutgers; giving multiple years of phenotypic data at both locations. Parameters measured to estimate plant health during heat or drought include visual ratings, NDVI (normalized difference vegetation index), chlorophyll content, membrane stability, use of light boxes to take photos for digital image analysis, in additional to leaf water content during drought periods as well as canopy temperature depression during summer heat stress.

A large range of genotypic variations in for both drought and heat tolerance were found at both locations. In 2015 average turf quality ratings ranged from 1 – 6.3 during drought, and 1.3 – 6.2 during summer heat stress at UGA, while at Rutgers ratings ranged from 2.2 – 6.7 during summer heat stress, and 1 – 4.7 during the fall drought period (Fig. 1) Similar distributions can be see for membrane stability demonstrating a large range of stress tolerance in population (Fig. 2). Many experimental lines performed better than standard commercial cultivars which ranged from 17% of lines ranking better than the best commercial cultivars for the Georgia drought trial to 42% of lines ranking better than the best commercial cultivar during the Rutgers heat trial. Several lines performed extremely well across both locations for both heat and drought stress such as S11-8675-2 and S11-8712-10 which were always ranked in the top 10%.

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. Over 22 markers have been fully screened and tested for associations between markers and physiological traits linked to drought or heat tolerance in different years and locations.

Summary Points:

- A total of 144 new lines/cultivars were evaluated for summer heat tolerance and drought tolerance in both Georgia and New Jersey giving multiple years of field data at both locations to use for molecular marker analysis
- Genetic variations for both heat and drought tolerance were found at both locations, with several elite lines outperforming standard commercial cultivars at both locations under both heat and drought conditions.
- Screening of DNA markers continues with a large number of markers already being screened and shown to be polymorphic within the population, demonstrating that genetic difference for the previously selection markers exist.
- Top performing lines are being selected for more in depth characterization of mechanisms responsible improved drought or heat tolerance.

Figure 1:
Distributions of turf quality ratings for the University of Georgia drought stress sampling in June of 2015 (A), Rutgers drought stress sampling in September of 2015 (B), University of Georgia heat stress sampling in August of 2015 (C) Rutgers heat stress sampling in August of 2015 (D).

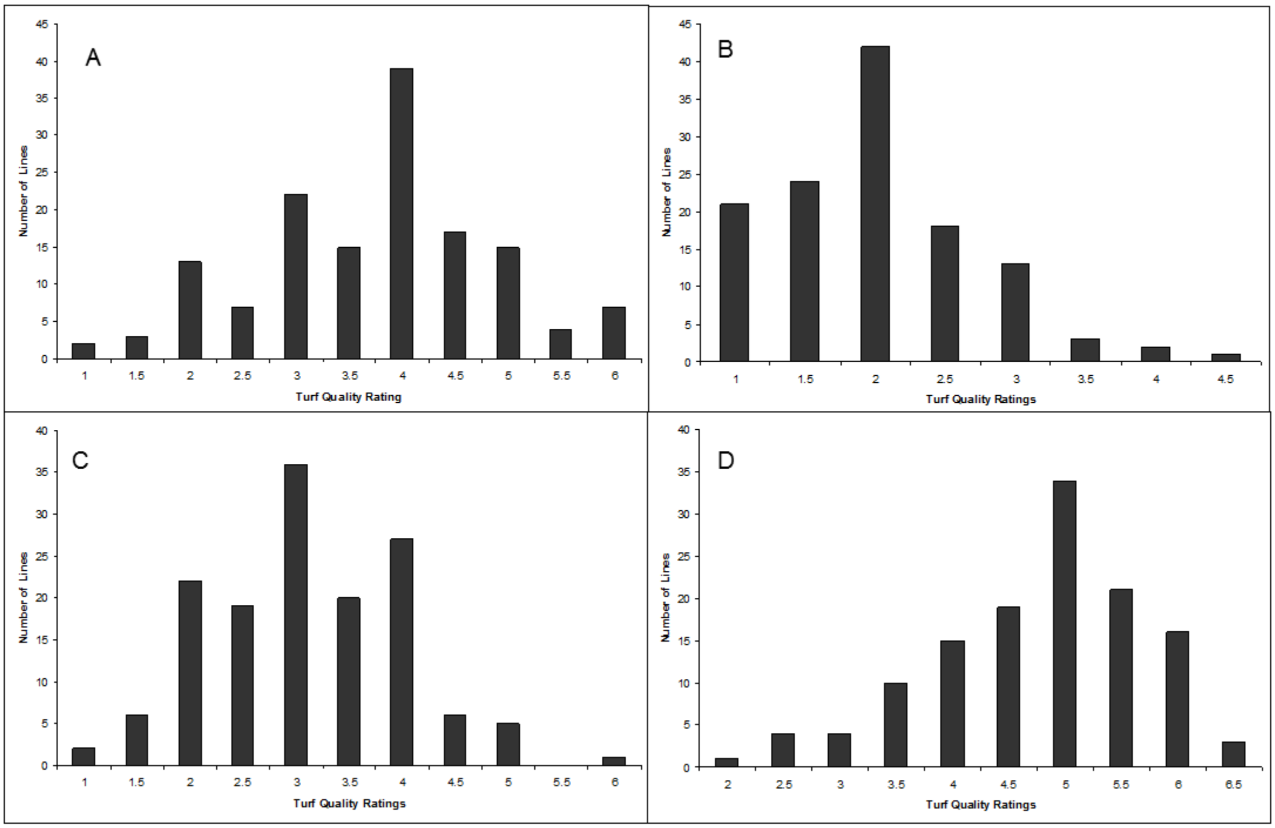

Figure 2:
Distributions of membrane stability as estimated by electrolyte leakage for the University of Georgia drought stress sampling in June of 2015 (A), Rutgers drought stress sampling in September of 2015 (B), University of Georgia heat stress sampling in August of 2015 (C) Rutgers heat stress sampling in August of 2015 (D).

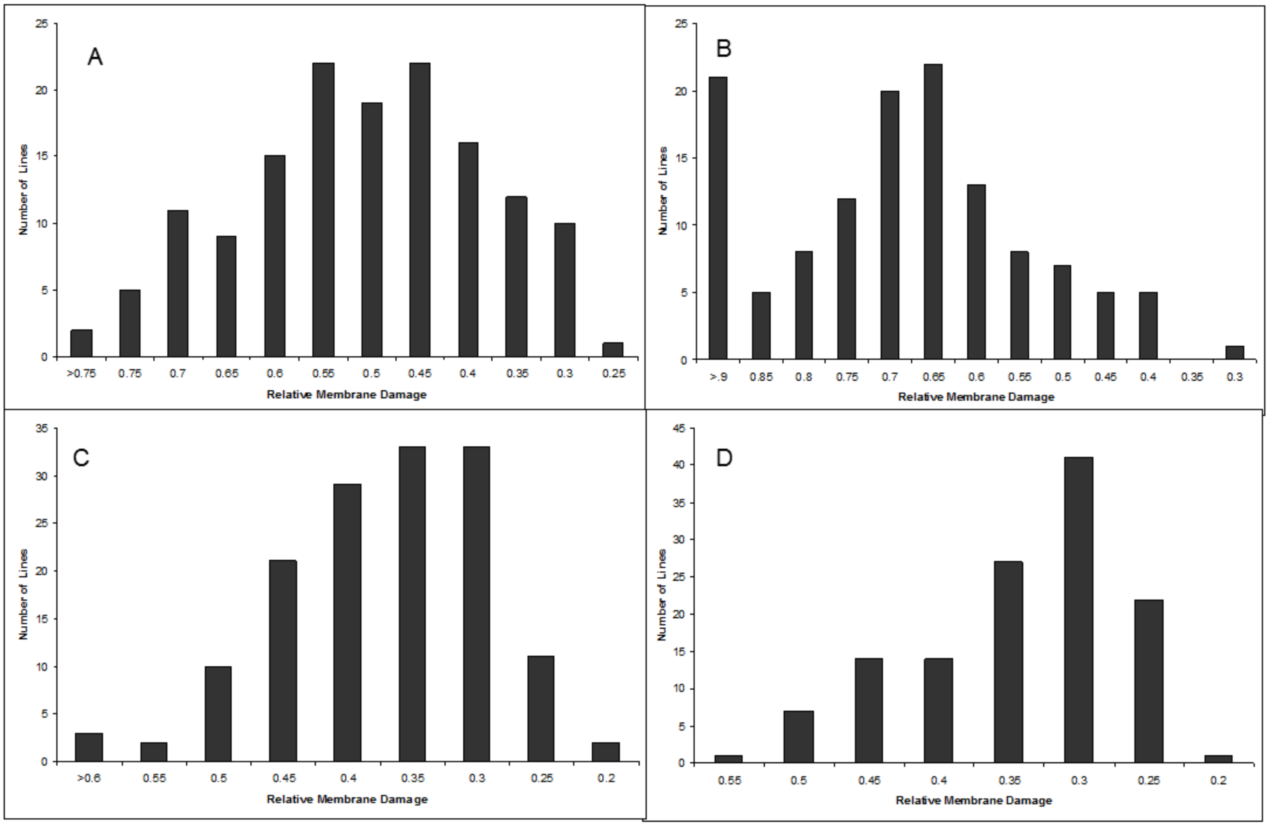

Figure 3:
Image taken at the University of Georgia during the 2015 drought stress period demonstrating the range of responses to drought stress with in the population.

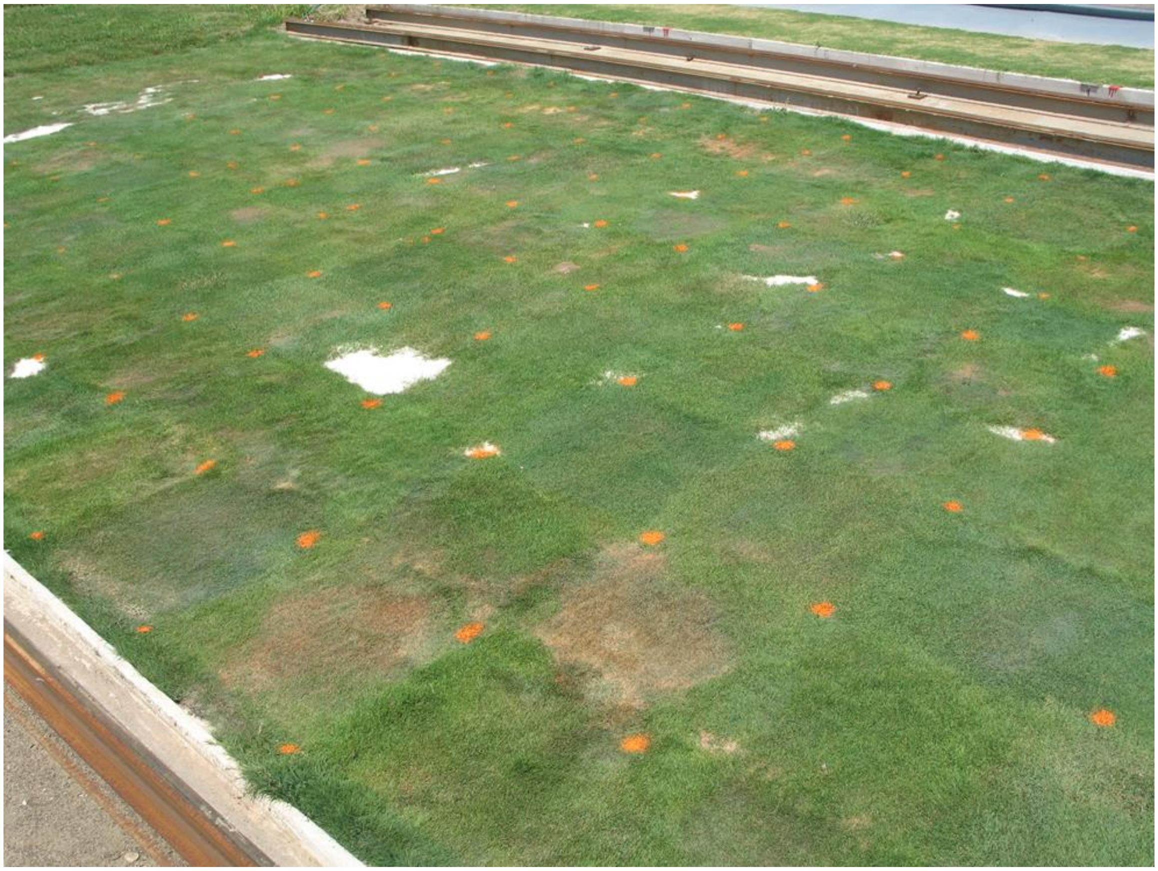

Figure 4:
Representative results from SSR marker screening using a capillary electrophoresis system, showing polymorphisms among three genotypes (A, B, C) for a heat-tolerance marker. Peaks are SSR marker products and orange dotted lines represent potential product sizes for the marker.