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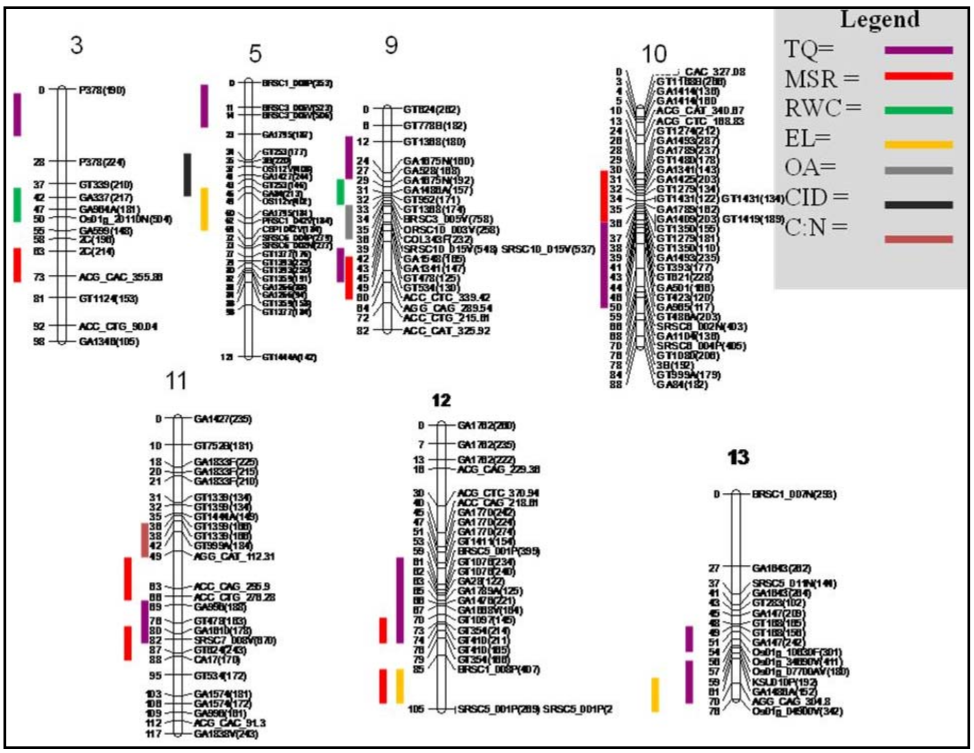

Rutgers University and USDA-ARS scientists continue to unravel the genetics of creeping bentgrass drought and heat tolerance. Major quantitative trait loci (QTLs) were found in several chromosomal regions which may be useful for future marker-assisted selection of both drought- and heat-tolerant creeping bentgrass cultivars.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 400 projects at a cost of $31 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Identification of Quantitative Trait Loci (QTL) Associated with Drought and Heat Tolerance in Bentgrass Species

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SUMMARY

Rutgers University and USDA-ARS scientists continue to unravel the genetics of creeping bentgrass drought and heat tolerance. To date, findings of this research include:

- Significant phenotypic variations in drought or heat tolerance were found in two populations of bentgrass species segregated for disease resistance. Phenotypic variations in drought and heat tolerance in both the creeping bentgrass and creeping and colonial bentgrass hybrid populations allowed for identification of potentially important QTL regions for drought and heat tolerance.
- Major QTLs were found in several chromosomal regions which were consistent across years and environments, such as mapping groups 5 and 11 for the bentgrass population and chromosomes 2A1 and 2A2 for the colonial/creeping bentgrass hybrid backcross population. These may be important chromosomal regions with QTL markers governing stress tolerance traits in bentgrass species.
- Regions of mapping groups 3 and 11 in the creeping population were significant for both heat and drought tolerance. The importance of these groups in tolerance to multiple stresses may be most useful in future studies for development of marker-assisted selection of both drought- and heat-tolerant creeping bentgrass cultivars.

Creeping bentgrass is one of the most widely used turfgrasses for greens, tees, and fairways on golf courses in cool, humid climatic regions. Over the past decade there has been an increase in the use of creeping bentgrass on golf courses, particularly fairways, because of disease epidemics in perennial ryegrass fairways. Its use has been extended into warmer climatic zones, but its use is limited by climatic conditions.

A primary environmental factor limiting growth of creeping bentgrass in warm climatic regions is heat stress. Maintaining high quality bentgrass during summer months is a major challenge for golf course superintendents. Creeping bentgrass starts to deteriorate when temperature increases to 27º C or higher (12). Heat stress is becoming a more significant problem as global warming continues. Another important challenge facing the turf industry is dealing with drought stress and water conservation as water availability for irrigation becomes increasingly limited. Creeping bentgrass quality declines within 5-7 days without adequate irrigation (7). However, golfers at private and public facilities express their displeasure when fairways and tees are no longer of the quality they expect when irrigation is restricted during summer months.

Currently, the majority of commercially available cultivars of creeping bentgrass does not have adequate drought or heat tolerance and cannot withstand prolonged periods of heat or drought stress. Therefore, there is an urgent need to improve heat and drought tolerance of bentgrass in order to maintain quality turf on golf courses.

Rainout shelter study at Rutgers University for drought and heat tolerance with a creeping bentgrass mapping population.

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Approaches and Strategies for Improving Bentgrass Stress Tolerance

One strategy to cope with drought stress or restricted water use is to develop heat- and drought-tolerant cultivars, which allow superintendents to maintain quality playing surfaces in hot and dry periods. Several approaches may be taken to improve drought and heat tolerance, including traditional breeding, molecular breeding, and biotechnology techniques. Much of the past breeding efforts have aimed primarily to generate cultivars that exhibit high turf quality under favorable environmental conditions.

In recent years, there has been some progress in improving abiotic stress tolerance through traditional breeding, but progress is limited. In addition, traditional breeding methods utilized to investigate the genetic control of quantitative traits, such as drought and heat tolerance, do not provide information on 1) chromosome regions regulating the variation of each trait, 2) the simultaneous effects of each chromosome region on other traits and the genetic control (pleiotropy or linkage) of the associated effects, and 3) the identification of specific candidate genes associated with stress-tolerance traits (5).

Some of these constraints can now be partially overcome by utilizing molecular markers, which can allow for the identification of quantitative trait loci (QTL) controlling a chosen phenotype and also enable scientists to assess the effects of the same QTL region on other traits. Breeders also can use QTLs for marker-assisted selection to improve selection efficiency.

It is generally accepted that QTL analysis is more useful in studying quantitative traits than classical genetics and provides a powerful tool for dissecting the complex inheritance of quantitative traits, and ultimately in identifying the genes underlying the QTLs for desired traits. QTL analysis has been successfully utilized to identify phenotypic traits and candidate genes associated with drought and heat stress tolerance in various agronomic crops providing useful molecular markers for marker-assisted breeding (13, 14).

This approach has recently been used in molecular breeding for disease resistance in turfgrass, mainly for dollar spot and snow mold in creeping bentgrass (4) and for gray leaf spot in perennial ryegrass (8). Bonos (4) identified three putative QTL loci for dollar spot (one in the susceptible parent and two in the resistant parent) using interval mapping in MapQTL 5.0. Jo and Jung (8) identified four QTLs for gray leaf spot resistance. Those QTL markers for disease resistance have a great potential to be utilized in breeding improvement for disease resistance.

This approach also may be effective for developing markers linked to abiotic stress tolerance in turfgrass. To our knowledge, however, there has been no research reported on the use of QTL analysis in improving turfgrass abiotic stress, such as heat or drought stress. The results reported here could have dramatic effects on the ability of breeders to develop heat- and drought-tolerant bentgrass cultivars for stressful environments.

In order to identify QTLs for drought or heat tolerance, the first step is to choose phenotypic traits underlying the stress tolerance with potential value for breeding. Many traits are associated with drought and heat tolerance. Most widely used traits for the selection of drought tolerance in QTL analysis include osmotic adjustment, water use efficiency, cell membrane stability estimated by electrolyte leakage (EL), and leaf photochemical efficiency or chlorophyll fluorescence (Fv/Fm) (5, 13).

The latter two traits are also positively correlated with heat tolerance in various plant species, including turfgrass (1,10,11). Marcum (11) suggests that electrolyte leakage can be used to predict whole-plant tolerance to heat stress for Kentucky bluegrass cultivars. Markers for the above traits associated with drought or heat tolerance have been identified in many agronomic crops (see review by Bonos and Huang, 5), but QTL mapping for the traits linked to drought or heat tolerance are not available for any turfgrass species.

Identification of QTLs linked to drought or heat tolerance also requires an experimental population segregating for the trait of interest and

Significant phenotypic (whole-plant) variation observed in turf quality during drought stress field study (October, 2008).

a genetic linkage map based on molecular markers (13). Two experimental populations of creeping bentgrass segregating for dollar spot resistance have been developed at Rutgers University. One population, L-93-10 x 7418-3, is a creeping x creeping cross, consisting of 180 pseudo F_2 progenies and 450 F_3 and backcross progenies. A genetic linkage map was developed for each parent using JoinMap 3.0 (4). The 630 individuals were rated for drought tolerance in a field trial in 2006, and 102 F_2 progenies exhibited large variation in drought stress tolerance in un-irrigated field plots, with leaf wilting score ranging from 1 to 9. The 102 progenies also varied significantly in physiological parameters, leaf relative water content (RWC) and osmotic adjustment (OA), when exposed to drought stress under greenhouse conditions (unpublished data).

The other population is a creeping x colonial hybrid x creeping cross (Hybrid 15 x 9188). This population exhibits segregation for dollar spot resistance. Genetic linkage maps have also been created for this population. Variations in turf performance among the second population have been observed during dry periods at Rutgers field plots in June 2007. Leaf wilting rating ranged from 3 to 9 (9 = no wilting, 1 = complete dessication) (unpublished data).

Canopy minus air temperature or canopy temperature depression (CTD) is a widely used indicator of transpirational cooling. Positive values indicate complete stomatal closure due to water deficit while more negative values indicate active transpiration due to high cellular hydration. Our preliminary data demonstrated that the hybrid bentgrass population exhibited large genetic variability of transpirational cooling capacity, with some individuals having complete stomatal closure during drying while some individuals maintained fully turgid leaves and full capacity of transpirational cooling.

The objectives of this project are 1) to evaluate variations in drought and heat tolerance for two mapping populations of bentgrass segregating for disease resistance; 2) to identify phenotypic traits associated with drought and heat tolerance; and 3) identify QTL markers associated with drought and heat tolerance utilizing the available linkage maps. The ultimate goal is to dissect the complex inheritance of quantitative traits associated with drought and heat tolerance and to identify QTL markers, which could be potentially used in molecular marker-assisted selection for improving drought and heat tolerance in creeping bentgrass.

Materials and Methods

A creeping bentgrass mapping population (L93-10 x 7418-3; 3) and a creeping/colonial bentgrass hybrid backcross population (2), both segregating for dollar spot resistance, were evaluated for variations in drought or heat tolerance and for QTL localization. The creeping population consisted of a pseudo F_2 mapping population (180 individuals) that was generated from the intra-specific cross of a dollar spot resistant (L93-10) and a susceptible (7418-3) genotype in the spring of 2003. For all QTL analysis described here, a subset of the population (100 individuals of the F_2 progeny) and the parents were evaluated for heat and drought tolerance. This population was used

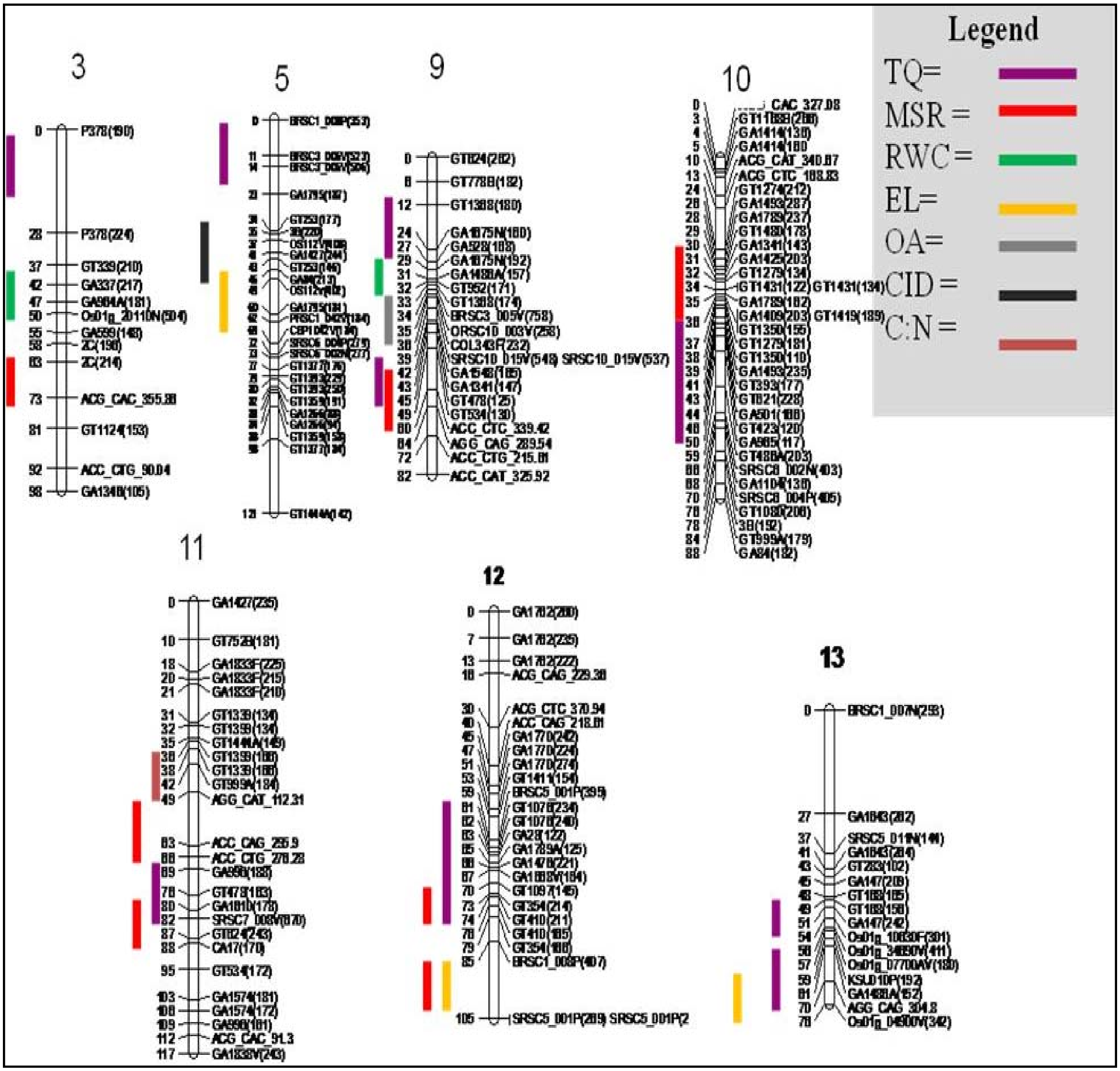

Figure 1. Researchers were able to link genomic regions of the mapping populations to traits associated with heat and drought tolerance. They included turfgrass quality (TQ), relative water content (RWC), electrolyte leakage (EL), and osmotic adjustment (OA).

in a previously funded USGA project to identify QTL markers associated with dollar spot resistance in creeping bentgrass.

A linkage map using microsatellite markers has already been developed, and markers for dollar spot resistance have been identified. The population was evaluated 4 times for phenotypic variation in drought tolerance in 3 different environments. Phenotypic screening for drought tolerance were done in a greenhouse (2007), the field (fall, 2008 and fall, 2009) and a growth chamber (2010). Evaluation for variation in heat stress was conducted in two separate growth chamber studies and in the field for two years (summer, 2008 and summer, 2009).

The hybrid bentgrass population was originally developed by crossing creeping (*Agrostis stolonifera* L.) and colonial bentgrass (*Agrostis capillaris* L.) by interspecific hybridization for the introgression of colonial genes for dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) resistance into creeping and for the development of colonial linkage map. The F_2 population, progeny of a creeping x colonial hybrid crossed with a creeping plant, was used for drought screening. The hybrid bentgrass population was evaluated for drought tolerance in a greenhouse in 2009, 2010, and in a growth chamber in 2010, as discussed in the respective sections below.

Results

Phenotypic Variations in Drought and Heat Tolerance

In order for QTLs to be detected, significant measurable variations must exist within the population to allow for the association of the phenotypic traits to the presence or absence of genomic regions associated with that trait (6). In response to drought stress, both populations exhibited significant variations in most physiological parameters evaluated. For example, the creeping population visual turf quality rating (TQ) ranged from 3 to 6 following 7 days of drought stress in the 2007 greenhouse study. In the same study, relative water content varied from 60% to 94% and osmotic adjustment ranged from 0 to 0.50. In the 2009 greenhouse study, the hybrid population exhibited variations after 5 days of drought, ranging from 3 to 9 for turfgrass quality, 13% to 77% for relative water content, and 0.4 to 0.8 for photochemical efficiency.

The greater variations observed in the hybrid population is likely due to the greater genetic divergence between the parents. In addition, the distribution around the population mean for the measured traits during drought stress were generally more normal in the hybrid population, which may result in greater significance of QTLs detected in hybrid population compared to the creeping population. Drought stress caused greater variation within both populations than heat stress, resulting in significant QTLs for more traits in response to drought.

QTL Analysis of the Creeping Bentgrass Population

Several possible QTLs were identified in plants within the creeping bentgrass population exposed to heat, drought, and after recovery from stress. Under drought stress, from the results of both the field and greenhouse studies, possible QTLs were identified on chromosomes 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, and 13. Of these, four chromosomes, groups 3, 5, 9, 10, 11, and 13 had multiple regions that could be possible QTLs or had overlapping regions for multiple traits (Figure 1). For instance, the QTL on group 3 was identified for both canopy green leaf biomass (expressed as normalized difference vegetation index or NDVI) and relative water content in years 2007 and 2008. The possible QTL region on group 11 was for turfgrass quality and carbon to nitrogen ratio, NDVI, and turfgrass quality. Turfgrass quality on this group mapped to the 70-80 cM region in both 2007 and 2008 during recovery from drought.

Group 5 may be a location determining turfgrass quality characteristics under stress conditions, since the same region (approximately 30-50 cM) was consistent across all years. Groups 5, 11, and 12 may be the most important groups for drought tolerance traits, since regions within these groups were significant across most environments and years 2007 to 2009. This could mean that these regions may have higher heritability and may not be as sensitive to environmental factors as the other QTLs. However, more data analysis and completion of the map will be performed to determine this.

For the creeping bentgrass population exposed to heat stress, potentially important regions of the genome include chromosome groups 2, 3, 6, 9, 10, 11, 12, and 13 identified for the traits turfgrass quality, NDVI, leaf area index, and Fv/Fm. QTLs on groups 2, 3, 9, 11, and 12 accounted for traits over multiple years, indicating that these could also be important chromosomal locations to look at for future use in marker assisted selection. In the field studies of 2008 and 2009, groups 2, 9, 11, and 12 may have important regions controlling visual quality during heat stress as measured by turfgrass quality ratings, NDVI, and leaf area index.

<u>QTL Analysis of the Creeping and Colonial Hybrid Population</u>

Important linkage groups including possible QTLs for drought tolerance in this population

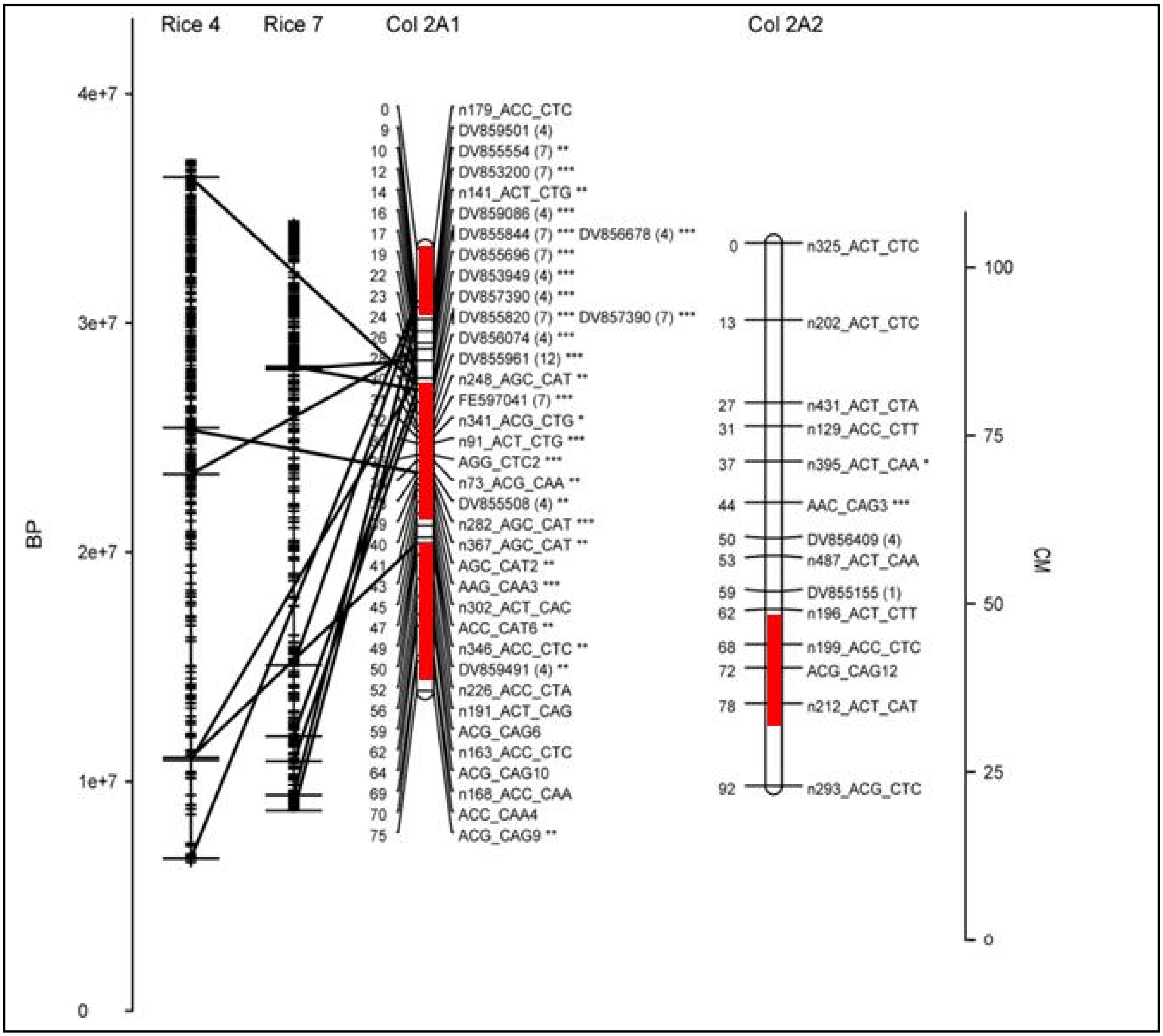

Figure 2. In the creeping and colonial hybrid population, several major QTLs were located in the same group region such as turfgrass quality and canopy temperature depression.

were 1A1, 2A1, 2A2, 5A1, 5A2, and 6A2. Greenhouse evaluation of variations in drought tolerance in 2009 resulted in significant QTLs on three different groups 2A1, 2A2, and 6A2. The location of the QTL on group 2A1 overlapped for the two traits, electrolyte leakage and canopy temperature depression. Turfgrass quality mapped to 3 locations, 2 on group 2A2 and 1 on group 6A2.

In 2010, the greenhouse and growth chamber evaluations revealed possible QTLs on groups 1A1, 1A2, 2A1, 2A2, 5A1, and 5A2. Several major QTLs were located in the same group region such as TQ and CTD on groups 2A1 and 2A2 (Figure 2) and TQ and chlorophyll content (CHL) (group 5A2), which may be particularly useful for marker-assisted selection. In addition, since the most consistent QTLs across years and environments were groups 2A1 and 2A2, traits in these regions may contribute greatly to the variation in drought tolerance seen within the hybrid population.

The linkage group assignments for the colonial bentgrass map (15) were based on the Triticeae system using the established rice-wheat chromosomal relationships (9). It is interesting that the QTLs for drought tolerance were found on the colonial bentgrass group 2 homoeologous linkage groups. It was recently reported that velvet bentgrass was the diploid donor of the A2 genome of allotetraploid colonial bentgrass (15). The diploid donor of the A1 genome has not yet been identified.

This population also exhibited significant variations in heat tolerance, as demonstrated by turfgrass quality, electrolyte leakage, and Fv/Fm in a growth chamber study. QTL analysis of the hybrid population for heat tolerance is currently being conducted. We anticipate the identification of few major QTLs associated with heat tolerance in this population based on the significant genotypic variations within this population.

Future Research and Utilization of QTL Markers

QTLs associated with drought or heat tolerance, particularly encompass multiple traits for both drought and heat tolerance, may be transferred into high quality creeping bentgrass cultivars using marker assisted backcrossing. The resulting crosses can then be used directly to breed drought and heat tolerant cultivars. In addition, backcrossing between elite parents may be used to verify the QTLs obtained, identify new QTLs, determine their stability over a range of test cross parents and environments, and assess the involvement of physiological traits in drought and heat tolerance.

Since drought and heat screening typically requires significant labor intensive measurements only a limited number of plants can be evaluated, so unfortunately, screening for these traits is not done extensively in breeding programs. But the identification of markers for heat and drought tolerance reported here is a major breakthrough for current turfgrass breeding programs. This could result in hastened breeding and the development of cultivars with more dramatic improvements in both heat and drought tolerance. Additionally, these markers when used with markers for other traits, such as disease resistance, can be used to develop cultivars with multiple stress tolerance. This research will help to jump start marker-assisted selection for drought and heat tolerance in turfgrasses.

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