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Volume 9, Number 14
July 15, 2010

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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Exploring Molecular Mechanisms of Heat Tolerance in Bentgrass Species

Bingru Huang

SUMMARY

Rutgers University scientists continue to investigate the genetic control of heat tolerance of bentgrass. They compared thermal *Agrostis scabra* collected in high-temperature sites near thermal vents in Yellowstone National Park with heat-tolerant and heat-sensitive cultivars of creeping bentgrass. Results of their studies include:

- Differential display and suppression subtraction hybridization analysis identified some important genes associated with heat tolerance from thermal *A. scabra*.
- Heat-responsive genes identified in these studies mainly control protein metabolism, carbon metabolism, stress defenses and signaling, and cell wall synthesis.
- The largest group of heat-responsive genes is involved in stress defense, which is followed by the group of genes related to carbon and energy metabolism, and protein synthesis and degradation.
- The expression in one of the heat-responsive genes, expansin, was confirmed to be positively related to heat tolerance in creeping bentgrass.
- PCR-based SSR markers are being developed from heat-responsive genes, which could be used in marker-assisted selection of heat-tolerant bentgrass and other cool-season turfgrass species.

The decline in turf growth and quality of creeping bentgrass has been a major challenge for maintaining bentgrass putting green and fairways on many golf courses in warm climatic regions. Heat stress is a primary factor causing summer bentgrass decline, as the optimal temperature for the growth of cool-season grasses is 15-24° C for shoots and 10-18° C for roots, but air and soil temperatures often exceed these levels during summer in many areas. Improving heat tolerance is becoming increasingly important, especially as its use is extended to further south. Therefore, there is an increasing demand on heat-tolerant bentgrass germplams.

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Several approaches can be taken to improve heat tolerance of creeping bentgrass, including traditional breeding and genetic engineering. Over the years, breeders have aimed primarily to generate cultivars with high turf quality. The progress using either approach to improve heat tolerance has been restricted by the lack of heat-tolerant germplasms or the genes that control heat tolerance. An effective and efficient solution to improve heat tolerance is to find genes and gene products (proteins) controlling heat-tolerant traits in plants that are capable of surviving at extremely high temperatures. Such information would lead to the creation of heat-tolerant plants through genetic engineering or breeding.

In recent years, breeders have become increasingly interested in exploiting genetic markers such as 'marker assisted selection' (MAS) as a new breeding methodology for genotypic selection. Marker-assisted selection is becoming increasingly useful in the development of newer germplasms with improved stress tolerance, which has the potential to dramatically enhance the pace and efficiency of genetic improvement. Once genetic markers have been developed for a particular trait, markers can be used to evaluate the variation available to breeders. From these data, more informed decisions can be made on parental combinations. Marker-assisted selection for stress tolerance, however, requires identification of gene networks that are responding to stress or controlling stress tolerance. The advent of whole genomic-related technology is providing the necessary tools to identify the key gene networks that respond to stresses and relating their regulation to adaptive events occurring during stress.

The genetic make-up of crop plants has been finely tuned by decades of breeding. It is unlikely that large improvements for abiotic stress tolerance can be made by altering the composition

USGA Turfgrass and Environmental Research Online 9(14):1-5.
TGIF Record Number: 166409

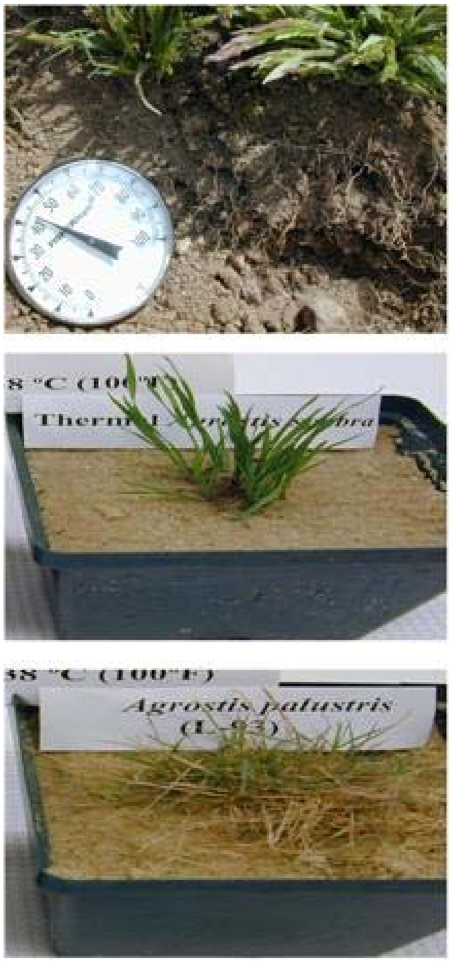

Figure 1. Thermal *A. scabra* near a thermal vent in Yellowstone National Park, showing active growing roots and shoots. Soil temperature at the top 10-cm soil is approximately 45° C. Picture taken July 2004. Thermal *A. scabra* (B, middle) and non-thermal *A. palustris* (C, bottom) exposed to soil temperature of 38° C for 28 days in a growth chamber, showing death of non-thermal plants, but healthy plants of the thermal grass.

of developmental genes. Stress tolerance genes linked to essential agronomic traits need to be identified if realistic improvements of plants for abiotic stress tolerance are to be made. This has spurred the search for sources of such variation among wild relatives of cultivated plants. Wild species offer a rich source of genes of enormous potential for crop improvement. We have discovered an *Agrostis* species, thermal *Agrostis scabra*, in the thermal sites at Yellowstone National Park, which demonstrates superior tolerance to high soil temperatures (45 – 50° C at the surface 3-5 cm soil and 60-70° C below 10 cm of soil).

The thermal *Agrostis* species is closely related to creeping bentgrass; the genetic similarity between the two species is about 0.70 (on a scale of 0-1). We found that *A. scabra* plants were able to maintain much higher quality and physiological functions compared to creeping bentgrass under heat stress (35-40° C) (Figure 1). Various studies in our lab found that thermal *A. scabra* was able to maintain new root production and significantly higher root viability and various physiological and metabolic activities in both roots and shoots under high temperatures whereas *A. stolonifera* failed to produce new roots and exhibited high root mortality and dramatic decline in both root and shoot growth and physiological activities at such high temperatures (1, 4, 5, 6, 8, 9).

Our physiological studies demonstrated that the ability of roots to continue growth and maintain viability in thermal A. scabra exposed to heat stress was associated with the maintenance of high cell membrane stability, cytokinin synthesis, and antioxidant scavenging ability, which contributed to higher photosynthetic activities and overall shoot growth (1,2, 9). Root thermotolerance of thermal *A. scabra* was also associated with a greater capacity to control high-temperature-induced increases in respiration through both short-term and long-term respiratory acclimation (3). This was mainly due to a greater ability to prevent increases in root maintenance respiration, leading to reduction in carbon expenditure and extended root survivability in high temperature soils (4, 6).

The next logical question is what the molecular factors are controlling these physiological traits linked to heat tolerance in the thermal bentgrass species that allow survival and even grow in extremely high temperatures.

Identification of Heat-responsive or Heat-tolerance Genes in Bentgrass Species

To gain insights into molecular mechanisms of grass tolerance to heat stress, we used two approaches, differential display and suppression subtractive hybridization (SSH) analysis, to identify heat-responsive or heat-sensitive genes in thermal *A. scabra* (7, 8, 9). Identification of genes differentially regulated during different kinds of stresses can give clues to what defense mechanisms and biochemical pathways are regulated under these circumstances.

Plants were exposed to 20º C (control) or 35º C for 12 days. Differentially-expressed cDNA fragments were cloned to screen the heat up-regulated library. The SSH analysis identified 120 non-redundant putative heat-responsive cDNAs out of 1,180 clones. Among them, 55 clones showed high homologs to other genes or proteins with known functions. The most interesting finding in our studies was that 31 novel genes were identified in thermal *A. scabra* that have not been reported as being regulated by heat stress in other plant species. This suggests that there might be unique mechanisms associated with the superior ability of long-term adaptation of thermal *A. scabra* to high temperatures up to 45º C. Northern blot analysis of 12 selected genes confirmed their increases in transcripts under heat stress. Transcripts of 8 of the 12 genes were strongly enhanced and induced in shoots, while 2 out of the 12 selected genes were induced only in roots under heat stress.

The differential display technique was also used to identify genes differentially expressed in shoots and roots in thermal *A. scabra* compared to heat-sensitive *A. stolonifera* at 20 and 40º C (8). Differential display analysis revealed that 21 gene fragments were down-regulated while 18 fragments were up-regulated in heat-stressed *A. stolonifera*. For thermal *A. scabra*, 38 gene fragments were down-regulated and 22 fragments were up-regulated under heat stress. Putative functional analysis with BLASTX found that most of up-regulated genes in both species were involved in stress defense pathways, cell wall elasticity, carbon metabolism, regulatory functions, and protein synthesis and degradation.

Cloning and Functional Characterization of Heat-responsive and Tolerance Genes in Thermal Bentgrass

Cloning the full length cDNAs and characterization of the expression pattern of selected genes with known functions were performed in thermal *A. scabra* and genotypes of *A. stolonifera* differing in heat tolerance to determine whether these heat-responsive genes isolated from roots of the thermal grass species could be detected in heat-tolerant genotypes or could be used as potential molecular marker to select heat-tolerant germplasm.

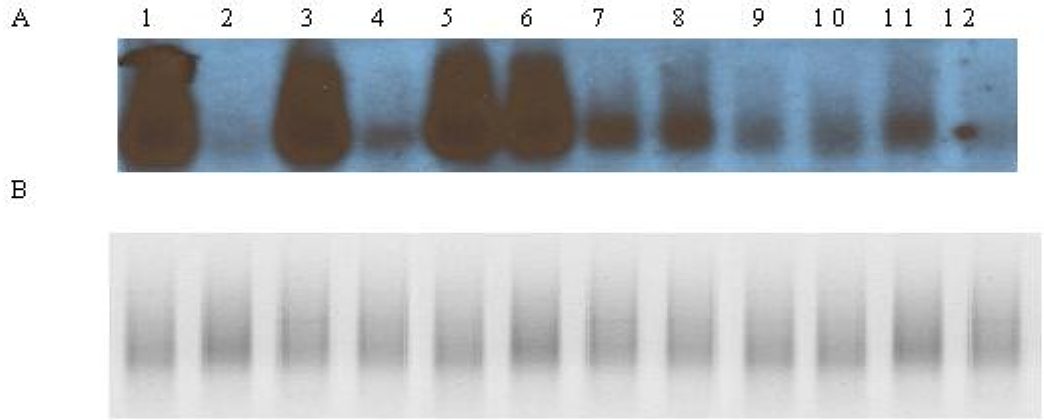

Figure 2. Expression level of expansin gene (AsEXP1) correlated to the level of heat tolerance in creeping bentgrass. A virtual northern hybridized with labeled AsEXP1 cDNA of total cDNA from thermal *A. scabra* and 11 cultivars of creeping bentgrass. (A) Lane 1. *A. scabra* (NTAS); 2, ‘Backspin’; 3, ‘Penn A-4’; 4, ‘Independence’; 5, *A. scabra* (FTAS); 6, ‘Declaration’; 7, ‘Shark’; 8, ‘L-93’; 9, ‘Putter’; 10, ‘Kingpin’; 11, ‘Century’, 12, ‘Pennlinks’. (B) background control.

We isolated and cloned the full length cDNA of some heat up-regulated genes, including several genes encoding heat shock proteins. Heat shock proteins (HSPs) are a family of functionally-related proteins whose expression is typically increased under heat stress. Heat shock proteins function as intra-cellular chaperons for other proteins, which play important roles in prevent damages to other proteins or help other proteins to maintain their conformation for proper functions.

RT-PCR analysis indicated that small molecular weight HSPs (16 kDa) were induced in roots by heat stress and larger molecular weight HSPs (70 kDa) were up-regulated and more so in thermal *A. scabra* and more heat tolerant genotypes of *A. stolonifera* ('Penn A-4' and 'Declaration') than the heat-sensitive genotype 'Penncross'. These analyses suggest that genes encoding HSPs, particularly small HSPs may play roles in heat tolerance.

One of the most interesting genes identified associated with heat tolerance is expansin gene which encodes proteins that control cell expansion and elongation. The expression level of the expansin gene was correlated to the level of heat tolerance in creeping bentgrass cultivars (Figure 2). The more tolerant cultivars, 'Declaration' and 'Penn A-4' had strongest expression of the expansin gene, while those ranked lower in heat tolerance rating such as 'Putter', 'Kingpin', 'Century', 'Bengal', 'Backspin', and 'Pennlinks' had weak or no expression. 'Shark', 'L-93', and 'Independence' that are ranked intermediate for heat tolerance showed intermediate level of expression.

Our study was the first to identify a heat-related expansin gene in grass species and demonstrate the involvement of expansin in grass adaptation to heat stress that were not previously reported in the literature. These heat-responsive genes could be used as potential candidate genes for molecular marker development.

Acknowledgements

We wish to thank the USGA Turfgrass and Environmenteal Research Program for provding funding for this research. It is noteworthy that all publications listed below are the result from projects funded by the USGA Turfgrass and Environmental Research Program.

Literature Cited

1. Lyons, E. M., J. Pote, M. DaCosta, and B. Huang. 2007. Whole-plant carbon relations and root respiration associated with root tolerance to high soil temperature for *Agrostis* grasses. *Environ. Exp. Bot.* 59:307-313. (TGIF Record 124345)

2. Pote, J., Z. Wang, and B. Huang. 2006. Timing and temperature of physiological decline for creeping bentgrass. *J. Amer. Soc. Hort. Sci.* 131:608-615. (TGIF Record 117129)

3. Rachmilevitch, S., H. Lambers, and B. Huang. 2008. Short-term and long-term root respiratory acclimation to elevated temperatures associated with root thermotolerance for two *Agrostis* grass species. *J. Exp. Bot.* 59:3803-3809. (TGIF Record 166317)

4. Rachmilevitch, S., Y. Xu, M. A. Gonzalez-Meler, B. Huang, and H. Lambers. 2007. Cytochrome and alterative pathway activity in roots of thermal and non-thermal *Agrostis* species in response to high soil temperatures. *Physiol. Plant.* 129(1):163-170. (TGIF Record 166326)

5. Rachmilevitch, S., H. Lambers, and B. Huang. 2006. Root respiratory characteristics associated with plant adaptation to high soil temperature for geothermal and turf-type *Agrostis* species. *J. Exp. Bot.* 57:623-631. (TGIF Record 124348)

6. Rachvillesmitch, S., B. Huang, and H. Lambers. 2006. Assimilation and allocation of carbon and nitrogen of thermal and nonthermal

Agrostis species in response to high soil temperature. *New Phytologist* 170:479-490. (TGIF Record 124346)

7. Tian, J., F. Belanger, and B. Huang. 2009. Identification of heat stress-responsive genes in heat-adapted thermal *Agrostis scabra* by suppression subtractive hybridization. *J. Plant Physiol.* 166:588-601. (TGIF Record 147023)

8. Xu, J., F. Belanger, and B. Huang. 2008. Differential gene expression in shoots and roots under heat stress for a geothermal and non-thermal *Agrostis* grass species contrasting in heat tolerance. *Environ. Exp. Bot*. 63:240-247. (TGIF Record 143777)

9. Xu, J., J. Tian, F. Belanger, and B. Huang. 2007. Identification and characterization of an expansin gene AsEXP1 associated with heat tolerance in C3 *Agrostis* grass species. *J. Exp. Bot.* 58:3789–3796. (TGIF Record 166321)