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Full Length Research Paper

Response to heat stress in warm season and cool season turf grass cultivars

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In this study, we investigated differences between cool-season and warm season grasses when exposed to heat stress. The results show that Kentucky blue seed germination rate was the highest at 25°C (40-50%), decreased significantly at 30°C (23-30%) and dropped to 0 at 40°C. For the Bermuda cultivars, seed germination rate was two fold higher at 30°C then 25°C, and reached the highest at 40°C. The cool season perennial rye and Bent grass seeds germinated well between 25 and 30°C. Their germination rate was significantly lower at 40°C. After exposure to 38°C for 2 d, the chlorophyll content decreased significantly for Bermuda cultivars, remained stable for Bent grass and perennial rye, and increased to the greatest extent for Kentucky blue. Leaf soluble protein contents increased significantly for Kentucky Blue, but remained stable for most of the other cultivars. Western blot with Hsp70 and dehydrin antibodies identified that the Bermuda cultivars had a dynamic changes in the profile of the heat and dehydration stress-related proteins. No similar changes were detected in Kenblue cultivars. High temperature induced dehydrin homolog proteins, but not the Hsp 70 in Perennial rye and Bent grass cultivars. Findings from this research can be used to differentiate cool season and warm season grass cultivars in terms of germination and survival ability under high temperature.

Key words: seed germination, chlorophyll content, leaf soluble protein, heat shock protein, dehydrin protein

INTRODUCTION

Temperatures above optimum growth range can cause damages to sensitive plant species by altering patterns of gene expression, inducing changes in cellular structures, and impairing membrane function (Bensaude et al., 1996; Gibson and Palsen, 1999; Bray et al., 2000). Elevated temperature and the associated physiological dehydration have been identified to accelerate senescence, diminish photosynthetic activities, and reduce yields and quality (Mutters et al., 1989; Wigley et al., 1994; Mullarkey and Jones, 2000).

The turf grass ranks as the major plant species that covers the exposed lands in urban and suburban areas. With annual revenue of $35 billion, turf industry plays an important role both for economic stability, and environmental improvement and protection in the United States. To meet the requirements of microenvironment, different warm season and cool season varieties have been selected and bred from a wide range of native and introduced species (Wallner et al., 1982; Sleper, 1985; Zwonitzer and Mian, 2002). However, high temperature and the associated damage has been a major issue for the turf industry. Extensive researches have been performed to understand the molecular, physiological and biochemical changes induced by heat stress (Park et al., 1996; Salvucci et al., 2001; Vani et et al., 2001; Vargas-Suarez et al., 2004). Higher plants exposed to excess heat exhibit a characteristic set of cellular and metabolic responses, including a decrease in the synthe-sis of normal proteins and an accelerated transcription and translation of heat shock proteins (HSPs) (Key et al., 1983; Boston, 1996; Guy, 1999; Hong et al., 2003). In fescue, genes encoding low molecular weight heat shock proteins or small heat shock proteins (smHSPs, 16.9- and HSP26.7kD) (Vierling, 1991) are induced stronger in heat-sensitive genotype compared to the heat-tolerant

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Table 1. Seed germination rate (%) of different turf cultivars at different temperatures

cultivar	25°C	35°C	40°C
Bentgrass "Crenshaw"	10.0[a*]	62.3[b]	20.0[c]
Bentgrass "Penn Links"	60.0[a]	56.7[a]	56.7[a]
Kentuky Blue "Ken Blue"	41.7[a]	23.4[b]	0[c]
Kentuky Blue "Midnight"	48.4[a]	30[b]	0[c]
Perennial rye "Linn"	71.7[a]	58.4[b]	53.0[b]
Perennial rye "Mach I"	68.4[a]	66.7[a]	51.7[b]
Burmuda Yukon	16.7[a]	40[b]	58.4[c]
Burmuda Arizona Common	6.7[a]	21.7[b]	40[c]
Creeping bent grass "jasper II"	58.4[a]	56.7[a]	11.7[b]
Creeping bent grass "Boreal"	66.7[a]	48.4[b]	38.4[c]

*Mean values within rows not carrying same letters are significantly different at the 95% confidence level using ANOVA test

genotype with heat treatment (Zhang et al., 2004; 2005). Decreased activities of antioxidant enzymes, photosynthetic capacity, and decreased leaf chlorophyll content are all described to be associated with heat stress for the cool season grasses (Liu and Huang, 2000). This study was conducted to identify the characteristic changes common to different cool and warm season varieties. Different parameters including seed germination, leaf chlorophyll content, soluble protein content and accumulation of heat-induced proteins were compared. The goal was to identify the physiological capacity for survival of high temperature in different types of grasses.

MATERIALS AND METHODS

Cool season grass cultivars included the Kentucky bluegrass (*Poa pratensis* L. cv. "Ken blue" and "Midnight"); Bent grass (*Agrostis palustris* Huds. cv. "Crenshaw", "Penn links", "Boreal" and "Jasper II"); Perennial ryegrasses (*Lolium perenne* L. cv. "Linn" and "Mach I"). The warm season grass was Bermuda grass (*Cynodon dactylon* var. *dactylon* (L.) Pers. cv. "Yukon" and "Arizona Common"). The cool season grasses are normally adapted to 15.5 to 23.9°C. The warm season grass cultivars begin ggrowth at temperatures above 15°C, and their optimum growth temperature is between 24 to 37°C.

Seed germination test: 20 seeds were placed on five layers of water soaked filter paper in each Petri dish. Three replicates were included for each cultivar. There were three temperature treatments at 25, 30 and 40°C. The number of seeds germinated in each dish was counted after12 d. Germination rate was calculated as % = (germinated seeds/ 20) X 100.

Plant growth and heat treatment: Seeds were germinated in flats in a greenhouse. When the leaves reached 3 cm in height, the seedlings were transferred to incubators for heat treatment at 38°C. To ensure water supply in the potting media, the flats were sit in plastic containers filled with 2 cm deep water. Leaf samples were collected at 8, 24, 32 and 48 h. Two controls were included. One was leaf tissues prior to heat treatment and the other one was leaves collected from plants incubated at 26°C for 48 h. The experiment was performed in total darkness condition.

Leaf chlorophyll extraction and content determination: Leaf chlorophyll was extracted by homogenizing 1 g of leaf tissues in 30 ml of 80% acetone (v/v) under liquid N_2 and followed by incubation in darkness overnight. After filtration to remove the debris, the absorbance of the pigment extract was measured at 662 and 645 nm on a spectrophotometer (Spectronic 601 instrument, Milton Roy, Penn). The chlorophyll "a" (Ca) and Chlorophyll "b" (Cb) contents of the leaves were calculated on a fresh weight basis using the following formulas (Dere et al., 1998): Ca = 11.75A662-2.350A645; Cb = 18.61A645-3.960A662.

Leaf soluble protein extraction and content determination: Leaf tissue was manually ground to a fine powder under liquid N_2 and mixed in a buffer (1:3; w/v) containing 50 mM Tris, pH7.5, 200 mMDTT and 0.3%SDS. The protein was extracted by incubating the mixture at 4°C for 24 h on a rotary shaker. After centrifugation at 13,000 rpm for 10 min, the supernatants were collected and considered as the soluble leaf protein extracts. Protein concentration was determined by absorbance at 662 nm (Bradford, 1976). A standard curve was prepared with Bovin γ globulin (Biorad, Hercules and Calif).

Electrophoresis of leaf soluble protein and immunoblot assay: 150 ng of total protein was loaded on to a 12.5% Tris precast gel (Biorad) and electrophoresis was performed at 200 V for 45 min. After electro-blotting into PVDF membrane, the total protein was probed with antibodies to dehydrin and 70 kD heat shock protein cognate (mouse monoclonal Hsp70 (ab6535) and rabbit polyclonal anhydrin (ab681) (Abcam, Cambridge, Mass.). The antibody-antigen complexes were detected using goat-anti mouse, or rabbit antibodies tagged with alkaline phosphatase (Biorad).

Statistics analysis: For all the quantitative measurements, triplicates of biological repeats were included for each treatment and control. Results were presented as mean ± SD. Differences among sample means were separated by ANOVA at 0.05 probability level.

RESULTS

Effect of temperature on seed germination: Seed germination at different temperatures is dependent on the characteristics of the cultivars (Table 1). For the two warm season cultivars in the Bermuda series, high temperature is necessary for seeds to germinate. The seed germination rate was very low at 25°C and increased significantly as temperature went up to 30 and 40°C. For the cold season Kentucky blue series, Kenblue and Midnight are very sensitive to high temperature. Kentucky blue seed germination rate was the highest at 25°C, and then decr-

Table 2a. Chlorophyll a content of different turf grass cultivars under heat stress (µg/g FW).

cultivar	Prior to treatment	2d26°C	2d38°C
Creeping bent grass "Boreal"	39.36±1.25[a*]	38.12±2.13[a]	46.32±0.54[b]
Bermuda Arizona Common	30.86±2.08[a]	31.49±1.76[a]	15.06±1.15[b]
Bermuda Yukon	41.02±0.20[a]	38.43±0.43[a]	11.72±0.09[b]
Perennial rye "Mach I"	48.35±1.83[a]	42.17±3.53[ab]	38.45±2.20[b]
Kentuky Blue "Midnight"	47.39±2.23[a]	50.53±2.68[a]	84.54±3.40[b]
Kentuky Blue "Ken Blue"	37.09±2.48[a]	47.94±2.58[b]	75.25±4.76[c]
Bentgrass "Penn Links"	31.30±9.33[ab]	38.69±1.47[a]	24.22±1.31[b]
Bentgrass "Crenshaw"	30.00±5.86[a]	43.07±3.70[b]	30.56±5.21[a]

* mean ± SD. Mean values within rows not carrying same letters are significantly different at the 95% confidence level using ANOVA test.

Table 2b. Chlorophyll b content of different turf grass cultivars under heat stress (µg/g FW) (mean ± SD)

cultivar	Prior to treatment	2d26°C	2d38°C
Creeping bent grass "Boreal"	14.71±0.82[a*]	14.15±0.40[a]	21.62±0.07[b]
Bermuda Arizona Common	10.60±0.42[a]	10.87±0.96[a]	5.69±0.66[b]
Bermuda Yukon	16.98±0.28[a]	13.77±0.36[a]	5.20±0.04[b]
Perennial rye "Mach I"	25.47±2.68[a]	22.80±2.76[a]	34.59±2.75[b]
Kentucky Blue "Midnight"	19.76±1.17[a]	24.86±1.66[b]	31.26±0.37[c]
Kentucky Blue "Ken Blue"	15.41±1.18[a]	21.61±2.00[b]	33.18±3.19[c]
Bentgrass "Penn Links"	13.85±5.14[a]	16.94±0.91[b]	11.31±0.76[a]
Bentgrass "Crenshaw"	13.48±2.74[a]	18.79±2.06[b]	13.59±2.31[a]

- mean value ± SD. Mean values within rows not carrying same letters are significantly different at the 95% confidence level using ANOVA test

Table 3. Leaf soluble protein content of different turf grass cultivars under heat stress.

Cultivar	Treatment	Protein content (µg/g FW)	Cultivar	Treatment	Protein content (µg/g FW)
Bentgrass "Crenshaw"	38°C-0h	6.06±0.23[a*]	Kentuky Blue "Ken Blue"	38°C-0h	6.35±0.25[a]
	38°C-8h	6.15±0.35[a]		38°C-8h	13.17±0.31[b]
	38°C-24h0	6.24±0.23[a]		38°C-24h	9.42±0.13[c]
	38°C-32h	9.80±0.21[b]		38°C-32h	12.84±0.25[b]
	38C°-48h	7.38±0.95[ab]		38°C-48h	9.56±0.21[c]
	26°C-48h	6.42±0.13[a]		26°C-48h	7.83±0.13[a]
Bentgrass "Pennlinks"	38°C-0h	6.06±0.12[a]	Kentuky Blue "Midnight"	38°C-0h	6.10±0.15[a]
	38°C-8h	n/a		38°C-8h	6.18±0.22[a]

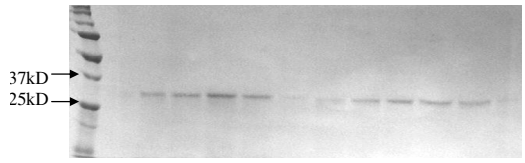
eased significantly at 30°C; germination rate was 0 at 40°C. For the Perennial rye and Bent grass, seeds of "Penn Links" germinated equally well at 25, 30 and 40°C.All the other cultivars including Crenshaw, Linn, Mach I, Jasper II, Boreal were sensitive to heat. The seed germination rates were significantly decreased when the temperature increased to 40°C.

Effect of temperature on chlorophyll content: Changes in chlorophyll content are presented in Table 2. Exposure to high temperature resulted in a 2-4 fold decreases in

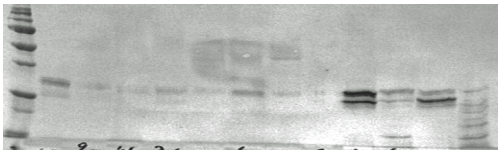
Table 3. Contd.

	38°C-24h	n/a		38°C-24h	9.29±0.21[b]
	38°C-32h	6.22±0.21[a]		38°C-32h	7.44±0.23[ab]
	38°C-48h	6.42±0.25[a]		38°C-48h	6.75±0.19[a]
	26°C-48h	6.20±0.10[a]		26°C-48h	7.01±0.25[ab]
Perennial rye "Linn"	38°C-0h	6.09±0.11[a]	Burmuda Yukon	38°C-0h	6.05±0.11[a]
	38°C-8h	n/a		38°C-8h	n/a
	38°C-24h	n/a		38°C-24h	n/a
	38°C-32h	n/a		38°C-32h	6.65±0.13[a]
	38°C-48h	n/a		38°C-48h	6.06±0.14[a]
	26°C-48h	6.15±0.13[a]		26°C-48h	6.52±0.13[a]
Perennial rye "Mach I"	38°C-0h	6.05±0.13[a]	Burmuda Arizona Common	38°C-0h	6.11±0.12[a]
	38°C-8h	6.05±0.15[a]		38°C-8h	6.06±0.11[a]
	38°C-24h	7.96±1.25[a]		38°C-24h	6.06±0.12[a]
	38°C-32h	6.12±0.22[a]		38°C-32h	6.05±0.15[a]
	38°C-48h	6.07±0.25[a]		38°C-48h	6.23±0.26[a]
	26°C-48h	6.44±0.24[a]		26°C-48h	6.06±0.13[a]
Creeping bent grass "Boreal"	38°C-8h	6.14±0.13[a]			
	38°C-24h	6.05±0.26[a]			
	38°C-32h	6.77±0.23[a]			
	38°C-48h	6.34±0.32[a]			
	26°C-48h	6.89±0.12[a]			

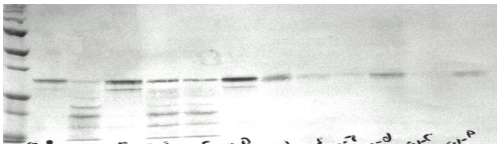
• mean value ± SD. Mean values within column for the same variety not carrying same letters are significantly different at the 95% confidence level using ANOVA test.



Prior 8h 1d 32h 2d ctr Prior 8h 1d 32h 2d ctr
Kenblue Midnight
Kentucky Blue cultivars
Prior: previous to heat treatment; ctr: 26°C for 2, 8, 1, 32 and 2d indicate time period of heat treatment under 38°C.



x x prior 8h 1d 32h 2d ctr prior 1d 2d ctr
Perennial rye"Mach I" Bermuda "Yukon"
Prior: previous to heat treatment; ctr: 26oC for 2d; 8h, 1d, 32h and 2d indicate time period of heat treatment under 38°C.



Prior 8h 1d 32h 2d ctr Prior 8h 1d 32h 2d ctr
Bermuda "Arizona Common" Bent grass "Boreal"
Prior: previous to heat treatment; ctr: 26oC for 2d; 8h, 1d, 32h and 2d indicate time period of heat treatment under 38°C.

Figure 1. Accumulation of hsp70 associated protein in leaf tissues of different grass cultivars under heat stress. 150 ng of total protein was loaded on to the 12.5% precast gel (Biorad) and electro-blotted onto PVDF membrane. The primary antibody was a mouse monoclonal HSP70 antibody (ABCAM, ab6535); detection was using the alkaline phosphatase conjugate substrate (Biorad). The Bermuda cultivars showed a stronger band (ca. 35kD and a few smaller molecular weight protein bands after heat treatments. Only the one protein band (ca. 35kD) was detected in Kentucky blue, perennial rye and Bent grass cultivars

chlorophyll content for Bermuda cultivars. In contrast, the two Kenblue Blue cultivars had a 2 fold increases at 38°C compared to 26°C. The two Perennial rye cultivars responded differently, "Linn" had a 2 fold increase and "Mach I" was relatively stable. In the case of Bent grass, heat induced a significantly decrease for "Penn Links" and remained similar for "Crenshaw".

Changes in leaf soluble protein content: The most obvious changes in leaf soluble protein content were detected in Kenblue cultivars (Table 3). It increased significantly after 8 and 48 h for "Ken Blue", while "Mid-night" had a transient high level at 24 h and then started to decrease. Protein content remained stable for the two Bermuda cultivars. The perennial rye and Bent grass cultivars basically showed no significant fluctuation in protein content, except for Bent grass "Crenshaw", which had a higher value at 32 h treatment.

Changes in heat induced proteins. Western blot with the Hsp70 and dehydrin antibodies detected that dynamic changes in protein profiles were happening during heat treatments for the two Burmuda cultivars (Figures 1 and

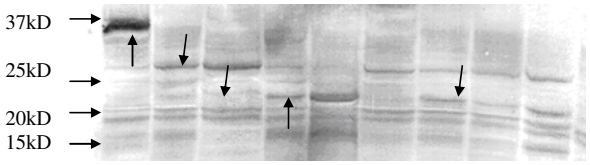


Figure 2. Accumulation of dehydrin cognate protein in leaf tissues of Burmuda cultivars.150 ng of total protein was loaded on to the 12.5% precast gel (Biorad) and electro-blotted onto PVDF membrane. The first antibody was rabbit polyclonal anhydrin antibody (ABCAM, ab22681); the detection was using the alkaline phosphatase conjugate substrate (Biorad). Different protein bands appeared in the two cultivars of Bermuda turf grass after heat treatments.

Figure 3. Detection of the 70 kD heat shock protein using the antibody for the 70 kD heat shock protein cognate (Hsp70).

2). Figure 1 shows that for Burmuda "Yukon", two strong bands were detected prior to heat treatment; these two bands were changing in density after exposure to heat treatment. In Bermuda "Arizona Common", a minimum of four bands (15 – 25 kD) appeared after heat stress (38°C) while these bands were not detectable prior to heat treatment and at 26°C (Figure 1-2, 1-3). In addition, while some dehydrin homolog protein disappeared, new protein bands appeared after heat treatment (Figure 2). These new proteins bands also appeared in the 2 d 26°C control, indicating they may be more related to dehydration than heat stress. The Kentucky blue cultivars showed no changes in protein profile after heat treatment when detected by the HSP70 antibody (Figure 1-1). The Perennial rye and Bentgrass cultivars showed no new hsp70 associated proteins (Figure 1-2, Figure 1-3) after exposure to heat stress. In addition, we were able to see heat-induced new protein bands detected by dehydrin antibody in perennial rye and bent grass cultivars.

However, the data was not presented here due to poor quality of the image.

DISCUSSION

Heat stress is a major factor limiting the growth of turf grasses, especially, for the cool season grasses. This will continue to be the primary concern in turf grass management, as temperature increases with global warming. Through this research, we have tested seed germination, chlorophyll content, soluble protein metabolism and heat induced changes in stress related proteins. The results show that each parameter has a big difference between Bermuda warm season grass and the Kentucky blue cool season grasses. The Bermuda cultivars showed a dynamic change in the proteins that are associated with heat shock and dehydration processes which are absent in the cool season grass cultivars.

The Hsp70 antibody was raised against a full length native protein (purified) (Bovine brain) (Abcam). We could observe a band at about 710 kD in Bentgrass and Perennial rye, but not in Kentucky blue and Bermuda. Although the band is very weak, nevertheless, the result can validate that the 70 kD HSP antibody can recognize homolog proteins in the turf grasses (Figure 3). We also probed the leaf total proteins with Hsp100 antibody, the two Bermuda cultivars showed two bands at ca.100 kD, and only one band appeared in the cool season varieties. However, because the bands were very fain, we are not able to present the result in this report. In the western blot with the Hsp70 antibody, the most abundant protein has molecular weight of ca 35 kD and there are a few more bands at lower molecular weight range (15-35 kD) in the two Bermuda cultivars. These proteins could be the products of proteolytic products of the 70 kD HSPs, or low molecular weight heat shock proteins (LMWHSPs) that have epitopes recognizable by the Hsp70 antibody. In either case, this dynamic change in the HSP associated protein after exposure to heat stress in the heat-tolerant cultivars suggest its significance in heat tolerance.

Dehydrins are multigene family proteins, known as Group II late-embryogenesis abundant (LEA). The proteins are commonly induced by environmental stresses associated with low temperature or dehydration and during seed maturation drying (Ismail et al., 1999), as well as high temperature (Rizhsky et al., 2002). These proteins have high hydrophilicity and thermostability, and function with chaperone-like properties to protect protein or membranes in plant tissues under abiotic stress such as desiccation or low temperature (Close, 1997; Borovskii et al., 2002). In this study, we performed the heat treatment with sufficient water supply in the potting media.

However, high environmental temperature can cause a simultaneous increase in the transpiration of leaf tissues. Associated with the rapid water loss and temperature increases in the leaves, is a delay in water absorption by the roots. The imbalance between water availability and demand will result in a physiological dehydration status in leaf tissues. The enhanced accumulation of dehydrin homolog proteins in the Bermuda cultivars may function to mitigate this type of stress. The same response mechanism also exists in the Perennial rye and Bent grass. The accumulation of the dehydrin proteins might help to protect intact cellular structures under heat stress so that the leaves can maintain relatively stable level in the chlo-

rophyll and protein content.

In this experiment, we detected an increase in chlorophyll content in the cool season grasses and a decrease in the warm season varieties after exposure to 38°C. However, higher chlorophyll content under heat stress is considered as an indicator of heat tolerance in warm season grasses (Liu and Huang, 2000). This contradictory result is caused by the difference of the heat treatment between these two studies. Liu and Huang (2000) performed the heat treatment as 35°C/25°C (L/D), which gives the plants an opportunity to reverse the heat damage and slowly adopt to higher temperature. In the current study, the cool season grasses were subjected to continuous high temperature. Extended exposure to 38°C led to water loss, which resulted in the apparent increase in protein and chlorophyll content in the leaf tissues.

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