Changes in Carbohydrate Metabolism in Two Kentucky Bluegrass Cultivars during Drought Stress and Recovery

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ABSTRACT. Carbohydrate metabolism is important for plant adaptation to drought stress. The objective of this study was to examine major forms of carbohydrates associated with superior drought tolerance and post-drought recovery in kentucky bluegrass (*Poa pratensis*) by comparing responses of different forms of carbohydrates with drought stress and re-watering in two cultivars contrasting in drought tolerance. Plants of drought-tolerant ‘Midnight’ and drought-sensitive ‘Brilliant’ were maintained well watered or subjected to drought stress for 10 days by withholding irrigation, and drought-stressed plants were re-watered for 3 days. Physiological analysis (turf quality, relative water content, and electrolyte leakage) confirmed the genetic variability of the two cultivars in drought tolerance. The two cultivars exhibited differential responses to drought stress and re-watering for the content of water-soluble sugars (sucrose, fructose, and glucose) and storage carbohydrates (starch and fructan), and ‘Midnight’ maintained higher sucrose content at 10 days of drought stress and more fructan at 3 days of re-watering. The greater accumulation of sucrose in ‘Midnight’ under drought stress corresponded with higher activities of two sucrose-synthesizing enzymes (sucrose phosphate synthase and sucrose synthase) but was not related to the sucrose-degrading enzyme activity (acid invertase). These results suggested that increased sucrose accumulation resulting from the maintenance of active sucrose synthesis could be associated with superior turf performance during drought stress, whereas increased fructan accumulation could contribute to rapid re-growth and post-drought recovery on re-watering in kentucky bluegrass.

Drought stress can be detrimental to the growth of annual and perennial plant species. For annual crops, the maintenance of yield production during drought is important, but for perennial grasses used as turfgrass or forage grasses, the ability of plants to survive severe drought and to recover rapidly from drought damages is an important strategy (Volaire and Lelievre, 2001; Volaire et al., 1998). Understanding the underlying mechanisms of drought survival and post-drought recovery after severe drought stress would facilitate the development of perennial grass species for use as turfgrass in areas with extended periods of drought stress or with limited irrigation.

Plant adaptation to drought stress involves changes in various metabolic processes, including photosynthesis, respiration, and carbohydrate accumulation (Fry and Huang, 2004; Nilsen and Orcutt, 1996). Carbohydrates in different forms are known to serve in diverse functions. For example, starch and fructan are major sources of energy reserves, whereas sucrose, fructose, and glucose are solutes for cell turgor maintenance and sucrose also may function in stabilizing proteins and membranes from stress damages (Kaur et al., 2007; Livingston et al., 2009; Santarius, 1973; Zhou and Yu, 2010). Drought stress alters the accumulation of water-soluble carbohydrates [WSC (i.e., sucrose, fructose, and glucose)] and storage carbohydrates [SC (i.e., fructan and starch)] (Kaur et al., 2007; Spollen and Nelson, 1994). Responses of carbohydrate metabolism to

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drought stress vary with forms of carbohydrates, plant species, and stress duration or severity. Decline in sucrose and starch content with short-term drought stress and increases in sucrose and starch have been observed with prolonged duration of drought stress in a C_4 grass *Setaria sphacelata* (Da Silva and Arrabaca, 2004). The accumulation of WSC has been considered one of the major traits for improving drought resistance in some plant species such as reported in durum wheat [*Triticum durum* (Al Hakimi et al., 1995)]. Other studies reported that SC are important for plant growth under drought stress such as found in tall fescue [*Festuca arundinacea* (Spollen and Nelson, 1994)] and orchardgrass [*Dactylis glomerata* (Volaire et al., 1998)]. Busso et al. (1990) reported that total nonstructural carbohydrates (TNC) accumulation in perennial grasses exposed to prolonged periods of drought contributed to plant regrowth on re-watering. Previous studies in several C_3 turfgrass species found that TNC content increased with drought stress (DaCosta and Huang, 2006; Fu and Dernoeden, 2008; Huang and Fu, 2000, 2001; Huang and Gao, 1999). Although various studies have shown the general importance of carbohydrates for drought resistance, limited information is available on the specific form of carbohydrates (i.e., sucrose, fructose, glucose, starch, or fructan) and underlying catalytic enzymes associated with genetic variability in drought tolerance that impart drought survivability and post-drought recovery, particularly for C_3 perennial turfgrass species exposed to extended periods of drought stress. For example, sucrose metabolism is controlled by multiple enzymes with sucrose phosphate synthase (SPS) and sucrose synthase controlling the synthesis of sucrose and acid invertase (AI) breaking down sucrose (Fu et al., 2010; Turner and Turner, 1975). However, how changes in these enzymes are related to genetic variations in sucrose response to drought stress are not well documented in C_3 perennial grass species.

Kentucky bluegrass is a widely used cool-season perennial turfgrass with a wide range of genetic viability in drought resistance, which is a good model material for exploring drought resistance mechanisms in C_3 perennial grass species (Bonos and Murphy, 1999; Richardson et al., 2008). Previous studies have demonstrated that activation of antioxidant enzyme activity and gene expression and abscisic acid metabolism as well as increased fatty acid saturation were important metabolic factors contributing to superior drought resistance in Kentucky bluegrass (Wang et al., 2004; Xu et al., 2011a, 2011b). The objectives of the present study were to determine major forms of carbohydrate (sucrose, fructose, glucose, starch, and fructan) as well as carbohydrate metabolism enzymes associated with plant tolerance to severe drought stress and post-drought recovery in two turfgrass cultivars (Midnight and Brilliant) known to differ in drought resistance as reported in previous studies (Chai et al., 2010; Wang et al., 2004; Xu et al., 2011a, 2011b).

Materials and Methods

PLANT MATERIALS AND GROWING CONDITIONS. Sod pieces of two Kentucky bluegrass cultivars (Midnight and Brilliant) were taken from three-year-old field plots and transplanted into plastic pots (16 cm diameter, 60 cm deep) filled with a mixture of fine sand and soil (1:1, v/v). Plants were maintained in a greenhouse located in New Brunswick, NJ, for 70 d (June to Oct. 2011) with an average day/night temperature of 22/16 °C and 11-h photoperiod of natural sunlight and then moved to

growth chambers where treatments were conducted. The growth chamber was set at 20/15 °C (day/night), 70% relative humidity, 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation at the canopy level, and a 12-h photoperiod.

TREATMENTS AND EXPERIMENTAL DESIGN. The experiment consisted of two cultivars and three water treatments (well-watered control, drought, and post-drought re-watering treatments), which were arranged as a completely randomized design within a growth chamber. Each treatment consisted of four replicates (four pots) per cultivar, which were placed in four growth chambers. Well-watered control plants were watered every other day to maintain soil volumetric water content (SWC) at the pot capacity of 28% (v/v). Drought stress was imposed by withholding irrigation for 10 d until SWC declined to 4% (v/v) for both cultivars and when leaves were completely wilted. Drought-stressed plants were then re-watered every day and SWC was maintained at 28% (v/v) during re-watering to evaluate post-drought recovery. The SWC in the 0- to 20-cm soil layer of each pot was measured using time domain reflectometry (Soilmoisture Equipment Corp., Santa Barbara, CA) by inserting a 20-cm-long probe into the soil.

MEASUREMENTS. Turf quality (TQ) was visually rated on a scale of 1 to 9 with a rating of 1 being a completely desiccated and brown turf canopy, a rating of 6 being the minimal acceptable TQ level, and a rating of 9 representing healthy plants with dark green, turgid leaf blades and a dense turf canopy (Turgeon, 1996).

Leaf relative water content (RWC) was measured to assess the level of leaf dehydration or water deficit. Leaf RWC was determined from fresh weight (FW), turgid weight (TW), and dry weight (DW) using the following formula: $\text{RWC (percent)} = [(FW - DW)/(TW - DW)] \times 100$. Leaves were immediately weighed for FW after being collected from the plants and then immersed into test tubes filled with deionized water for 12 h in the dark at 4 °C. Leaf samples were then blotted dry and immediately weighed for TW. Samples were then dried in an oven at 80 °C for at least 72 h and again weighed for DW (Barrs and Weatherley, 1962).

Leaf cellular membrane stability was evaluated by measuring electrolyte leakage (EL) of leaves (Blum and Ebercon, 1981). Fresh leaves (0.1 g) were placed in a test tube containing 20 mL deionized water and shaken for 24 h at room temperature (≈ 23 °C) to measure the initial conductivity of the solution (C_{initial}) using a conductivity meter (YSI, Yellow Springs, OH). Leaves were then killed by autoclaving at 140 °C for 20 min. The conductivity of the solution containing the killed tissues (C_{max}) was measured after samples were cooled down to room temperature. The EL was calculated as the percentage of C_{initial} over C_{max} (Blum and Ebercon, 1981).

For carbohydrate quantification, the procedure was conducted following the method used by Fu and Dernoeden (2009). Fresh leaves (0.1 to 0.2 g) were collected and dried in an oven at 80 °C. For the analysis of WSC and SC, 50 mg dry tissue was extracted in 1.0 mL of 92% ethanol and centrifuged at 20,000 g_n for 10 min. The supernatant was used to measure content of reducing sugars (glucose and fructose) and sucrose, and the residue was obtained for SC analysis. A 1-mL aliquot of supernatant was combined with 1.25 mL ferricyanide reagent and placed in a water bath at 100 °C for 10 min. After cooling tubes to room temperature, 2.5 mL of 2.0 N H_2SO_4 was added followed by 1.0 mL of arsenomolybdate reagent. The absorbance

of the solution was measured at 515 nm using a spectrophotometer (Helios Alpha; Thermospectronic, Rochester, NY), and reducing sugar content was calculated based on a glucose standard curve as described by Ting (1956). For sucrose hydrolysis, a 2-mL aliquot of supernatant was incubated in 2 mL of 4% H₂SO₄ (w/v) in a boiling water bath for 15 min to hydrolyze sucrose into reducing sugars, which was neutralized with 1.0 mL of 1 N NaOH. Ferricyanide and arsenomolybdate reagents were added to each test tube as described previously, and the absorbance of the solution was determined at 515 nm using a spectrophotometer. Fructan and starch content was measured using the method described by Smith (1981). A 0.5-mL aliquot of distilled water was added to each microtube containing the previously described SC residue. After incubation on a heating block at 100 °C for 10 min, 0.4 mL of 200 mM acetate buffer (pH 5.1) and 0.1 mL of enzyme solution (0.2 units of amyloglucosidase and 40 units of alpha amylase) were added to each microtube. The solution was incubated at 55 °C for 24 h and then centrifuged at 20,000 g_n for 10 min after cooling to room temperature. The extract was further hydrolyzed in 0.1 mL of 1.0 N H₂SO₄ to convert fructan to fructose. The resulting solution was heated on a heat block at 100 °C for 15 min and neutralized with 0.1 mL of 1.0 N NaOH after cooling to room temperature. Starch and fructan content were calculated based on the reducing sugar content as described previously.

The activity of SPS, sucrose synthase (SS), and AI was determined using the method described in Fu et al. (2010) and Khayat and Naftaly (1987). For the extraction of enzymes, 0.5-g frozen leaf samples were ground to powder in liquid nitrogen and placed in 5 mL of extraction medium containing 50 mM Hepes-NaOH buffer (pH 7.5), 0.5 mM MgCl₂, 1 mM Na₂EDTA, 2 mM diethyldithiocarbamic acid, 2.5 mM dithiothreitol, 1% bovine serum albumin, and 2% polyvinylpyrrolidone. After centrifuging for 20 min at 12,000 g, the supernatant was collected and added into reaction solution to analyze the activity of SPS, SS, and AI. The reaction solution for the sucrose-synthesizing activity of SS included glucose and fructose as a substrate for the enzyme. The SPS reaction solution was composed of 15 mM UDP-glucose, 15 mM fructose 6-P, 5 mM MgCl₂·6H₂O, 5 mM NaF, 5 mM Na₂MoO₄·2H₂O, and 50 mM Hepes-NaOH buffer (pH 7.5). The blank reaction solution did not contact UDP-glucose. The reaction was stopped by the addition of 70 mL of 1 N NaOH. The activity of SPS and SS was measured using the resorcinol colorimetric method (Ruffy and Huber, 1983). For AI activity, 200 mL of plant extracts were incubated for 30 min at 37 °C with an equal quantity of 1 M sucrose and 600 mL phosphate citrate buffer (pH 5.0). A 1-mL aliquot of Sumner reagent (containing 8.8 g of iron SO₄·7 H₂O in 100 mL 3.75 M sulfuric acid and 900 mL of water) (Sumner, 1925) was added into the reaction solution to stop the reaction. Activity of SPS, SS, and AI was expressed as micromoles sucrose per gram FW per hour.

STATISTICAL ANALYSIS. Statistical significance of data was tested using the analysis of variance procedure with SAS (Version 9.0; SAS Institute, Cary, NC). Differences between treatment means were separated by Fisher's protected least significance difference test at the 0.05 *P* level.

Results

GENOTYPIC VARIATION IN TURF QUALITY AND PHYSIOLOGICAL RESPONSES TO DROUGHT AND RE-WATERING. Turf quality declined

in both cultivars in response to drought stress, and 'Midnight' plants had higher TQ compared with Brilliant (Fig. 1A). For 'Brilliant', TQ decreased below 5 × 10 d of drought stress, whereas TQ of 'Midnight' was maintained at the minimum acceptable level of 6.0. After re-watering treatment, TQ of 'Midnight' recovered to the level of well-watered control plants, whereas TQ of 'Brilliant' did not show any recovery as exhibited by a TQ rating of ≈4.0.

Leaf RWC declined from ≈80% under well-watered conditions to below 40% for both cultivars by 10 d of drought stress

Fig. 1. (A) Turf quality (TQ), (B) relative water content (RWC), and (C) electrolyte leakage (EL) in 'Midnight' and 'Brilliant' kentucky bluegrass at pre-stress (0 d) or well-watered conditions, 10 d of drought stress, and 3 d of re-watering (R3). TQ was visually rated on a scale from 1 (completely dead plants) to 9 (green and dense canopy). BC = well-watered 'Brilliant' control; BD = drought-stressed 'Brilliant'; MC = well-watered 'Midnight' control; MD = drought-stressed 'Midnight'. Vertical bars indicate least significant different values (*P* ≤ 0.05) for all treatments comparison at a given treatment.

(Fig. 1B). ‘Midnight’ maintained higher RWC than ‘Brilliant’ under drought stress and recovered from 39% at 10 d to 90% at 3 d re-watering compared with well-watered control. However, RWC of ‘Brilliant’ did not fully recover after re-watering.

Electrolyte leakage increased in response to drought stress for both cultivars but was higher for ‘Brilliant’ regardless of drought or re-watering treatments (Fig. 1C). Although EL of ‘Midnight’ was increased by drought stress, the levels decreased to that of well-watered control treatment within 3 d of re-watering. The EL of ‘Brilliant’ did not return to the control level on re-watering.

GENOTYPIC VARIATIONS IN CARBOHYDRATE RESPONSES TO DROUGHT AND RE-WATERING. ‘Brilliant’ exhibited higher total SC content compared with ‘Midnight’ under well-watered conditions (Fig. 2A). Drought stress resulted in rapid decreases in SC in both cultivars. After re-watering treatment, SC content recovered to some extent but did not reach the levels of control plants. SC content did not differ between two cultivars under drought or re-watering conditions. Fructan content was higher in ‘Brilliant’ than ‘Midnight’ under well-watered conditions and the cultivar differences diminished under drought stress (Fig. 2B). After 10 d of drought stress, fructan content of both ‘Brilliant’ and ‘Midnight’ dramatically declined by 56% and 39%, respectively, compared with pre-stressed plants. Post-drought re-watering induced full recovery in fructan content in ‘Midnight’ but not in ‘Brilliant’, which only recovered 12% from the drought-stressed condition. Drought stress led to higher accumulation of starch in ‘Brilliant’ than in control plants but not in ‘Midnight’ (Fig. 2C). No significant differences in starch content were found between ‘Midnight’ and ‘Brilliant’ in drought-stressed plants. After 3 d of re-watering treatment, starch content was greater in ‘Brilliant’ than in ‘Midnight’.

The content of WSC (Fig. 3A), sucrose (Fig. 3B), and reducing sugars (Fig. 3C) remained relative constant during the duration of well-watered treatment for both cultivars. At 10 d of drought stress, WSC and sucrose content increased to a higher level than their respective well-watered controls for both cultivars, but ‘Midnight’ had a much greater amount of WSC and sucrose but lower content of reducing sugars than ‘Brilliant’. After 3 d of re-watering, WSC and sucrose content returned to the well-watered control level in both cultivars and reducing sugar content recovered in ‘Midnight’, whereas reducing sugar content of ‘Brilliant’ increased above the well-watered control level. In addition, ‘Midnight’ had higher sucrose but lower reducing sugar content than ‘Brilliant’ at 3 d of re-watering.

ACTIVITY OF SUCROSE METABOLIC ENZYMES RESPONSES TO DROUGHT AND RE-WATERING. ‘Brilliant’ had higher activities of SPS (Fig. 4A), SS (Fig. 4B), and AI (Fig. 4C) than ‘Midnight’ in the well-watered control treatment. Under drought stress, SPS and SS activities increased in ‘Midnight’, which was greater than those in ‘Brilliant’. After re-watering, the activities of SPS and SS showed significant decreases to the same level as the well-watered control for ‘Midnight’, but for ‘Brilliant’, the activities of SPS and SS of previously drought-stressed plants were lower than the well-watered control. In addition, at re-watering, ‘Midnight’ maintained higher SPS and SS activities than ‘Brilliant’. ‘Brilliant’ also had higher AI activity under well-watered conditions, but after drought stress, AI activity decreased to the same level in both cultivars. After re-watering, the activity of AI resumed to the level of well-watered control

Fig. 2. Content of (A) water-soluble carbohydrates (WSC), (B) sucrose, and (C) reducing sugars in ‘Midnight’ and ‘Brilliant’ kentucky bluegrass at pre-stress (0 d) or well-watered conditions, 10 d of drought stress, and 3 d of re-watering (R3). BC = well-watered ‘Brilliant’ control; BD = drought-stressed ‘Brilliant’; MC = well-watered ‘Midnight’ control; MD = drought-stressed ‘Midnight’. Vertical bars indicate least significant different values ($P \leq 0.05$) for all treatments comparison at a given treatment.

plants in ‘Midnight’ but did not recover in ‘Brilliant’, which was still lower than well-watered plants.

Discussion

Physiological analysis confirmed that ‘Midnight’ had superior drought tolerance or survivability over ‘Brilliant’, as manifested by greater TQ, greater RWC, and lower EL at the same level of SWC (4% at 10 d of drought stress) and greater post-drought recovery of these parameters on 3 d of re-watering. The results in the differential drought tolerance between these

Fig. 3. Content of (A) storage carbohydrates (SC), (B) fructan, and (C) starch in 'Midnight' and 'Brilliant' kentucky bluegrass at pre-stress (0 d) or well-watered conditions, 10 d of drought stress, and 3 d of re-watering (R3). BC = well-watered 'Brilliant' control; BD = drought-stressed 'Brilliant'; MC = well-watered 'Midnight' control; MD = drought-stressed 'Midnight'. Vertical bars indicate least significant different values ($P \leq 0.05$) for all treatments comparison at a given treatment.

two cultivars were consistent with previous studies (Hu et al., 2010; Merewitz et al., 2010; Xu et al., 2011a, 2011b). The underlying mechanisms for genetic variability in drought tolerance in kentucky bluegrass and cool-season turfgrass species in general are far from being completely understood. The superior leaf dehydration tolerance has been attributed to greater cellular membrane stability associated with accumulation of saturated fatty acids [palmitic acid and stearic acid (Xu et al., 2011a)] and stronger antioxidant defense mechanisms, particularly active enzymes involved in H_2O_2 scavenging (Xu

Fig. 4. Activities of (A) sucrose phosphate synthase (SPS), (B) sucrose synthase (SS), and (C) acid invertase (AI) in 'Midnight' and 'Brilliant' kentucky bluegrass at pre-stress (0 d) or well-watered conditions, 10 d of drought stress, and 3 d of re-watering (R3). BC = well-watered 'Brilliant' control; BD = drought-stressed 'Brilliant'; MC = well-watered 'Midnight' control; MD = drought-stressed 'Midnight'. Enzyme activities were expressed on the basis of fresh weight. Vertical bars indicate least significant different values ($P \leq 0.05$) for all treatments comparison at a given treatment.

et al., 2011b). The recuperative ability from drought damages after re-watering has been associated with rapid membrane repairs and increases in antioxidant enzyme activities (Wang and Huang, 2004; Xu et al., 2011a, 2011b). DaCosta and Huang (2006) reported that increased accumulation of TNC in leaves and stems of bentgrass species may contribute to osmotic adjustment and energy reserves, enhancing rapid regrowth of plants after re-watering. This study found that the differential changes in different types of carbohydrates could be associated with the genetic variability of drought tolerance and

post-drought recovery in kentucky bluegrass, which are discussed below in terms of biological functions of specific carbohydrates.

The accumulation of soluble carbohydrates is a vital mechanism for plant adaptation to drought stress (Jiang and Huang, 2001; Volaire and Thomas, 1995; Zhou and Yu, 2010; Živković et al., 2005). Soluble sugars such as reducing sugars (fructose and glucose) and sucrose produced during photosynthesis are critical for various metabolic processes such as osmolytes for cellular turgor maintenance and energy sources, and sucrose also plays protective roles in proteins and membranes from stress damages (Couée et al., 2006). Sucrose is the predominant disaccharide transported throughout the plant for carbon supply (Counce and Gravois, 2006). In this study, increased accumulation of WSC (including sucrose and reducing sugars) by 10 d of drought stress was observed in 'Midnight' and 'Brilliant' compared with pre-stressed control plants, but to a greater extent in 'Midnight'. The increase in WSC with drought stress and the greater accumulation of WSC in 'Midnight' were mainly the result of the accumulation of sucrose, but they were not related to reducing sugar content, because reducing sugar content decreased in 'Midnight' at 10 d of drought stress. Increased sucrose availability during drought stress might be used for maintaining plant growth of the drought-tolerant cultivar. The accumulation of sucrose in response to drought stress has been reported in various other plant species (Fu et al., 2010; Kaur et al., 2007; Zrenner and Stitt, 1991). After 3 d of re-watering, the content of WSC, sucrose, and reducing sugars content of plants previously exposed to drought stress returned back to the well-watered control level in 'Midnight' but not in 'Brilliant'. The remarkable reduction in WSC and sucrose in 'Midnight' on re-watering may be the result of the increased consumption of carbohydrate for regrowth. These results suggested that sucrose could be a critical soluble sugar conferring drought tolerance and post-drought recovery in kentucky bluegrass.

The mechanism for sucrose response to changes in water availability could be confirmed by activity changes of SPS, SS, and AI, which are key enzymes controlling sucrose metabolism, although other enzymes such as UDP glucose pyrophosphorylase are also involved (Turner and Turner, 1975). SPS and SS were two pivotal enzymes responsible for the biosynthesis of sucrose, whereas AI catalyzes the breakdown of sucrose, which acts as a major regulator of cellular metabolism (Huber and Huber, 1996; Santarius, 1973). Corresponding to the increase in sucrose content after 10 d of drought stress, higher activities of SPS and SS and lower AI activity were also observed in drought-tolerant 'Midnight'. For drought-sensitive 'Brilliant', neither sucrose content nor the activities of sucrose-metabolizing enzymes changed in response to drought. The combined effects of SPS, SS, and AI activities could be responsible for sucrose accumulation in the drought-tolerant cultivar. Fu et al. (2010) reported that activities of leaf SPS and SS increased and AI activity declined with the increase in sucrose accumulation in tall fescue. Puebla et al. (1997) found the higher activities of leaf SPS and SS compared with that of the control plants after 7 d of drought treatment in *Bromus auleticus*. Sucrose accumulation and increases in activities of SPS and SS and decline in AI activity have also been found in drought-tolerant cultivars of wheat (*Triticum aestivum*), but not in drought-sensitive cultivars (Kaur et al., 2007). In this study, cultivars differed in SPS and SS activity, but not AI activity, at

10 d of drought stress. Our results suggested that sucrose synthesis could be a unique characteristic of the drought-tolerant cultivar, because both sucrose content and sucrose-synthesizing enzymatic activities were enhanced to higher levels in 'Midnight' under drought stress relative to drought-sensitive 'Brilliant'.

Fructans and starch are mainly used as storage carbohydrates for energy reserves under stressful conditions, and the former is the predominant form of SC in cool-season grass species (Fry and Huang, 2004). Fructan and SC content decreased rapidly with drought stress for 10 d, whereas starch content was relatively unchanged in both cultivars, suggesting drought-induced decline in SC was mainly the result of the decrease in fructan content in kentucky bluegrass, but not related to starch. Similarly, Spollen and Nelson (1994) reported that the content of fructan of water-stressed plants declined to 60% of well-watered control plants in tall fescue. The cultivar difference in SC content could also be the result of the difference in fructan content rather than starch, because 'Brilliant' has higher SC and fructan content than 'Midnight' under well-watered and drought stress conditions, whereas starch content did not differ between the two cultivars at 10 d of treatment. The decrease of fructan content might result from decreased synthesis and/or increased depolymerization (Spollen and Nelson, 1994). The increased sucrose content in contrast to the decline in fructan at 10 d of drought stress for 'Midnight' indicates that fructan synthesis could be inhibited at the first step catalyzed by sucrose:sucrose fructosyl transferase (SST), which converts two molecules of sucrose into fructan trisaccharide. Drought inhibited SST activity in tall fescue (Spollen and Nelson, 1994). The decline in fructan could be associated with the accumulation of sucrose, which could be used as readily available sugars for the support of plant growth under drought stress in the drought-tolerant cultivar. In contrast to the accumulation of soluble sugars, the content of SC and fructan decreased after re-watering in 'Midnight', which could be reflective of the resumption of carbohydrate storage capacity when plants resumed growth at re-watering. The differential changes of fructan in response to drought and re-watering for drought-tolerant 'Midnight' suggested that fructan could be associated with re-growth when water was available after drought rather than with sustained growth during a drought period, as previously reported in perennial ryegrass [*Lolium perenne* (Thomas and James, 1999)].

In summary, this study demonstrated that superior drought resistance for 'Midnight' kentucky bluegrass could be characterized by the accumulation of sucrose in association with increased activity of sucrose-synthesizing enzymes (SPS and SS). Decreased accumulation of fructan could also contribute to the accumulation of sucrose under drought stress, imparting drought resistance in 'Midnight'. Post-drought recovery in 'Midnight' was associated with increases in the availability of fructan, which could facilitate plant re-growth after re-watering. Sucrose accumulation and fructan availability could be used as a biomarker in the selection for improved drought tolerance and post-drought recovery, respectively, in cool-season perennial grasses.

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