Osmotic Potential, Sucrose Level, and Activity of Sucrose Metabolic Enzymes in Tall Fescue in Response to Deficit Irrigation

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ABSTRACT. Effects of deficit irrigation applied to home lawns, used as means of water conservation, are an important issue. However, the impact of deficit irrigation on sucrose metabolism in tall fescue (*Festuca arundinacea*) is unknown and important because sucrose is the dominant form of carbohydrate transported to developing plant organs. The objectives of this study were to investigate the effects of deficit irrigation on leaf water content, osmotic potential (ψ_s), sucrose level, and the activity of sucrose phosphate synthase (SPS; EC 2.4.1.14), sucrose synthase (SS; EC 2.4.1.13), and acid invertase (AI; EC 3.2.1.26) in tall fescue leaves. Sods of 'Falcon II' tall fescue were established in polyvinylchloride (PVC) tubes (10 cm diameter $\times$ 40 cm long) filled with a mixture of sand and fritted clay [9:1 (v:v)] and then placed in growth chambers. Reference evapotranspiration rate [ET_o (millimeters of water per day)] was determined by weighing the PVC tubes containing well-watered turfgrass every 3 days to determine water loss on a daily basis as ET_o. Deficit irrigation treatments were applied as follows: well-watered control, mild drought stress (60% ET_o), and severe drought stress (20% ET_o). Leaf water content was lower at 6, 12, and 20 days of treatment for the 20% ET_o treatment and 20 days after treatment began for the 60% ET_o treatment. Compared with the well-watered control, ψ_s was lower in the 60% ET_o treatment on all three measurement dates. Sucrose was higher at 8 and 14 days after treatment began in the 60% ET_o treatment and on all three measurement dates in the 20% ET_o treatment relative to the well-watered control. No difference in sucrose level was observed between the 20% ET_o and 60% ET_o irrigation regimes at 8 and 14 days of treatment. Beginning 14 days after treatment, tall fescue had a higher level of SPS in the 60% ET_o and 20% ET_o treatments compared with the well-watered treatment. Tall fescue receiving 60% or 20% ET_o had a lower level of AI activity on all measurement dates. Results suggest that the decrease in ψ_s was accompanied by higher sucrose levels, which were the result of the increased level of SPS and SS activity and a decline in AI activity.

Proper irrigation management is critical to growing quality turfgrass with limited water in arid and semiarid regions. Deficit irrigation, defined as applying water in amounts less than the reference evapotranspiration rate, is an irrigation management practice that could result in water savings (Fu et al., 2007). The ET_o, measured as amount of daily water loss from a canopy under non-water-limiting conditions, often is used to estimate water requirements in turfgrass irrigation. Reduction in irrigation application not only can reduce costs associated with water consumption and can improve environmental stress tolerance, but it also prevents turfgrass from the injury of mechanical stresses, cyanobacteria, and diseases (Beard, 1973; Dernoeden, 2002; Turgeon, 2008).

Some researchers have found that turfgrass is able to tolerate moderate drought (DaCosta and Huang, 2006; Fu et al., 2004; Gilbeault et al., 1985). Tall fescue, bermudagrass (*Cynodon dactylon*), and zoysiagrass (*Zoysia japonica*) irrigated to 60% or 80% of ET_o exhibited similar turfgrass quality when

compared with well-watered turfgrass (Fu et al., 2004). Gilbeault et al. (1985) also observed that tall fescue, kentucky bluegrass (*Poa pratensis*), and perennial ryegrass (*Lolium perenne*) had only a slightly lower level of quality when irrigated at 80% ET_o relative to 100% ET_o.

Availability of total nonstructural carbohydrate (TNC) has been widely used as a physiological measure of stress tolerance, because carbohydrates provide energy and solutes for osmotic adjustment. Sucrose, an important component of TNC, is the dominant form of carbohydrate transported to developing plant organs and is one of the sugars stored in higher plants (Khayat and Zieslin, 1987). Sucrose also serves as an osmotic solute (Premachandra et al., 1992; Rekika et al., 1998; Tan et al., 1992; Zhang and Archbold, 1993). The effect of water deficits on sucrose levels has been reported in some plants. For example, improved responses of bean (*Phaseolus vulgaris*) to water deficits were associated with sucrose metabolism (Castrillo, 1992; Vasey et al., 1991). McManus et al. (2000) found that after white clover (*Trifolium repens*) was exposed to a period of moderate drought stress, leaf sucrose content increased significantly. Leaf sucrose level increased by 300% at the end of an

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8-d-long drought period in sugarbeet (*Beta vulgaris*) (Harn and Daie, 1992).

Because sucrose may serve an important role in drought tolerance, understanding the enzyme activity affecting sucrose metabolism is critical. Sucrose synthesis can be regulated by rapid changes in the activity of sucrose phosphate synthase, sucrose synthase, and acid invertase (Castrillo, 1992; Hawker, 1985; Huber and Huber, 1996). Sucrose phosphate synthase catalyzes the synthesis of sucrose-phosphate from uracil-diphosphate (UDP)-glucose and fructose-6-phosphate, and this reaction occurs predominantly in the cytosol of sucrose-source leaf tissue. Leaf SPS activity is often correlated with the rate of sucrose synthesis and export (Huber and Israel, 1982; Rocher et al., 1989; Stitt et al., 1988). Some researchers found that water deficits lead to an increase in SPS activity in potato tubers [*Solanum tuberosum* (Geigenberger et al., 1997)], soybean leaves [*Glycine max* (Cheikh and Brenner, 1992)], and pigeon-pea leaves [*Cajanus cajan* (Keller and Ludlow, 1993)]. However, Castrillo (1992) reported that the values of total (substrate-saturating conditions) and Pi-insensitive (substrate limiting conditions plus inorganic phosphate) SPS activity in *P. vulgaris* were reduced by drought stress. These conflicting results suggest that effects of drought stress on SPS activity depend on experimental conditions. Sucrose synthase catalyzes both the synthesis and cleavage of sucrose. Yang et al. (2001) reported that water deficits enhanced SS activity in the cleavage direction, but the activity of SS in the synthesis direction was not measured. Castrillo (1992) reported that the synthesis activity of SS was increased by water deficit. In contrast, the effects of drought on AI appear to be negative. AI catalyzes the hydrolysis of sucrose into glucose and fructose. Dorion et al. (1996) observed that the activity of soluble AI in wheat (*Triticum aestivum*) declined fourfold during a drought stress period and never recovered.

Effects of drought stress on sucrose metabolism have been examined intensively, mainly in annual crops, as discussed previously. However, how different levels of deficit irrigation influence sucrose metabolism and associated enzymes are not well understood, because plants are often subjected to different levels of irrigation regimes or levels of drought stress. The objectives of this study were to address the question by examining the influence of deficit irrigation on leaf ψ_s , sucrose level, and sucrose metabolic enzymes in a cool-season grass species, tall fescue. This species is a widely used species as turfgrass, which has superior drought resistance to many other cool-season grass species (Fry and Huang, 2004). Tall fescue is widely used in temperate climates, because it tolerates heat and drought well compared with other cool-season turfgrasses (Fry and Huang, 2004). Greater knowledge of these responses might provide insights into drought resistance mechanisms of cool-season grass species in areas with varying irrigation availability.

Materials and Methods

PLANT GROWTH CONDITIONS. Sod pieces of 'Falcon II' tall fescue were collected from the field on 4 Nov. 2001 and planted on a mixture of sand and fritted clay [9:1 (v:v); Profile Products, Deerfield, IL] contained in PVC tubes (10 cm diameter $\times$ 40 cm long). The grasses were grown for $\approx$ 90 d in growth chambers with a temperature of 25/20 °C (day/night) and a 14-h photoperiod with a photosynthetic active radiation of 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. During the 90-d period of plant establishment,

the turfgrass was well irrigated until drainage occurred from the bottom of the containers. It was fertilized weekly with half-strength Hoagland's solution (Hoagland and Arnon, 1950), and it was mowed every other day at a 5 to 6 cm height with an electric clipper.

IRRIGATION TREATMENTS AND EXPERIMENTAL DESIGN. The tall fescue was subjected to three irrigation regimes: 1) fully irrigated control to replace 100% water loss measured as ETo; 2) moderate deficit irrigation to replace 60% of ETo; and 3) severe deficit irrigation to replace 20% of ETo. Reference evapotranspiration rate was measured using the water balance method described by Fu et al. (2004). It was determined by weighing, between 0900 and 1000 HR, PVC tubes containing well-watered turfgrass at 3 d after treatment began to determine daily water loss (expressed as millimeters per day ETo). For the different irrigation regimes, the amount of water needed to provide 20%, 60%, or 100% of ETo was added to each container corresponding to each treatment. Reference evapotranspiration was measured and the amount of water needed was added to each container every other 3 d based on the previously described methods. The study was set up as a randomized block design. Each treatment was replicated four times with one replication in each growth chamber (Convion, Winnipeg, Canada).

MEASUREMENTS. To measure leaf water content (LWC), $\approx$ 0.5 g of fresh leaves was collected and weighed immediately. Then, the leaf samples were killed at 105 °C, dried at 75 °C in a convection oven until the sample weight became constant, and reweighed. Leaf water content was calculated based on the difference between leaf fresh weight and dry weight. Dry leaves were used for sugar analysis.

Leaf ψ_s was measured on fully expanded fresh leaves using the method described by Qian and Fry (1997). Excised fresh leaves were placed in a microcentrifuge tube, immediately frozen in liquid nitrogen, and stored in -20 °C until analyzed. Frozen leaves were thawed for 30 min and cell sap was pressed using a laboratory press (Fred S. Carver, Wabash, IN). A 10- μL aliquot of the expressed sap was injected onto a filter paper disc that was placed in the sampling chamber of an osmometer (Wescor, Logan, UT). Osmolarity of cell sap was converted from millimoles per kilogram to ψ_s (megapascals) using the formula: $\text{MPa} = -c \times 2.58 \times 10^{-3}$, where c is osmolarity (millimoles per kilogram).

Concentration of sucrose was determined using the method of Liu and Huang (2000). Dry leaves were incubated in 10 mL 0.1 M phosphate buffer (pH 5.4) for 24 h at 22 °C. A 0.2-mL sample of supernatant was mixed with 1.0 mL invertase (10 U/mL) or distilled water and incubated in a water bath for 1 h at 50 °C. The difference in reducing sugar concentrations between the incubation solution with and without invertase was used to calculate sucrose concentration (Ting, 1956). The amount of reducing sugar was determined using the method of Ting (1956). One milliliter of the extraction solution was transferred to a 100-mL flask, and 5 mL of ferricyanide reagent was added. Flasks were placed in a boiling water bath for 10 min. After heating, the flasks were cooled quickly in running water. The solutions were neutralized partially with 10 mL of a 1 M H_2SO_4 solution and mixed thoroughly until no more gas was evolved. Four milliliters of arsenomolybdate were added, and solutions were mixed again and then diluted to volume. The absorbance of the solution was measured at 515 nm with a spectrophotometer (Spectronic Instruments, Rochester, NY). The amount of

sugar in the solution was calculated using a glucose standard curve as described by Ting (1956).

For enzyme analysis, 0.5-g fresh leaf samples were placed in liquid nitrogen and stored in a freezer at -80°C . To extract enzymes, frozen leaf samples were ground to powder using a mortar and pestle in liquid nitrogen. To the ground sample was added 5 mL of extraction medium containing 50 mM Hepes-NaOH buffer (pH 7.5), 0.5 mM MgCl_2 , 1 mM Na_2EDTA , 2 mM diethyldithiocarbamic acid, 2.5 mM dithiothreitol, 1% bovine serum albumin, and 2% polyvinylpyrrolidone. The extract was centrifuged for 20 min at $12,000\text{ g}$. The supernatant was assayed for SPS, SS, and AI activity. Sucrose synthase catalyzes both sucrose synthesis and sucrose degradation. The catalytic activity of SS for sucrose synthesis was measured using glucose and fructose as a substrate for the enzyme. Assays for SPS and SS were performed by incubating (70 μL) tissue extracts for 30 min at 37°C with an equal volume of reaction mixture according to Khayat and Zieslin (1987). The SPS assay contained 15 mM UDP-glucose, 15 mM fructose 6-P, 5 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5 mM NaF, 5 mM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, and 50 mM Hepes-NaOH buffer (pH 7.5). For the SS assay, fructose 6-P was replaced by fructose. The reaction was ended by the addition of 70 μL of 1 N NaOH, and sucrose formation was determined by the resorcinol colorimetric method (Rufty and Huber, 1983). Enzymatic activity was determined by reference to a blank reaction mixture without UDP. Acid invertase activity was determined according to Khayat and Zieslin (1987). Aliquots (200 μL) of plant extracts were incubated for 30 min at 37°C with an equal quantity of 1 M sucrose and 600 μL phosphate citrate buffer (pH 5.0). The reaction was ended by the addition of 1 mL of Sumner reagent (containing 8.8 g of iron $\text{SO}_4 \cdot 7\text{H}_2\text{O}$ in 100 mL 3.75 M sulfuric acid and 900 mL of water) (Sumner, 1925). Enzyme blanks were incubated in Sumner reagent. Activity was expressed as the quantities of reducing sugars (i.e., micromoles of sucrose per gram fresh weight per hour) formed by sucrose hydrolysis (Khayat and Zieslin, 1987). Levels of SPS, SS, and AI were expressed as micromoles sucrose per gram fresh weight per hour.

STATISTICAL ANALYSIS. Treatment effects were determined by analysis of variance using the general linear model procedure of SAS (Version 9.1.3; SAS Institute, Cary, NC). Mean separation was performed using Fisher's protected least significant difference test ($P \leq 0.05$). The analysis of variance revealed an interaction between irrigation levels and sampling dates. Therefore, data from each date are shown separately.

Results

Compared with the well-watered regime, LWC was lower on all three dates for tall fescue subjected to the 20% ETo irrigation level and lower at 20 d after treatment began for the 60% ETo irrigated level (Table 1). Osmotic potential was lower in the 20% or 60% ETo treatment compared with the well-watered control on all three measurement dates. Among the three irrigation regimes, the lowest level of ψ_s was found in the 20% ETo treatment. When measured at 20 d of treatment, ψ_s was 36.6% and 171.4% lower in the 60% or 20% ETo treatments, respectively, relative to the well-watered tall fescue.

For the 60% or 20% ETo treatment 8 d after irrigation water was withheld, sucrose content in tall fescue leaves increased above that in the well-watered control (Fig. 1). When measured

Table 1. Leaf water content and osmotic potential in 'Falcon II' tall fescue subjected to three irrigation levels.

Irrigation (% ETo) ^z	Duration of irrigation treatment (d)		
	6	12	20
	Leaf water content (%)		
100	73.3 a ^y	72.7 a	72.7 a
60	70.8 a	70.0 a	68.3 b
20	60.1 b	54.5 b	58.9 c
	Osmotic potential (MPa)		
100	-1.95 c	-1.76 c	-1.75 c
60	-2.21 b	-2.31 b	-2.39 b
20	-3.03 a	-4.49 a	-4.75 a

^zReference evapotranspiration (ET_o) of well-watered turfgrass.

^yMeans in a column for each day followed by the same letter are not significantly different based on Fisher's protected least significant difference test at $P \leq 0.05$.

Fig. 1. Sucrose content in 'Falcon II' tall fescue leaves subjected to 20%, 60%, or 100% of reference evapotranspiration. Means in a column for each day followed by the same letter are not significantly different based on Fisher's protected least significant difference test at $P \leq 0.05$.

at 23 d of treatment, sucrose content in the plants subjected to the 60% ETo irrigation declined to a level similar to the well-watered control. However, at 23 d of treatment, sucrose content was 16.3% higher in 20% ETo treatment when compared with the well-watered control.

The enzymes involved in the metabolism of sucrose were also affected by the irrigation treatment. The tall fescue subjected to the 60% ETo irrigation and the 20% ETo irrigation exhibited an activity of SPS and SS that was similar at 8 d of treatment; however, leaves from the same treatment had higher SPS and SS activity levels at 14 and 23 d of treatment relative to the well-watered control (Table 2). At 23 d of treatment, SPS level was 26.3% higher in the 20% ETo treatment and 9.0% higher in the 60% ETo treatment relative to the well-watered control, respectively. Tall fescue subjected to 23 d of 20% and 60% ETo treatments exhibited an 11.3% and 16.6% higher SS activity, respectively, compared with the well-watered plants (Table 2). On all three measurement dates, AI activity was lower in the 20% or 60% ETo treatment relative to the well-watered control (Table 2). At 23 d of treatment, AI activity was 6.3% and 16.1% lower in the 60% and 20% ETo treatments, respectively, than that of the well-watered control.

Table 2. Activity of sucrose phosphate synthase (SPS), sucrose synthase (SS), and acid invertase (AI) in 'Falcon II' tall fescue leaves subjected to three irrigation levels.

Irrigation (% ETo) ^z	Duration of irrigation treatment (d)		
	8	14	23
	SPS activity [sucrose ($\mu\text{mol}\cdot\text{g}^{-1}$ fresh wt per hour)]		
100	256.0 a ^y	198.3 b	220.2 c
60	231.1 a	235.3 a	240.1 b
20	232.9 a	246.3 a	278.1 a
	SS activity [sucrose ($\mu\text{mol}\cdot\text{g}^{-1}$ fresh wt per hour)]		
100	208.9 a	165.9 c	228.6 b
60	186.3 a	248.3 a	254.5 a
20	186.7 a	199.0 b	266.5 a
	AI activity [sucrose ($\mu\text{mol}\cdot\text{g}^{-1}$ fresh wt per hour)]		
100	66.6 a	55.1 a	57.3 a
60	58.9 b	47.0 b	53.7 b
20	42.3 c	39.7 c	48.1 c

^zReference evapotranspiration (ET_o) of well-watered turfgrass.

^yMeans in a column for each day followed by the same letter are not significantly different based on Fisher's protected least significant difference test at $P \leq 0.05$.

Discussion

Lowering of ψ_s during drought contributes to enhanced drought tolerance because it maintains a favorable water status for plants with limited water availability (Zhou and Yu, 2010). In this study, a greater reduction in ψ_s was observed in tall fescue receiving 60% ETo (28.7% lower ψ_s) and 20% ETo (136.9% lower ψ_s) compared with 100% ETo treatment when data were averaged over three measurement dates. This finding is in agreement with previous reports (DaCosta and Huang, 2006; Qian and Fry, 1997). For example, Qian and Fry (1997) reported that when soil water content declined to 18% for tall fescue and 16% for warm-season grasses [i.e., zoysiagrass, bermudagrass, and buffalograss (*Buchloe dactyloides*)], ψ_s dropped rapidly. Eight to 15 d after irrigation was stopped, drought stress resulted in increased osmotic adjustment for both creeping bentgrass (*Agrostis stolonifera*) and velvet bentgrass (*Agrostis canina*) (DaCosta and Huang, 2006).

Decreased ψ_s is accompanied by the accumulation of sucrose. Morgan (1984) reported that the accumulation of solutes such as water-soluble carbohydrate [WSC (i.e., glucose, fructose, and sucrose)] is associated with active osmotic adjustment when plants are subjected to soil water deficits. DaCosta and Huang (2006) reported that creeping bentgrass plants osmotically adjusted to dehydration stress by accumulating WSC. Similarly, Jiang and Huang (2001) observed that drought-preconditioned kentucky bluegrass had 21% to 44% higher leaf WSC than non-preconditioned plants. Spollen and Nelson (1994) found that sucrose content increased 258% in the leaf base of tall fescue when averaged over 78, 102, and 126 h after water was withheld. In our study, sucrose content was significantly higher than that of the well-watered plants at 100% ETo irrigation at 8 d of deficit irrigation treatments. Sucrose content under long-term deficit irrigation was not different from that in well-watered fall fescue. The early increase in sucrose content may be an adaptive response to a short-term water deficit, because the water deficit may induce a shift in the partitioning of carbon in favor of sucrose synthesis (Castrillo, 1992; Daie, 1988).

Sucrose phosphate synthase, SS, and AI are major enzymes involved in sucrose metabolism (Mendicino, 1960; Rufty and Huber, 1983). In this study, tall fescue subjected to the 20% and the 60% ETo treatment had a higher level of SPS activity than plants with the 100% ETo. This may indicate that the demand for sucrose as an osmotic solute activates SPS activity under drought stress conditions. Sucrose synthase catalyzes both sucrose synthesis and degradation. In the present study, SS was measured as an enzyme for sucrose synthesis. Our data indicated that short-term deficit irrigation at 60% or 20% ETo enhanced SS activity and reduced AI activity in tall fescue. The changes became more pronounced with prolonging treatment duration. The effects of water deficit on SS and AI have been reported in other species (Castrillo, 1992; Dorion et al., 1996; Naya et al., 2007). Castrillo (1992) reported that leaf SS activity for sucrose synthesis was greater when *P. vulgaris* plants were exposed to water deficit than when they were well-watered. However, Vassey et al. (1991) did not find a higher level of SS activity when *P. vulgaris* plants were subjected to water deficit. No changes in SS activity were observed in alfalfa (*Medicago sativa*) nodules subjected to a moderate drought (leaf water potential of -1.3 MPa) (Naya et al., 2007). Acid invertase is involved in sucrose hydrolysis. In contrast to SS, AI activity in this study was reduced under long-term deficit irrigation. These results are similar to other studies. For example, at anthesis of wheat, AI activity in anthers from water-stressed plants was half that observed in well-watered controls (Dorion et al., 1996). Stancato et al. (2001) reported that activities of AI were reduced in leaves of drought-stressed epiphytic orchids (*Cattleya forbesii* $\times$ *Laelia tenebrosa*). These results implied that an increase in SS and a decrease in AI could partially contribute to the sucrose increase that occurred early in our experiment (before the first measurement day, 8 d after water was withheld). Kaur et al. (2007) reported higher sucrose content accompanied by a higher SPS and a lower AI and SS (in the cleavage direction) activities in wheat roots under water deficit, and this could be responsible for drought tolerance in wheat.

In summary, we demonstrated that tall fescue subjected to deficit irrigation generally exhibited a reduced LWC and ψ_s for tall fescue. Plants exposed to short-term (8 d) deficit irrigation had higher leaf sucrose content than well-irrigated controls. Higher sucrose levels in the leaves of water-stressed tall fescue might contribute to improved drought tolerance by decreasing ψ_s in leaves in response to prolonged periods of water stress. Sucrose phosphate synthase, SS, and AI enzymes play an important role in sucrose accumulation.

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