Metabolic Responses of Hybrid Bermudagrass to Short-term and Long-term Drought Stress

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ABSTRACT. The accumulation of different types of metabolites may reflect variations in plant adaptation to different severities or durations of drought stress. The objectives of this project are to examine changes in metabolomic profiles and determine predominant metabolites in response to short-term (6 days) and long-term (18 days) drought stress with gas chromatography–mass spectrometry analysis in a C₄ perennial grass species. Plants of hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis* cv. Tifdwarf) were unirrigated for 18 days to induce drought stress in growth chambers. Physiological responses to drought stress were evaluated by visual rating of grass quality, relative water content, photochemical efficiency, and electrolyte leakage (EL). All parameters decreased significantly at 6 and 18 days of drought stress, except EL, which increased with the duration of drought stress. Under short-term drought stress (6 days), the content did not change significantly for most metabolites, except methionine, serine, γ -aminobutyric acid (GABA), isoleucine, and mannose. Most metabolites showed higher accumulation under long-term drought stress compared with that under the well-watered conditions, including three organic acids (malic acid, galacturonic acid, and succinic acid), 10 amino acids (proline, asparagine, phenylalanine, methionine, serine, 5-hydroxynorvaline, GABA, glycine, theanine, valine), seven sugars (sucrose, glucose, galactose, fructose, mannose, maltose, xylose), one nitrogen compound (ethanolamine), and two-sugar alcohol (myo-inositol). The accumulation of those metabolites, especially malic acid, proline, and sucrose, could be associated with drought adaptation of C₄ hybrid bermudagrass to long-term or severe drought stress.

Water stress is one of the most widespread abiotic stresses limiting plant growth in many areas in the world (Neill et al., 2008). Drought stress affects numerous metabolic processes in plants, including synthesis of primary metabolites such as carbohydrates, amino acids, and organic acids (Hu et al., 2010; Levitt, 1980; Seki et al., 2007). Organic acid, sugars, sugar alcohols, amino acids, and amines are major solutes responsive to drought stress, which are known to play critical roles in plant adaptation to drought stress, because these primary metabolites may serve as energy reserves, osmolytes to maintain cell turgor, antioxidants, byproducts of stress, or as signal transduction molecules (Bartels and Sunkars, 2005; Seki et al., 2007; Shulaev et al., 2008).

Different types of metabolites may accumulate in response to drought stress in different plant species, depending on plant species varying in drought tolerance and the severity of drought stress in different studies. Rizhsky et al. (2004) found high expression of citric acid, β -alanine, proline (Pro), tyrosine (Tyr), valine (Val), 1,3-diaminopropane, ethanolamine, putrescine, galactose, glucose, and others, especially Pro, under mild drought stress (6 to 7 d without irrigation) in arabidopsis

(*Arabidopsis thaliana*). Ashraf and Iram (2005) demonstrated that long-time drought stress induced free amino acid increase in two legume species (*Phaseolus vulgaris* and *Sesbania aculeate*). Significant increases of amino acid and soluble sugar levels were found at 7 d of drought stress in alfalfa [*Medicago sativa* (Aranjuelo et al., 2011)]. Different from most results, Vasquez-Robinet et al. (2008) believed that differences in drought tolerance of two Andean potato genotypes (*Solanum tuberosum* subspecies) could not be explained by protective roles of compatible solutes at prolonged drought stress.

Most work in drought-responsive metabolites has been performed in annual crops or arabidopsis, which were exposed to mild or a moderate level of drought stress (Evers et al., 2010; Rizhsky et al., 2004; Roessner et al., 2000; Skirycz et al., 2010; Vasquez-Robinet et al., 2008). Perennial grass species used as turfgrass, particularly C₄ species such as hybrid bermudagrass, exhibited superior tolerance to severe drought stress (Hanna, 1998). Understanding differential metabolic changes in response short-term or mild stress and long-term or severe drought stress for drought-tolerant perennial grass species may provide further insights into metabolites or metabolic pathways involved in drought adaptation mechanisms. The objectives of this study were to examine changes in metabolites in response to short-term and long-term drought stress using gas chromatography–mass spectrometry (GC-MS) analysis and to identify predominant metabolites responsive to each level of drought stress in hybrid bermudagrass.

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Materials and Methods

PLANT MATERIALS, GROWING CONDITIONS, AND TREATMENTS.

Two-year-old mature sods of hybrid bermudagrass (cv. Tifdwarf) were transplanted from field plots to plastic pots (30 cm in diameter and 40 cm deep) filled with sandy loam soil (fine-loamy, mixed mesic Typic Hapludult). Plants were maintained in a greenhouse for 60 d in the Turfgrass Experiment Farm at Shanghai Jiao Tong University, Shanghai, China. Grasses were watered every 3 d and fertilized once per week with a controlled-release fertilizer (15N–6.5P–8.3K) at a total amount of 57 kg·ha⁻¹ nitrogen at 2 g per pot. Then plants were transferred to growth chambers (HP1500 GS-B; Wuhan Ruihua Instrument and Equipment, Wuhan, China) with temperature of 30/25 °C, 70% to 80% relative humidity, a 14-h photoperiod, and a photosynthetically active radiation of 510 μmol·m⁻²·s⁻¹. Plants were maintained in growth chambers for 7 d before drought treatments were imposed to allow acclimation of plants to the growth chamber conditions.

Treatments included a well-watered control and drought stress. Well-watered plants were irrigated every 2 d to maintain soil water content at field capacity. Soil volumetric water content (SWC) was measured using a soil moisture meter (TDR 200; Spectrum Technologies, Plainfield, IL). In the drought stress treatment, plants were not watered for 18 d. There were four pots of plants as four replicates for each treatment. The plants in the two treatments were randomly placed inside a growth chamber and the experimental design was a completely randomized plot design.

PHYSIOLOGICAL ANALYSIS. Turf quality (TQ) was visually rated based on turf color and density on a 1 to 9 scale with 9 being the best (a fully turgid and green turf canopy) based on shoot color and density (Turgeon, 2008). The minimum acceptable level was 6. Leaf relative water content (RWC) was determined with 10 to 15 first and second fully expanded leaves per pot using the method described in Barrs and Weatherley (1962). Leaves were clipped and weighed [fresh weight (FW)] and placed in a tea bag in petri dishes filled with water to ensure both sides of leaves fully absorb water. They were soaked in water for ≈24 h at room temperature and then weighed immediately after excess moisture was removed with paper towels [turgid weight (TW)]. The leaves were then dried in an oven at 80 °C for 72 h to determine dry weight (DW). Leaf RWC was calculated as (FW – DW)/(TW – DW) × 100. Leaf photochemical efficiency was determined by measuring chlorophyll fluorescence of leaves, the ratio of variable fluorescence to maximal fluorescence (Fv/Fm), with a leaf photochemical efficiency analyzer (OS 1FL; Opti-Sciences, Hudson, NH) after dark adaptation for 30 min.

Cell membrane stability of leaves was estimated using the measurement of electrolyte leakage from leaf cells (Blum and Ebercon, 1981). EL of leaves was measured following the method described in DaCosta et al. (2004) with modifications. First and second fully expanded leaves with FW of 0.3 g per pot were excised and cut into 1-cm segments. After being rinsed three times with distilled water, leaf segments were placed into 50-mL vials containing 20 mL distilled water. After shaking for 24 h, initial conductivity (C_i) of the bathing solution was measured with a conductivity meter (DDS-11C; Tianjing Huike Instrument and Equipment, Tianjin, China). Leaves were then killed in an autoclave at 121 °C for 30 min and placed on a shaker for 24 h before final conductivity (C_f) of the bathing solution was measured. The EL was calculated as C_i/C_f × 100%.

METABOLITE EXTRACTION AND CONTENT ANALYSIS. For analysis of metabolite content in leaves under drought stress, leaves at 0, 6, and 18 d were collected and immediately frozen in liquid nitrogen and stored at –80 °C until further analysis. The extraction protocol was modified from Rizhsky et al. (2004) and Roessner et al. (2000). One hundred milligrams of leaves were ground to a fine powder with liquid nitrogen and were extracted in 1.4 mL of 80% (v/v) aqueous methanol and shaken at 200 g_r for 2 h in a shaking water bath (SHZ-88A; Suzhou Pui Ying Experimental Equipment, Suzhou, China) at ambient temperature (with 100 μL of 2 mg·mL⁻¹ ribitol solution as the internal standard). Then the samples were transferred in a water bath at 70 °C for 15 min. After centrifuging, the supernatant were extracted with 1.4 mL of water and 0.75 mL of chloroform. Three hundred microliters of the polar phase was decanted into high-performance liquid chromatography vials and dried in a Centrивap benchtop centrifugal concentrator (Labconco, Kansas City, MO). The dried polar phase was methoximated for 90 min at 30 °C with 80 μL of 20 mg·mL⁻¹ methoxyamine hydrochloride in pyridine and was trimethylsilylated with 80 μL N-methyl-N-(trimethylsilyl) trifluoroacetamide (with 1% trimethylchlorosilane) and 80 μL pyridine for 1 h at 70 °C. After methoximation and trimethylsilylation, the extracts were analyzed according Du et al. (2011) with a gas chromatograph coupled with a mass spectrometer (TurboMass-Autosystem XL; PerkinElmer, Waltham, MA). The metabolites detected were identified by Turbomass 4.1.1 software (PerkinElmer) coupled with commercially available compound libraries: NIST 98 (PerkinElmer) and Wiley 7.0 (John Wiley & Sons, Hoboken, NJ). The quantity of metabolites was expressed as relative content, which was calculated as the ratio of a peak area for a given metabolite relative to the internal standard (ribitol).

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS. Differences in physiological measurements and relative contents of

Table 1. Effects of the drought stress on soil volumetric water content, turf quality, relative water content, photochemical efficiency, and electrolyte leakage for hybrid bermudagrass (cv. Tifdwarf) for the well-watered control and after 6 and 18 d of drought.

Treatment	Soil volumetric water content [mean ± SE (%)]	Turf quality [mean ± SE (1–9 scale)] ^a	Relative water content [mean ± SE (%)]	Photochemical efficiency [mean ± SE (Fv/Fm)]	Electrolyte leakage [mean ± SE (%)]
Well-watered control	28.77 a ^y ± 0.60	8.50 a ± 0.00	89.62 a ± 1.16	0.76 a ± 0.00	7.03 b ± 0.35
Drought stress 6 d	23.04 b ± 0.31	7.75 b ± 0.00	82.60 b ± 0.54	0.75 b ± 0.00	4.09 b ± 0.30
18 d	5.70 c ± 0.28	3.81 c ± 0.08	32.13 c ± 1.99	0.63 c ± 0.00	23.00 a ± 4.96

^aVisually rated based on turf color and density with 9 being the best (a fully turgid and green turf canopy).

^yMeans followed by different lower case letter indicate significant differences between days of treatment based on least significant difference test ($P = 0.05$, $n = 4$).

metabolites between different treatment times were assessed by the least significance difference at $P = 0.05$ level. Statistical significance was determined by one-way analysis of variance using SAS (Version 9.1; SAS Institute, Cary, NC). For GC-MS results, compounds were identified based on retention time and comparison with reference spectra in mass spectral libraries. Peak areas of compounds were integrated with the Genesis algorithm (Thermo Fisher Scientific, San Jose, CA).

Results and Discussion

SWC was measured as an indication of the level of soil water deficit. Under well-watered conditions, SWC was maintained at 28.77%. After irrigation was withheld for 6 and 18 d, SWC declined significantly, and they were 23.04% at 6 d and 5.70% at 18 d (Table 1). The low SWC indicated that 18 d of drought imposed a severe stress to the plants. Leaf RWC is an indicator of the internal water status during dehydration (Abraham et al., 2004; Matin et al., 1989). Leaf RWC decreased to 82.60% at 6 d and was only 32.13% at 18 d of stress (Table 1), suggesting that the plants suffered from severe water deficit by 18 d of drought stress. Previous studies have reported that RWC of $\approx 30\%$ is a critical deficit level below which kentucky bluegrass (*Poa pratensis*) plants exhibited permanent damage and could not recover even after rewatering (Xu et al., 2011).

Leaf chlorophyll fluorescence reflects photochemical efficiency of the PS II system in the light reaction of photosynthesis (Genty et al., 1987; Kaiser, 1987). At 6 and 18 d of drought stress, Fv/Fm decreased to 0.75 and 0.63, respectively, from 0.76 in the control plants. The differences between long-time drought-stressed plants and the control plants were significant (Table 1). Cell membrane stability plays a critical role for maintaining normal physiological functions in plants under drought stress (Bajji et al., 2002; Rachmilevitch et al., 2006). EL has been widely used to estimate cell membrane stability (Bajji et al., 2002). At 6 d of drought stress, EL did not change significantly. At 18 d of drought stress, EL increased by 227.17% compared with that of the control plants (Table 1). Turf quality is often used to evaluate overall turf performance. As shown in Table 1, TQ declined during drought stress, which remained at the acceptable level at 6 d of stress but decreased to only 3.81, which was significantly below the minimum acceptable level (6.0) by 18 d. The physiological and overall turf performance data indicated that

Fig. 1. Total relative content of organic acids, amino acids, sugars and other metabolite detected by gas chromatography-mass spectroscopy in polar extracts of hybrid bermudagrass (cv. Tifdwarf) leaves for the well-watered control and after 6 and 18 d of drought (A) and the percentage changes in the content of metabolites at 6 d (B) or 18 d (C) compared with the well-watered control level. Columns marked with a small letter indicate significant differences between days of treatment for hybrid bermudagrass based on least significant difference test ($P = 0.05$).

hybrid bermudagrass exposed to drought stress for 6 d suffered from mild water deficit, whereas plants subjected to 18 d of drought experienced severe cellular damage in this experiment.

It is very important to control cellular tolerance to dehydration by increasing the concentration of compatible solutes within the cell under drought stress (Skirycz et al., 2010). To examine the effects of drought stress on the accumulation of stress-associated solutes, we performed a GC-MS analysis of polar compounds extracted from leaves of hybrid bermudagrass under well-watered conditions and at short-term (6 d) and long-term (18 d) drought stress. A total of 96 metabolites were detected in leaves of hybrid bermudagrass. Among the 96 metabolites, 37 exhibited differential responses to short-term and long-term drought, including nine organic acids, 15 amino acids, 10 sugars, and two-sugar alcohols and one nitrogen compound (ethanolamine). The data for the 37 metabolites are presented in Figures 2 through 5.

The total amount of organic acids, amino acids, sugars, and sugar alcohols did not change significantly at 6 d of drought compared with the well-watered control (Fig. 1A–B). At 18 d of drought, the total content increased by 285.14%, 157.44%, 65.99%, and 28.30% for sugars, amino acids, organic acids, and sugar alcohols, respectively, compared with the well-watered control level (0 d) (Fig. 1C). Timpa et al. (1986) also found that the content of total organic acids increased in cotton (*Gossypium kirsatam*) under dryland conditions.

As shown in Figures 2 through 5, the metabolite profile of hybrid bermudagrass subjected to 6 d of drought stress was similar to that of the control plants. The content of all nine organic acids, most amino acids, sugars, sugar alcohol, and ethanolamine did not change significantly at 6 d of drought compared with the well-watered control (Figs. 2A–B, 3A–B, 4A–B, and 5A–B). The content of methionine (Met) (Fig. 3A–B) and mannose (Fig. 4A–B) increased significantly. Changes in these metabolites could reflect the early metabolic responses to mild or short-term drought stress (6 d). Previous research indicated that nitric oxide (NO) is an endogenous signal in plants and NO and its related species can modify proteins under abiotic stress. In short-time drought stress, the significant increase of Met might have resulted from oxidized proteins by the reaction of NO with superoxide (Neill et al., 2008). High expression of mannose was also found in arabidopsis under drought stress (Rizhsky et al., 2004). Drought induced expression of

Fig. 2. Relative content of organic acid detected by gas chromatography–mass spectroscopy in polar extracts of hybrid bermudagrass (cv. Tifdwarf) leaves for the well-watered control and after 6 and 18 d of drought (A) and the percentage changes in the content of metabolites at effects of drought stress on the other metabolites content changes at 6 d (B) or 18 d (C) compared with the well-watered control level. Columns marked with a small letter indicate significant differences between days of treatment for hybrid bermudagrass based on least significant difference test ($P = 0.05$).

Fig. 3. Relative content of amino acid detected by gas chromatography–mass spectroscopy in polar extracts of hybrid bermudagrass (cv. Tifdwarf) leaves for the well-watered control and after 6 and 18 d of drought at (A) and the percentage changes in the content of metabolites at effects of drought stress on the other metabolites content changes at 6 d (B) or 18 d (C) compared with the well-watered control level. Columns marked with a small letter indicate significant differences between days of treatment for hybrid bermudagrass based on least significant difference test ($P = 0.05$).

sucrose synthase (Sus) (Déjardin et al., 1999). Sus is the key enzyme in plant sucrose catabolism and catalyzes the reversible conversion of sucrose and uridine diphosphate (UDP) into fructose and UDP-glucose (Subbaiah et al., 2006). It has been found that mannose regulates the expression of Sus in arabidopsis (Ciereszko and Kleczkowski, 2002). No significant accumulation of glucose and fructose was found at 6 d of drought stress (Fig. 4A).

The amino acids exhibiting decline during the early phase of drought stress (6 d) included serine (Ser), GABA, and isoleucine (Ile) (Fig. 3A–B), but little is known of the involvement of these amino acids in drought responses. In plants, GABA is mainly metabolized by two mitochondrial enzymes, GABA transaminase and succinic semialdehyde dehydrogenase (SSADH) (Bouché and Fromm, 2004). Fait et al. (2005) showed a close relationship of GABA shunt deficiencies with the accumulation of reactive oxygen species by *ssadh* mutants in arabidopsis. The decrease of Ile content was found in arabidopsis (Rizhsky et al., 2004) and pea [*Pisum sativum* (Charlton et al., 2008)] under drought stress. Ser is the residue on the active site of serine protease. Hieng et al. (2004) found that the expression level of a protein analog with serine protease activity was downregulated during drought treatment in a drought-tolerant *P. vulgaris* cultivar and deduced that the function of serine protease was associated with a protection mechanism against premature drought-induced senescence. Dramé et al. (2007) found downregulation of a serine protease-related gene in the early stage of drought stress in s drought-tolerant peanut (*Arachis hypogaea*) cultivar. Nevertheless, how the decline in serine, GABA, and Ile in a drought-tolerant hybrid bermudagrass is associated with early drought responses may deserve further investigation.

After 18 d of drought stress, the relative content of organic acids either decreased or increased significantly compared with that in the control plants depending on the specific type of metabolites. Malic acid exhibited the greatest increases in response to 18-d drought stress among all organic acids. The relative content of malic acid increased by 214.79% at 18 d of drought stress compared with the well-watered control level (Fig. 2C). Malate is considered a strong osmoticum in higher plants (Guicherd et al., 1997). The accumulation of malic acid under water stress was found in various plant species such as cotton (Cutler et al., 1977), two tropical grasses [green panic (*Panicum maximum* var. *tuichoglume*) and spear grass (*Heteropogon contortus*) (Ford

Fig. 4. Relative content of sugar detected by gas chromatography–mass spectroscopy in polar extracts of hybrid bermudagrass (cv. Tifdwarf) leaves for the well-watered control and after 6 and 18 d of drought (A) and the percentage changes in the content of metabolites at effects of drought stress on the other metabolites content changes at 6 d (B) or 18 d (C) compared with the well-watered control level. Columns marked with a small letter indicate significant differences between days of treatment for hybrid bermudagrass based on least significant difference test ($P = 0.05$).

and Wilson, 1981)], alfalfa nodules (Naya et al., 2007), and *Fraxinus excelsior* (Guicherd et al., 1997).

The content of two other organic acids, galacturonic acid and succinic acid, also significantly increased compared with the control. At 18 d of drought stress, the relative content of galacturonic acid and succinic acid increased by 126.35% and 36.43%, respectively, compared with the well-watered control level (Fig. 2C). Galacturonic acid is an intermediate in the ascorbic acid (AsA) biosynthesis pathway (Agius et al., 2003). AsA is an important antioxidant in plants against oxidative stress under abiotic stress (Conklin, 2001; Nayyar and Gupta, 2006). Hemavathi et al. (2009) confirmed that overexpression of galacturonic acid reductase in potato genotypes (*S. tuberosum*) led to the accumulation of ascorbic acid and enhanced drought stress tolerance. The increase observed in galacturonic acid in hybrid bermudagrass suggested that leaves could have been subjected to oxidative stress at 18 d of drought stress and that the galacturonic acid increase could play an important role in averting oxidative damage under severe water stress. Succinate is a key component of the citric acid cycle, which plays a critical role in energy production through respiration (Steuer et al., 2007). Naya et al. (2007) found succinic acid accumulation in alfalfa nodules under drought stress. The increased accumulation of succinic acid may reflect enhanced respiratory activity in hybrid bermudagrass for stress responses.

The decline in the content of several organic acids was detected at 18 d of drought stress, including citric acid, isocitric acid, glyceric acid, and methyl malonic acid (Fig. 2A), which decreased by 36.41%, 49.91%, 55.47%, and 49.27%, respectively, compared with the well-watered control level (Fig. 1C). The relative content of gluconic acid and threonic acid did not change significantly (Fig. 1A). Organic acids are precursors of amino acid biosynthesis and play an important role in energy production and modulating plant adaptation to environmental stress (López-Bucio et al., 2000). Citric acid and isocitric acid are key metabolites in respiration. The decline in those organic acids may reflect inhibitory effects of long-term drought stress on energy production.

Among amino acids, alanine, Ile, and lysine contents significantly decreased and Tyr content did not change, but the content of other 11 amino acids [Pro, asparagine, phenylalanine (Phe), Met, Ser, aspartic acid (Asp), 5-hydroxynorvaline, GABA, glycine, threonine (Thr), Val] significantly increased at 18 d of drought stress (Fig. 3A). Pro exhibited the greatest increase among

Fig. 5. Relative content of other metabolite detected by gas chromatography–mass spectroscopy in polar extracts of hybrid bermudagrass (cv. Tifdwarf) leaves for the well-watered control and after 6 and 18 d of drought (A) and the percentage changes in the content of metabolites at 6 d (B) or 18 d (C) compared with the well-watered control level. Columns marked with a small letter indicate significant differences between days of treatment for hybrid bermudagrass based on least significant difference test ($P = 0.05$).

all amino acids, whereas others increased to a lesser extent. Pro has been shown to be a typical drought-induced metabolite (Chaves et al., 2003; Yamada et al., 2005). Pro content increased by 28.70-fold (Fig. 2C). The high accumulation of Pro was also found in perennial ryegrass [*Lolium perenne* (Thomas, 1991)] and sorghum [*Sorghum bicolor* (Sivaramakrishnan et al., 1988)] after severe drought stress. High proline levels under water stress were associated with osmotic regulation and antioxidant protective roles (Aranjuelo et al., 2011; Chaves et al., 2003). The compounds involved in osmotic adjustment differ widely in different species (Patakas et al., 2002; Rizhsky et al., 2004; Vasquez-Robinet et al., 2008). Showler (2002) found that Phe, Thr, Val, and total free amino acids significantly increased in cotton under water deficit. In maize (*Zea mays*) seedlings, drought stress caused an increase in total free amino acid and increased accumulation of Val, Asp, Ser, and Thr (Ranieri et al., 1989). Researchers have found high expression of Val in arabidopsis (Rizhsky et al., 2004) and Val, Phe, Met, and GABA in flatpea [*Lathyrus sylvestris* (Shen et al., 1989)] under drought-stressed conditions.

Sugars have long been known to increase in many plants under drought stress (Chaves, 1991; Chaves et al., 2009; Kameli and Lösel, 1993). Important soluble sugar species in plants such as sucrose, glucose, galactose, and fructose showed significant increases (Fig. 4A), especially sucrose, and its relative content increased ≈ 27 -fold compared with the control (Fig. 4C). Lu et al. (2009) also found a significant increase of total soluble sugar and sucrose in hybrid bermudagrass under drought stress. In general, soluble sugars tend to increase and starch content tends to decrease under drought stress (Chaves, 1991). The increase of soluble sugars (including sucrose, glucose, and fructose) might mainly come from starch breakdown from chloroplasts (Chaves et al., 2009). Significant accumulation of galactose was found in arabidopsis (Rizhsky et al., 2004), potato (Evers et al., 2010), and in chilling-tolerant rice (*Oryza sativa*) genotypes (Morsy et al., 2007) under drought stress. The increase of galactose could be related to the synthesis of other osmoprotectants (Morsy et al., 2007).

Other sugars, including mannose, maltose, and xylose, also increased significantly. At 18 d of drought stress, a high content of mannose might be related to the function of Sus as mentioned before. Ferrando and Spiess (2001) found that maltose presented a higher protective effect on cell membrane integrity and it stabilized cell functions during

osmotic treatment. Xylose is the major precursor in hemicellulose of the cell wall. Water induced the accumulation of hemicellulose in tobacco (*Nicotiana tabacum*) culture cells (Iraki et al., 1989), durum wheat [*Triticum durum* (Rascio et al., 1990)], wheat [*Triticum aestivum* (Wakabayashi et al., 1997)], and *Atriplex halimus* (Martinez et al., 2004).

The relative content of two sugars, ribose and melibiose, decreased at 18 d of drought stress (Fig. 4C). Ribose is a component of cyclic ADP-ribose (cADPR). cADPR was identified as a signaling molecule in the ABA response (Wu et al., 1997). ABA plays an important role in response to drought stress (Seki et al., 2007). At severe drought stress (18 d), the decrease in the relative content of ribose might relate to ABA response under drought stress. Melibiose is a non-permeating osmoticum in solution culture (Verslues et al., 1998). The decrease of melibiose content was also found in arabidopsis (Rizhsky et al., 2004) under drought stress. However, the relationship between lower melibiose content and drought stress was unknown.

The relative content of myo-inositol significantly increased after 18 d of drought stress (Figs. 5A and 5C). Evers et al. (2010) also found a significant accumulation of myo-inositol in potato under drought stress. Myo-inositol is synthesized from glycolytic glucose-6-phosphate (Loewus and Murthy, 2000). Many studies have showed that phosphorylated lipids and methylated derivatives of myo-inositol can be used to produce compatible solutes to increase a plant's drought tolerance or affect signalling processes that protect a plant against water stress (Keller and Ludlow, 1993; Munnik and Vermeer, 2010; Perera et al., 2008).

Rizhsky et al. (2004) reported that ethanolamine content increased in arabidopsis under drought stress. In our experiment, relative content of ethanolamine increased by 93.36% compared with control at 18 d of drought stress in hybrid bermudagrass. Ethanolamine moieties are crucial for membrane biogenesis and are precursors of some important osmoprotectants such as glycine betaine (Ashraf and Foolad, 2007; Rontein et al., 2003). The significant increase of ethanolamine at 18 d of drought stress in hybrid bermudagrass might be related to membrane protection and osmotic adjustment.

In summary, the metabolic profiling demonstrated that few metabolites (Met, Ser, GABA, Ile, and mannose) were affected by short-term drought stress (6 d) in hybrid bermudagrass when leaf RWC declined to $\approx 82\%$, which corresponded to less severe decline in TQ, Fv/Fm, and EL. Many metabolites either increased or decreased in their abundance in response to long-term drought (18 d) or severe drought stress (leaf RWC = 32%). Among all metabolites, malic acid, Pro, and sucrose accumulated to a higher content under severe drought stress, and they were the most predominant metabolites reflecting metabolic changes for hybrid bermudagrass adaptation to long-term drought stress. However, how the drought-responsive metabolites are directly correlated to drought tolerance in hybrid bermudagrass and other warm-season turfgrass species requires further investigation through modification of the endogenous content of the metabolites or the evaluation of genetic variations of differential metabolite profiles in response to drought stress.

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