

<https://doi.org/10.17221/426/2022-PSE>

Effects of drought stress on carbon metabolism of bermudagrass (*Cynodon dactylon* L.)

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Citation: Zhang Y.L., Bao Y.X., Li P.Y., Yu Q.K., Li W., Tang L.S., Sun X.F., Sun Z.J., Li S. (2023): Effects of drought stress on carbon metabolism of bermudagrass (*Cynodon dactylon* L.). Plant Soil Environ., 69: 269–281.

Abstract: The effect of drought stress on carbon metabolism in the leaves and roots of bermudagrass was investigated. Plants established in PVC tubes suffered from three water treatments for 10 days. C138 and Tifway (drought-tolerant) were found to have lower relative electrical conductivity and higher water use efficiency than C32 (drought-sensitive) under moderate drought by increasing carotenoid and soluble sugar content and rapidly decreasing leaf starch content. The sucrose synthase activity of leaves and roots, acid invertase and neutral invertase activity of C32 roots substantially decreased under severe drought, resulting in a slow sucrose decomposition rate and significantly lower fructose and glucose contents than C138 and Tifway. The activities of four carbon metabolism enzymes and sucrose content in the leaves were greater than those in the roots, while the fructose and glucose contents were on the contrary, indicating that bermudagrass transported fructose and glucose obtained from sucrose decomposition from leaves to roots under drought to reduce roots damage. The path analysis indicated that leaves neutral invertase activity, and roots soluble sugar content might be the key parameter of carbon metabolism in bermudagrass under drought.

Keywords: abiotic stress; differences in drought resistance; sucrose metabolism; nonstructural carbohydrate content; irrigation

Water is an important resource for human survival, and with climate change, population increase and urbanisation, the shortage and uneven distribution of water have become very serious (Qin et al. 2011), especially in arid and semi-arid regions. When water shortage, lawn irrigation is firstly restricted, thus revealing the drought resistance mechanism of grass and breeding drought-resistant cultivars become urgent problems for turf industry development.

Carbon metabolism is the most fundamental metabolic process in plants, including photosynthetic carbon assimilation, synthesis, decomposition and

carbohydrate interconversion, which is essential for plant growth and development and yield formation (Muller et al. 2011). The two key components of carbon metabolism are starch and sucrose (Dietze et al. 2014), they play opposite but complementary roles in carbon storage and transport, and their relative metabolism drives most of the carbon fluxes in most plants (Smith and Stitt 2007). Starch is stored as nutrition, while soluble sugars are produced for rapid plant utilisation in physiological activities, particularly maintaining turgor pressure for drought resistance (Hummel et al. 2010). A study highlighted that starch is usually

Supported by the National Natural Science Foundation of China, Grant No. 31960362, and by the Xinjiang Agricultural University Graduate Student Innovation, Project No. XJAUGRI2021022.

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stored in roots under stress or can be broken down and transported to leaves, where it forms soluble sugars to maintain turgor (Martínez-Vilalta et al. 2016). Plant carbon metabolism is regulated by carbon metabolism enzyme activity. When the activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS) are high, plant carbon metabolism is vigorous, and the rate and accumulation of organic matter synthesis are fast and high, which can provide carbon skeletons and facilitate the formation of other compounds (Xu et al. 2015). In addition, acid invertase (AI) and neutral invertase (NI) can catalyse sucrose decomposition into glucose and fructose, involving plant response to environmental stress (Zhu et al. 2021).

Plants' response to drought is usually affected by stress level, duration, genotype and developmental stage (Anjum et al. 2011). During long-term adaptation to different environments, the plant has formed different drought adaptation mechanisms. Drought-tolerant lines had higher yield and quality than drought-sensitive lines due to relatively higher osmotic adjustment ability (Martínez et al. 2007), antioxidant system (Fu et al. 2011) and developed root systems (Katuwal et al. 2019) under drought stress. Furthermore, although sugars indirectly affect plant growth and development by regulating carbohydrate metabolism under drought stress, it is still essential (Gupta and Kaur 2005). For example, drought-tolerant coffee increased sucrose and acid invertase enzyme activities under drought stress, while sucrose synthase activity in drought-sensitive coffee decreased (Praxedes et al. 2006). The contents of glucose, fructose and sucrose in flag leaves and grains of drought-sensitive wheat decreased significantly under severe water stress due to a sharp decrease of invertase, however, invertase activity in flag leaves for drought-tolerant wheat increased transiently (Saeed and Foad 2011). A study on responses of carbon metabolism of different drought-tolerant germplasms will help understand the intrinsic mechanism responsible for drought tolerance differences and drought-tolerant cultivars breeding. So far, few studies on carbon metabolism response to drought in grasses have been done, and the key parameter affecting carbon metabolism under drought are still unclear.

Bermudagrass is a perennial grass of the gramineous family, which has better drought tolerance than many other warm-season grasses such as zoysiagrass (*Zoysia* spp.), centipedegrass (*Eremochloa ophiuroides*

(Munro) Hack.) and augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) (Zhao et al. 2011, Zhou et al. 2013). Studies on the physiological mechanisms of bermudagrass under drought mainly focus on root morphology, osmoregulation and antioxidant systems (Zhou et al. 2013, Katuwal et al. 2019). Carbon metabolism response is less reported, and studies on changes in leaf and root nonstructural carbohydrate content and carbon metabolism enzyme activities of bermudagrass under drought have not been reported. Therefore, this study was conducted to determine the contents of nonstructural carbohydrate and carbon metabolism enzyme activities in leaves and roots of two types of bermudagrass under different drought stress, (1) exploring the response of bermudagrass leaves and roots carbon metabolism to drought stress, (2) to determine the response differences of carbon metabolism of different bermudagrass lines and to clarify key parameter about carbon metabolism under drought as can enrich the bermudagrass drought mechanism of carbon metabolism and provide a reference for new cultivars breeding.

MATERIAL AND METHODS

Test materials and drought treatment. C32 (drought-sensitive line) and C138 (drought-tolerant line) screened by our group in a previous study (Zeng et al. 2020) were used as test material, and Tifway (drought-tolerant cultivar, gifted by Nanjing Agricultural University) as control. One-year-old of single phytome propagation sod pieces of 5 cm diameter with 5 cm root retained were taken from the test field plots and transplanted into PVC tubes (10 cm diameter, 40 cm deep, 5.8 kg mixture of soil, vermiculite and 2 mm sieved sand (2:1:1, v/v), each material transplanted 18 tubes, the soil nutritional characteristics were: 15.26% soil organic carbon, 191.73 mg/kg available nitrogen, 10.54 mg/kg available phosphorus, 184.87 mg/kg available potassium, and the drought stress test was started after 30 days cultivation.

From August 15 to September 3, 2021 (a total of 20 days), a drought test was carried out at the outdoor test site of Xinjiang Agricultural University. As shown in Figure 1, the average maximum and minimum temperatures during drought were 27.9 °C and 16.6 °C, of which 13 days were cloudy, 6 days were sunny, and 1 day was light rain (covered by awnings). The weather and temperature are derived from the China Meteorological Network. CK – normal irriga-

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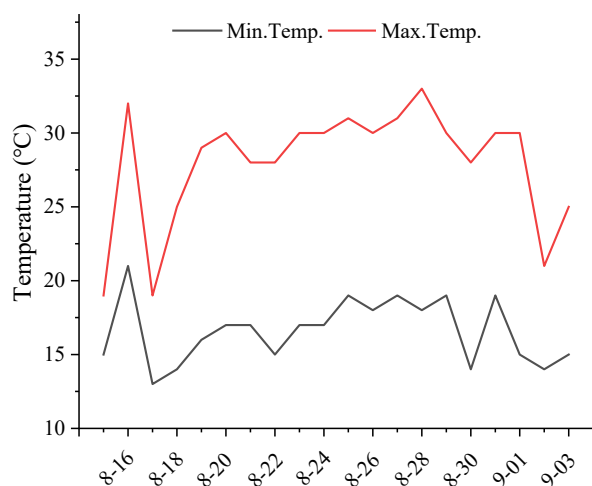


Figure 1. Temperature during drought treatment

tion ($75 \pm 5\%$ of field capacity, respectively), MD – moderate drought stress ($45 \pm 5\%$) and SD – severe drought stress ($25 \pm 5\%$) were the three experimental treatments, each with six tubes of bermudagrass. A randomised complete block design was used to minimise the impact of environmental conditions. Each tube was irrigated sufficiently to saturated field capacity, and then water evaporated naturally; when the soil water content of three treatments reached setting values, the experiment started, and the water loss was replenished by daily weighing to keep soil water content within the set range, after 10 days, the following indicators were measured.

Soil water content (SWC). W_1 (aluminium box weight + fresh soil weight); W_2 (weight after drying at 105°C for 48 h to constant weight); W_3 (the weight of the aluminium box). According to the formula: $\text{SWC} (\%) = (W_1 - W_2) / (W_2 - W_3) \times 100$, VWC was measured by MP-508 soil moisture analyser (Handan Ruiyan Zhihua Electronic Technology Co., Ltd., Handan, China). The VWC content was measured quickly after each soil sample was taken. The fitting equation of SWC and VWC calculated the SWC.

Relative electrical conductivity (REC), relative water content (RWC). DZS-706 conductivity meter (Shanghai Thundermagnetic, Shanghai, China) was used to measure the initial conductivity (R_1) after soaking for 12 h and the conductivity (R_2) after being heated in a boiling water bath for 30 min, cooled to room temperature, and then slightly vibrated. The formula is $\text{REC} (\%) = R_1 / R_2 \times 100$. RWC was determined from fresh weight (FW), turgid weight (TW) and dry weight (DW) using the following formula:

$$\text{RWC} (\%) = [(FW - DW) / (TW - DW)] \times 100.$$

Photosynthetic pigments, photosynthetic indexes. The dimethyl sulfoxide (DMSO, purity 99.5%, Jiangsu Debang Pharmaceutical Co., Ltd., Lianyungang, China) method (Wellburn et al. 1994) was used to determine photosynthetic pigment content. The OD value was read at 665 nm, 649 nm and 480 nm. The pigment concentration was calculated according to the following formula: $C_a = 12.19 A_{665} - 3.45 A_{649}$, $C_b = 21.99 A_{649} - 5.32 A_{665}$, $C_{x+c} = (1000 A_{480} - 2.14 C_a - 70.16 C_b) / 220$ (total carotenoids), $C_t = C_a + C_b$ (total chlorophylls). The net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r) and intercellular CO_2 concentration (C_i) were measured with a TPS-2 portable photosynthesis (PP Systems, Boston, USA) on the apical third fully mature leaf of bermudagrass shoots, and three times repeat for per tube, and the mean values were taken. The measurements were performed from 8:00 a.m. to 12:00 p.m.; leaf temperature was 25°C , light intensity was $1\,000\ \mu\text{mol}/\text{m}^2/\text{s}$, relative air humidity was $70.0 \pm 3.5\%$, and ambient CO_2 concentration was $400 \pm 5\ \mu\text{mol}/\text{mol}$. Water use efficiency (WUE) = P_n / T_r .

Nonstructural carbohydrate content. Soluble sugars (Ss) and starch were extracted according to the method of Buysse and Merckx (1993). The absorbance values of soluble sugars and starch were measured using a UV-Vis spectrophotometer (T6 New Century, Beijing, China), colourimetric at 630 nm, and concentrations of soluble sugar and starch were calculated, and expressed as mg/g dry weight. The accumulation of sugar or starch in each tissue was calculated by multiplying the concentration with the corresponding dry weight (mg/plant). Consequently, the sum of soluble sugar or starch was obtained. Then the proportion of sugar or starch in roots and leaves was calculated and expressed as a percentage. The sucrose of leaves and roots was extracted and quantified by a modified method of Xu et al. (2015). About 0.1 g of ground sample was extracted with 80% (v/v) ethanol (purity 99.7%, Qilu Petrochemical Co., Ltd., Zibo, China) at 80°C for 30 min, followed by centrifugation at $10\,000 \times g$ for 10 min. The residue was extracted two more times using 80% ethanol. The sucrose content was determined spectrophotometrically at 480 nm wavelength. 50 mg leaf was extracted in 80% ethanol with an 80°C water bath. After centrifugation, the extracted residue was washed with 80% ethanol three times. The combined supernatants were collected and used to assay for soluble carbohydrates, glucose, and fructose were measured according to Yu and Zhang (1999).

Carbon metabolism enzyme activities. The enzymes SPS (EC 2.4.1.14) and SS (EC 2.4.1.13) were extracted following Hubbard et al. (1989), and Lowell et al. (1989). 0.1 g frozen leaf or root sample was homogenised in cold extraction buffer containing 50 mmol Tris-HCl (pH 7.5), 1 mmol EDTA, 1 mmol $MgCl_2$, 12.5% (v/v) glycerin, 10% polyvinylpyrrolidone (PVP), and 10 mmol mercaptoethanol. The homogenate was centrifuged at $8\,000 \times g$ for 10 min at 4 °C.

The determination was determined according to the YX-C-B504/505 Shanghai Sino Best Biological Co, Ltd., Shanghai, China kits. The reaction buffer to measure SPS activity contained 12 mmol UDP-glucose, 40 mmol fructose-6-P, 200 mmol Tris-HCl (pH 7.0), 40 mmol $MgCl_2$ and 200 μ L extract. The reaction buffer to measure SS activity contained 12 mmol UDP, 40 mmol sucrose, 200 mmol Tris-HCl (pH 7.0), 40 mmol $MgCl_2$ and 200 μ L extract. The reaction was initiated by incubating the enzyme at 30 °C for 30 min and terminated using 100 μ L 2 mol/L of NaOH. Then the samples were immediately heated at 100 °C for 10 min to destroy untreated hexose and hexose phosphates. Samples were then cooled

to room temperature and mixed with 1 mL of 0.1% (w/v) resorcin in 95% (v/v) ethanol and 3.5 mL of 30% (w/v) HCl, and the solution was incubated for 10 min at 80 °C. Absorbance values were measured at 480 nm.

The acid and neutral invertase assays were performed as described by Lowell et al. (1989) and Vargas et al. (2007) with modification. The optimum pH of the buffer was 4.5 for acid invertase (AI (EC 3.4.21.68)) and 7.5 for neutral invertase (NI (EC 3.4.21.47)). The amount of glucose hydrolysed from sucrose was determined by colourimetric assay at 540 nm and compared to a standard curve. The enzyme activity was expressed by measuring the amount of reducing sugar produced.

Data analysis. Statistical analyses were performed using SPSS 26 (SPSS Inc., Chicago, USA). One-way analysis of variance was used to test the significance of different drought treatments and different materials. Significant differences were determined using Tukey's multiple comparison tests at a significance level 0.05. The Pearson method was used for correlation analysis. Path analysis was performed using

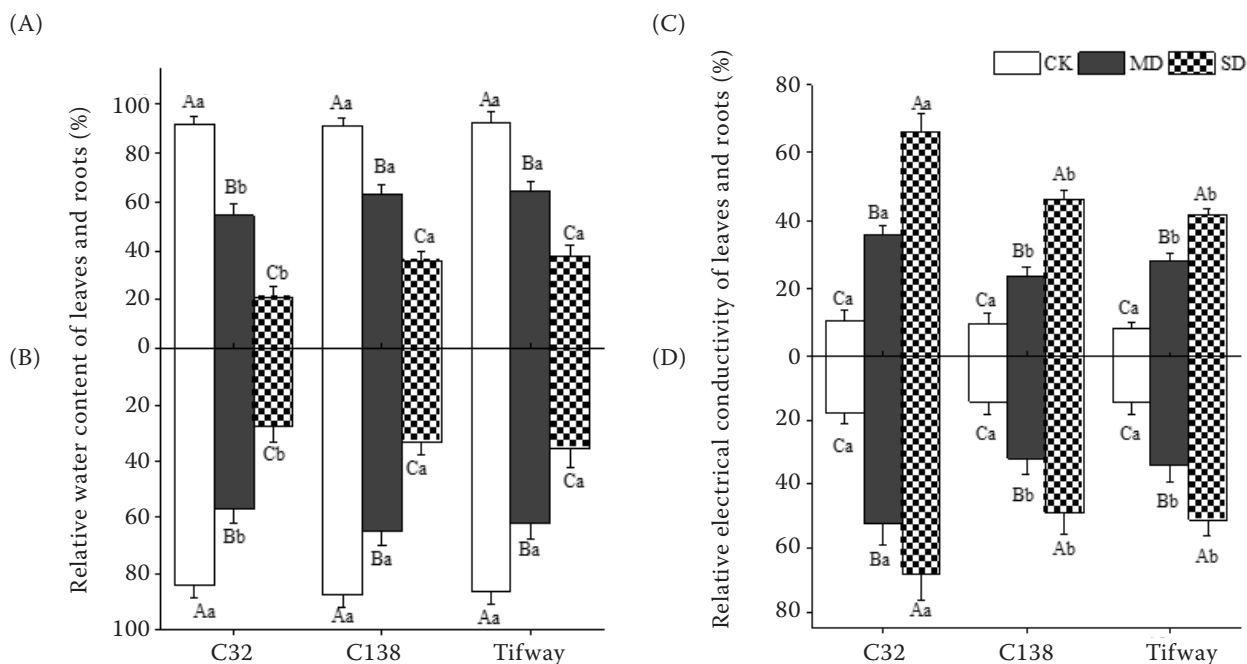


Figure 2. Effects of drought stress on relative water content and relative electrical conductivity of bermudagrass. In order to more intuitively show the comparison and change of leaves and roots indexes of each material under different drought treatments, the above X axis is regarded as leaves and the following is regarded as roots, the same as below. (A, B) the relative water content of leaves and roots, and (C, D) the relative electrical conductivity of leaves and roots. CK – normal irrigation; MD – moderate drought stress; SD – severe drought stress. Different lowercase letters indicate significant differences among different materials under the same treatment and different capital letters indicate significant differences among different treatments of the same material (Tukey's test, $P < 0.05$). Values are means \pm standard deviation

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SPSS 26. Using Origin 2018 (Originlab Company, Northampton, USA), figures were drawn. The data are presented as means \pm standard deviation (SD).

RESULTS

Effects of drought stress on bermudagrass's relative water content and relative electrical conductivity. In Figure 2A, we observed that the relative water contents of leaves and roots of C32 in MD were respectively 54.64% and 57.15%, significantly lower than those of other materials. The relative water content of leaves and roots of Tifway and C138 in SD was 70.21–78.03% and 19.29–27.54%, higher than those of C32. For Figure 2B, the relative electrical conductivity of C32 leaves and roots in MD increased by 2.47 and 1.94 times compared with CK, and the relative electrical conductivity of leaves and roots of SD materials increased by 371.86–539.60% and 248.27–285.59% compared with CK, and C32 showed the most serious damage.

Effects of drought stress on photosynthetic pigments of bermudagrass. Figure 3B showed that the chlorophyll *b* content of all materials increased at MD, and Tifway (56.10%) increased the most; the

chlorophyll *b* content of Tifway and C138 at SD was 2.46 times and 1.76 times higher than that of C32. For chlorophyll *a* (Figure 3A) and total chlorophyll content (Figure 3D), C32 was 65.59% and 67.67% lower than C138 at SD. In addition, the total carotenoid content of C138 and Tifway increased at MD, while that of C32 decreased by 46.43% (Figure 3C).

Effects of drought stress on gas exchange parameters of bermudagrass. Figures 4A and 4B showed that C32 had the lowest P_n and g_s under each treatment and no significant difference between C138 and Tifway. Figure 4D showed T_r of tested materials peaked at MD, and C138 had the lowest T_r at SD. C_i of C32 was higher than that of drought-resistant materials under drought (Figure 4C). Although drought significantly reduced the WUE of bermudagrass (Figure 4E), Tifway and C138 could maintain relatively higher WUE.

Effects of drought stress on soluble sugar and starch content of bermudagrass. We observed that the leaf-soluble sugar (Figure 5A) and root starch content (Figure 5D) of each material increased by 30.73–64.01% and 26.58–64.01%, respectively, compared with CK, the soluble sugar content of C138 leaves increased the most. At the same time, Tifway showed more root starch increment. The leaf-soluble

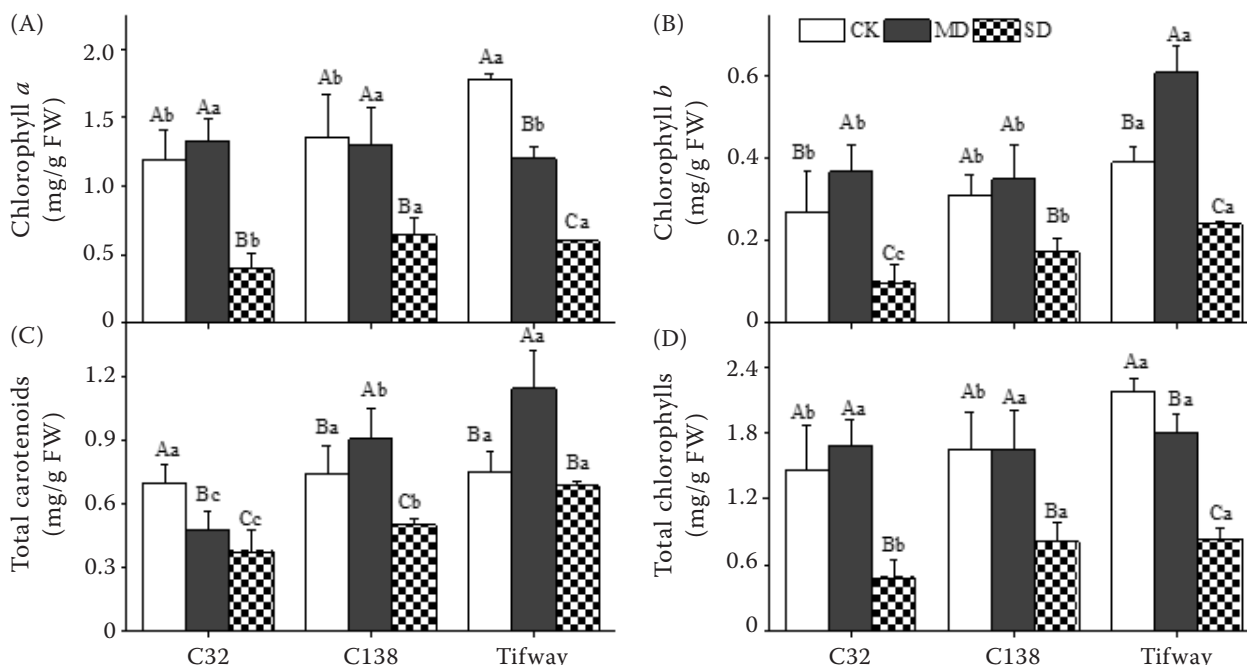


Figure 3. Effects of drought stress on photosynthetic pigments of bermudagrass. (A) chlorophyll *a*; (B) chlorophyll *b*; (C) total carotenoids, and (D) total chlorophylls. CK – normal irrigation; MD – moderate drought stress; SD – severe drought stress. Different lowercase letters indicate significant differences among different materials under the same treatment and different capital letters indicate significant differences among different treatments of the same material (Tukey's test, $P < 0.05$). Values are means \pm standard deviation; FW – fresh weight

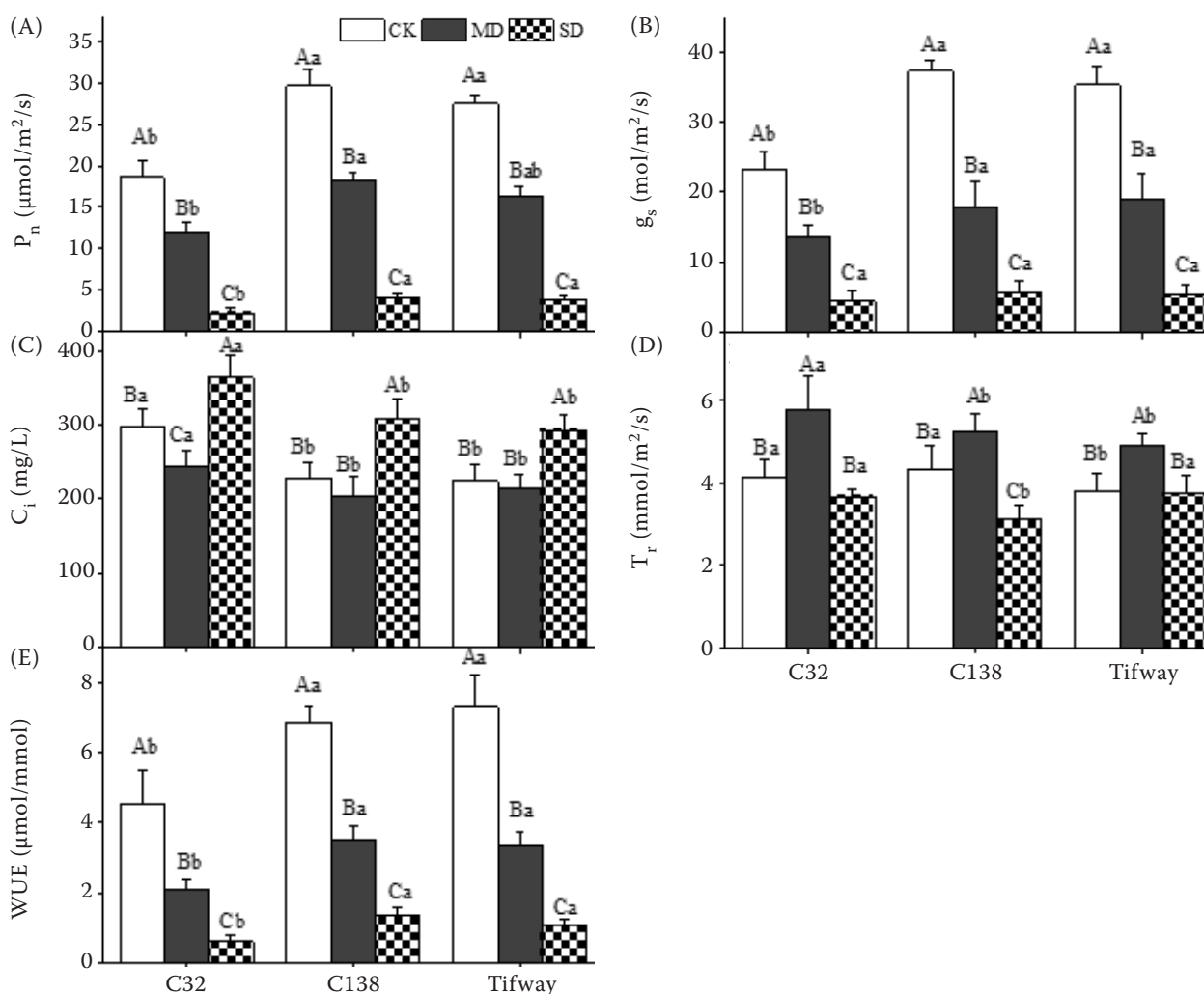


Figure 4. Effects of drought stress on gas exchange parameters of bermudagrass. (A) net photosynthetic rate (P_n); (B) stomatal conductance (g_s); (C) internal CO_2 concentration (C_i); (D) transpiration rate (T_r), and (E) water use efficiency (WUE). CK – normal irrigation; MD – moderate drought stress; SD – severe drought stress. Different lowercase letters indicate significant differences among different materials under the same treatment and different capital letters indicate significant differences among different treatments of the same material (Tukey's test, $P < 0.05$). Values are means \pm standard deviation

sugar and root starch content of Tifway increased by 87.82% and 56.70% at SD. The root-soluble sugar (Figure 5B) and leaf starch content (Figure 5C) of C138 were the highest in CK, which were 32.20% and 98.59% higher than C32. The content of soluble sugar in roots and starch in leaves of each material in MD decreased by 20.22–67.58% and 16.39–82.85% compared with CK; in SD, we observed that the above content of C32 decreased by 83.33% and 120.48%, which was 7.1 times and 2.1 times that of Tifway.

Effects of drought stress on sucrose, fructose and glucose contents of bermudagrass. Figure 6 showed that moderate drought could increase the content of sucrose, fructose and glucose in leaves and

roots of all materials, and the contents of sucrose (Figure 6A, B), fructose (Figure 6C, D) and glucose (Figure 6E, F) in leaves and roots of C32 under all treatments were significantly lower than those of drought-resistant materials, no significant difference between C138 and Tifway. In addition, the sucrose content in leaves was generally higher than that of roots under drought, but the fructose and glucose contents showed the opposite.

Effects of drought stress on the activities of carbon metabolising enzymes of bermudagrass. Under the same drought stress, SS activity in leaves (Figure 7A) was generally higher than in roots (Figure 7B). Compared with CK, the SS activity of leaves and

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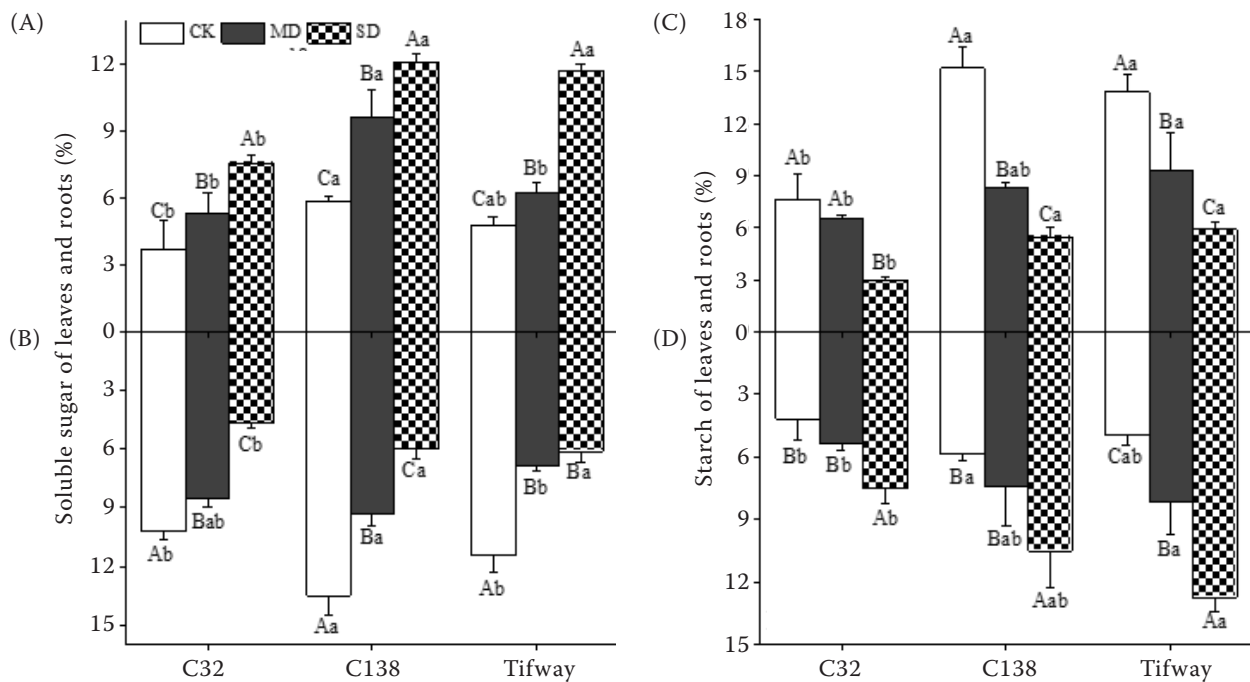


Figure 5. Effects of drought stress on soluble sugar and starch content of bermudagrass. (A, B) soluble sugar contents of leaves and roots, and (C, D) starch contents of leaves and roots. CK – normal irrigation; MD – moderate drought stress; SD – severe drought stress. Different lowercase letters indicate significant differences among different materials under the same treatment and different capital letters indicate significant differences among different treatments of the same material (Tukey's test, $P < 0.05$). Values are means \pm standard deviation

roots of each material increased by 25.08–40.00% and 15.38–25.00% under MD; SS activity of C32 decreased by 75.00% and 100.00% during SD. The SPS activity of drought-resistant materials in CK and MD (Figure 7C) was 38.46–46.15% and 36.36–45.45% higher than that of C32, respectively. The highest SPS activity in C138 leaves was 8.57 mg/g/h FW at SD. SPS activity of C32 roots (Figure 7D) was significantly lower than that of other materials, although the activity of C32 increased by 60.00% in MD, the drought-resistant materials only increased by 20.00–22.22%; the activity of C32 in SD was still 83.33–100.00% lower than that of C138 and Tifway. Figure 7E, F showed that the AI activity of leaves and roots of C32 was always lower than that of drought-resistant materials, and the AI activity of leaves was higher than that of roots. The decrease of leaf NI activity (30.71–31.68%, 34.93–42.25%) of drought-resistant materials during MD and SD was less than that of C32 (44.33–63.04%) (Figure 7G), in MD, the NI activity of C32 roots (Figure 7H) increased by 10.67%, and the drought-resistant materials increased by 16.67–18.13%. The activity of C32 decreased by 93.02% during SD, and its root NI activity was significantly lower than

that of drought-resistant materials under drought treatment.

Path analysis of leaf relative electrical conductivity and leaf and root carbon metabolism indicators of bermudagrass. The most direct appearance of turfgrass under stress is the growth state of leaves, and the relative electrical conductivity of leaves under stress could reflect the drought resistance of turfgrass. Based on this, we took the relative conductivity of bermudagrass leaves under drought as the independent variable, and the carbon metabolism indexes of leaves and roots as the dependent variable for path analysis (Table 1). The results showed that the direct effect of leaf NI activity on leaf relative conductivity was greater, and the direct path coefficient was -0.682 , while the indirect effect of root-soluble sugar (Ss) on leaf relative conductivity was greater, and the indirect path coefficient was -0.537 , mainly affected by leaf NI activity. The decision coefficient of leaf NI activity was the most, indicating that the comprehensive determination ability of leaf NI activity for leaf relative conductivity was the highest, and the leaf NI activity and root Ss of bermudagrass under drought stress played an important role in drought response.

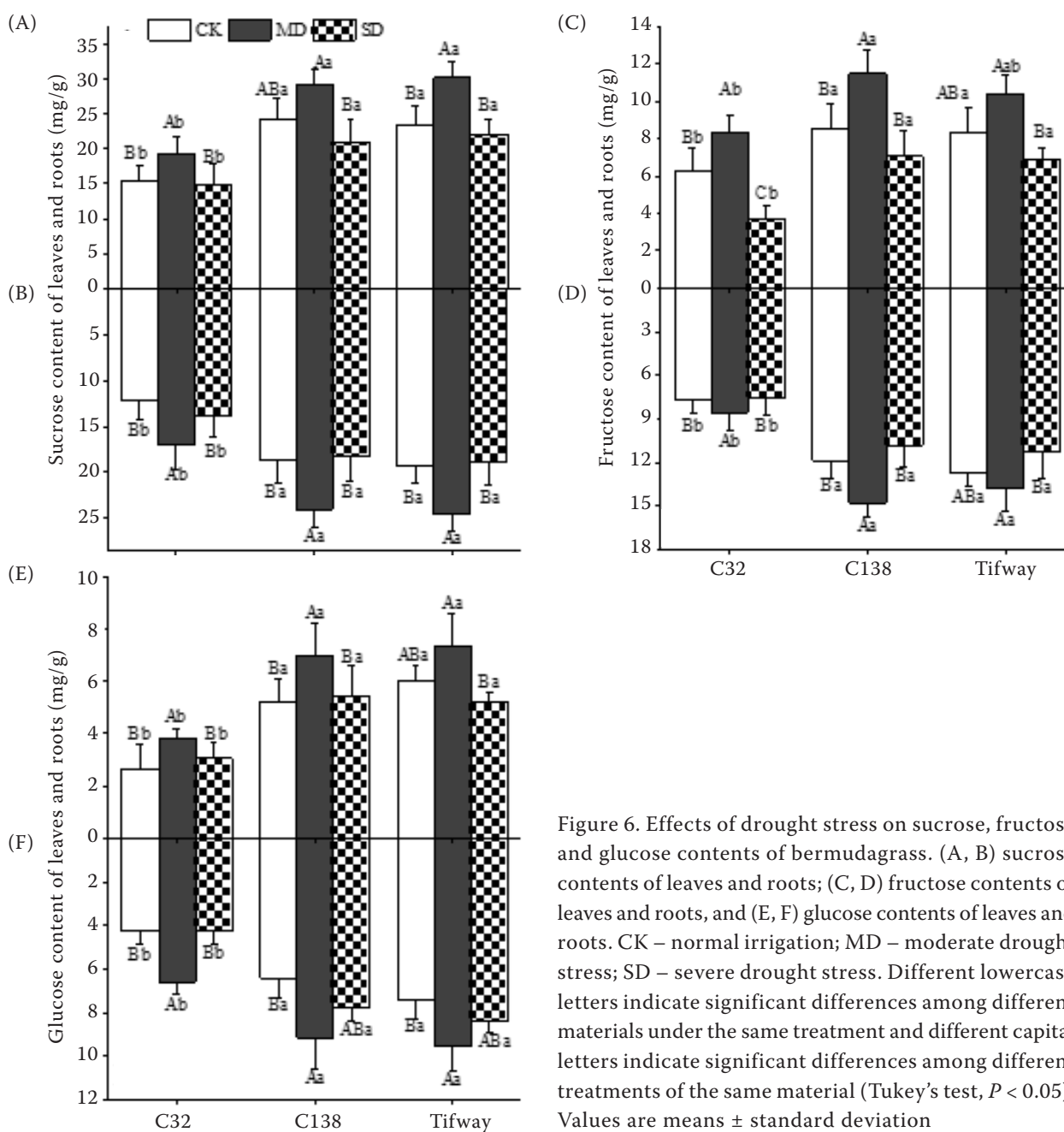


Figure 6. Effects of drought stress on sucrose, fructose and glucose contents of bermudagrass. (A, B) sucrose contents of leaves and roots; (C, D) fructose contents of leaves and roots, and (E, F) glucose contents of leaves and roots. CK – normal irrigation; MD – moderate drought stress; SD – severe drought stress. Different lowercase letters indicate significant differences among different materials under the same treatment and different capital letters indicate significant differences among different treatments of the same material (Tukey's test, $P < 0.05$). Values are means \pm standard deviation

DISCUSSION

Plant photosynthesis is greatly affected by drought stress, such as reducing stomatal conductance and reducing the diffusion rate of CO_2 to chloroplasts, which in turn leads to a decrease in leaf photochemical reaction and carbon metabolism. In this study, drought stress reduced the g_s of bermudagrass. The decrease of g_s of C138 was much larger than that of C32, indicating that drought-resistant materials responded more rapidly to drought stress, which was

consistent with the conclusion of Hu et al. (2010) in the study of bluegrass (*Poa annua* L.) with different drought tolerance. The rapid decrease of P_n and T_r and the rapid increase of C_i under severe drought indicated that non-stomatal factors were the main reason for the photosynthesis decrease of bermudagrass under drought stress, which was consistent with the results of Ohashi et al. (2006). In addition, Tifway and C138 showed relatively high P_n and relatively low T_r under drought stress, resulting in significantly higher WUE than C32; this is one of

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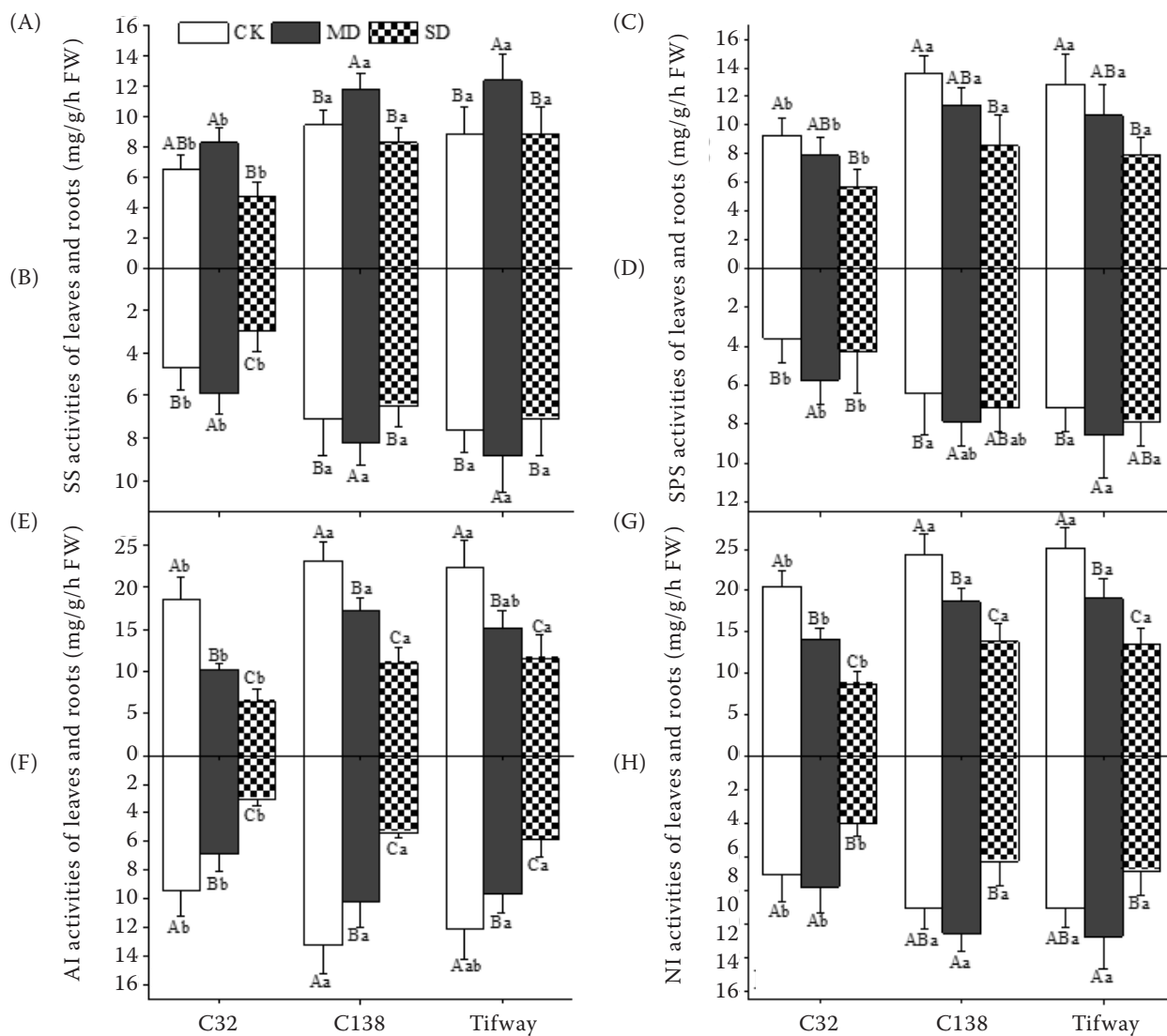


Figure 7. Effects of drought stress on the activities of carbon metabolising enzymes of bermudagrass. (A, B) SS – sucrose synthetase activities of leaves and roots; (C, D) SPS – sucrose phosphate synthase activities of leaves and roots; (E, F) AI – acid invertase activities of leaves and roots, and (G, H) NI – neutral invertases activities of leaves and roots. CK – normal irrigation; MD – moderate drought stress; SD – severe drought stress. Different lowercase letters indicate significant differences among different materials under the same treatment and different capital letters indicate significant differences among different treatments of the same material (Tukey's test, $P < 0.05$). Values are means \pm standard deviation; FW – fresh weight

the reasons why drought-resistant materials have stronger carbon assimilation ability. Carotenoids are important antioxidants in plants and play an important role in reducing and eliminating reactive

Table 1. Path analysis of leaf relative electrical conductivity and carbon metabolism indexes in roots and leaves of bermudagrass

Action factor	Correlation coefficient	Direct path coefficient	Indirect path coefficient		Total	Decision coefficient
			root Ss	leaf NI		
Root Ss	–0.818	–0.281		–0.537	–0.537	0.381
Leaf NI	–0.903	–0.682	–0.221		–0.221	0.767

Ss – soluble sugar; NI – neutral invertases

oxygen damage caused by drought and other stresses. We found that the carotenoid content of different germplasms showed different responses to drought stress; C32 decreased, while drought-resistant materials increased under MD and maintained relatively high carotenoid content under SD, which may be one of the reasons they had strong drought resistance.

Starch is an important energy and carbon storage compound in plants, and its metabolic balance will be affected under stress (Thalmann et al. 2016). Guo et al. (2020) showed that under drought conditions, the starch content in the buds of perennial ryegrass (*Lolium perenne* L.) decreased significantly, while the starch content in the roots did not change, indicating that starch not only preferentially allocated carbon to root growth under stress, but also preferentially allocated carbon to root storage. Duque and Setter (2013) found that starch content in cassava (*Manihot esculenta* Crantz) stems decreased significantly under drought, indicating that the starch of stems was decomposed and transported to other organs under drought, resulting in its content decrease. The starch content in all vegetative tissues of reed (*Phragmites australis* (Cav.) Trin. ex Steud.) increased under drought stress, but the starch content in the stem base was more than twice that in the upper stem (Kanai et al. 2007). This implies that changes in starch content under drought may vary depending on species and organs. We found that drought stress reduced starch content in bermudagrass leaves, as meant drought stress-regulated potential osmotic balance by promoting leaf starch hydrolysis and producing more soluble sugars. However, the starch content in roots showed an increasing trend. Under drought, bermudagrass decomposes starch in the aboveground part and preferentially transports it to the less damaged underground part for storage. On the other hand, it might be because the large increase in soluble sugar will cause starch accumulation. In addition, we found that the starch content in the leaves of C138 and Tifway decreased sharply by 48.14–82.85% under moderate stress, while that of C32 only decreased by 16.39%. The starch accumulation in roots was also significantly higher than that of C32; this indicates that drought-resistant bermudagrass has more active regulation of starch metabolism in response to drought stress.

Soluble sugars are important osmoprotectants and energy sources for plant cells under drought stress. Yan et al. (2020) think that increasing soluble sugar content in leaves under drought stress may be

a strategy for soybean (*Glycine max* L.) response to drought. This study showed that drought stress decreased the soluble sugar content in bermudagrass roots. At the same time, leaves accumulate a large amount of soluble sugar to reduce the potential osmotic disorder caused by drought, possibly due to bermudagrass roots converting more soluble sugar into starch for storage. The sucrose content of bermudagrass increased first and then decreased under drought stress. Sucrose was hydrolysed into fructose and glucose, which provided the energy for bermudagrass to resist drought. Both fructose and glucose had the largest accumulation under moderate drought, indicating that sucrose metabolism was more active under moderate drought. The sucrose content in the leaves was higher than that in the roots, indicating that sucrose synthesis and decomposition were more active in leaves under drought stress, while the glucose and fructose content in the roots was higher than that in the leaves, indicating that the fructose and glucose obtained from the decomposition in the leaves were preferentially transported to bermudagrass roots under drought stress; thus the roots could grow rapidly and repair damaged tissues (Yan et al. 2020), which is consistent with the results of path analysis, soluble sugar in roots plays an important role in drought resistance of bermudagrass. This study found that the contents of sucrose, glucose and fructose in drought-resistant bermudagrass were significantly higher than those in drought-sensitive bermudagrass under drought stress, indicating that sucrose metabolism might be related to drought resistance of bermudagrass.

The SS and SPS activity changes in different plants under drought were inconsistent. Drought stress enhanced the activities of SS and SPS in soybean leaves, increasing the conversion of sucrose to glucose and fructose and improving the osmotic adjustment ability under stress (Yan et al. 2020). The activity of SPS in wheat (*Triticum aestivum* L.) stems decreased under drought stress, and SS activity increased, resulting in a significant decrease in sucrose content (Abid et al. 2017). It was also reported that the activities of SS and SPS in drought-tolerant Kentucky bluegrass (*Poa pratensis* L.) increased with drought aggravation, while the activities of carbon metabolic enzymes in drought-sensitive Kentucky bluegrass did not change during drought (Yang et al. 2013). The inconsistency may be because plant accumulation and transport are determined by various enzymes (Yang et al. 2004). This study showed that the activity

<https://doi.org/10.17221/426/2022-PSE>

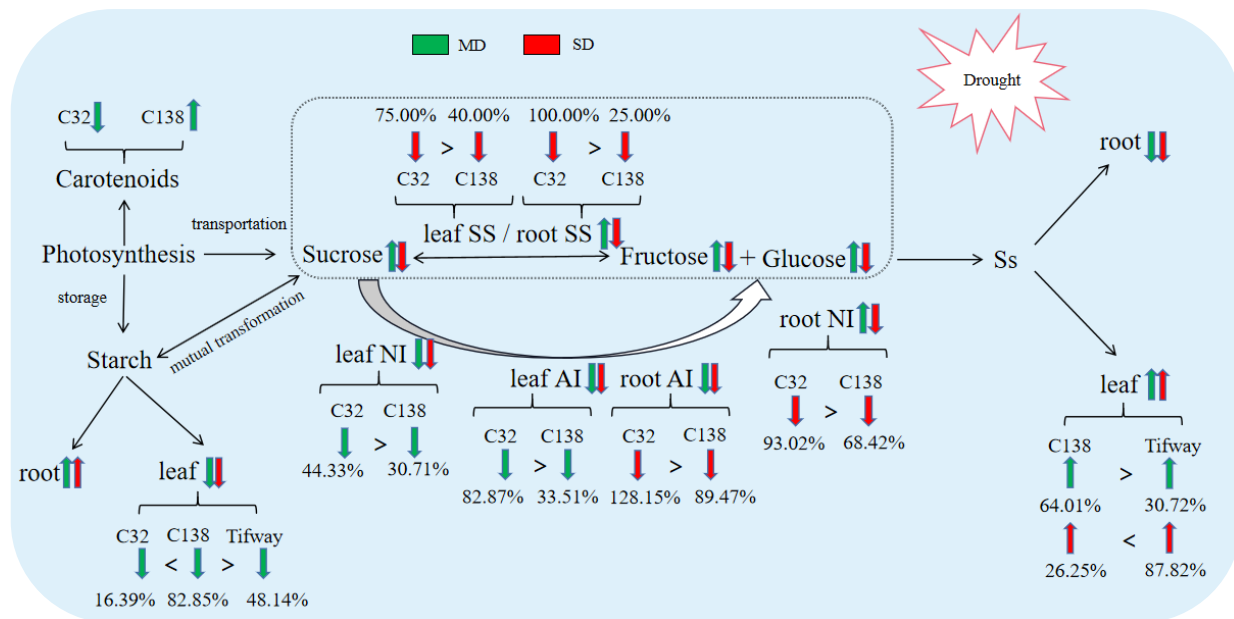


Figure 8. Response difference of carbon metabolism in leaves and roots of bermudagrass under drought stress. Green represents moderate drought (MD), red represents severe drought (SD), the up arrow represents an increase, the down arrow represents a decrease, and C32, C138 and Tifway represent test materials. Ss – soluble sugar; SS – sucrose synthase; SPS – sucrose phosphate synthase; AI – acid invertase; NI – neutral invertase. Percentage value of Arabic numbers indicating rise or fall

of SPS in bermudagrass leaves decreased gradually. SS activity increased first and then decreased under drought stress, while the activity of SS and SPS in bermudagrass roots increased first and then decreased under drought stress. The activity of SS and SPS in leaves was higher than that of roots, indicating that sucrose metabolism was more active in leaves than in roots. MD could increase SS activity in leaves and the activity of SS and SPS in roots of bermudagrass, inhibit SPS activity in the leaves, promote sucrose decomposition in the leaves, thus decreasing the osmotic potential of cells, promote water absorption and reduce plant damage. Fructose and sucrose decomposed from leaves were transported to roots. SS and SPS in roots were in dynamic balance, which promoted the synthesis and decomposition of sucrose, maintained normal cell osmotic potential and alleviated the damage to roots.

Our result showed that the invertase activity in the bermudagrass leaves decreased with drought stress aggravation, and the decrease was more significant under severe drought stress. The AI activity in roots also decreased, but the NI activity increased first and then decreased, indicating that MD increased roots' NI activity and enhanced the ability of roots to decompose sucrose. Still, SD caused serious damage

to roots and decreased NI activity. The activities of AI and NI in leaves were always higher than those in roots under drought stress. The contents of fructose and glucose in roots were higher than those in leaves, which again proved that fructose and glucose were preferentially allocated to roots and reduced their oxidative damage; path analysis also showed that leaf NI activity was an important indicator for drought resistance of bermudagrass. Barratt's et al. (2009) research showed that the role of sucrose synthase in plant sucrose metabolism is far lower than invertase. Similarly, this study also showed that the SS activity of leaves was significantly lower than invertase activity. Another study showed that the invertase activity of drought-resistant Kentucky bluegrass was higher than that of drought-sensitive cultivars (Yang et al. 2013), which was similar to the results of this study. In this study, drought-resistant materials' AI and NI activities were significantly higher than those of C32 at all treatments (Figure 8).

The balance between plant carbon assimilation and carbon consumption can be measured by the size of the NSC pool, and the ability of plants to respond to environmental stress is reflected by the selective supply of NSC in their organs. Qiu et al. (2019) thought that seedlings of three shrubs tended

to preferentially supply NSC to roots under drought stress, and increased NSC in roots could be used for root growth to increase water uptake and improve drought resistance. Similar rules also appear in this article; with drought aggravation, bermudagrass accumulated soluble sugar in leaves and starch in roots, as may be because, under drought, more NSC will be transformed into soluble sugars and other substances with the osmotic regulation ability to maintain the osmotic pressure balance of leaves under stress, and energy storage substances will be transferred to roots for preservation. On the other hand, the preferential distribution of NSC promotes the respiration of bermudagrass roots, thus increasing the opportunity for bermudagrass to obtain water during drought.

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Received: December 1, 2022

Accepted: May 23, 2023

Published online: June 6, 2023