EFFECT OF REGULATED DEFICIT IRRIGATION ON THE MORPHOLOGY, PHYSIOLOGY, CARBON ALLOCATION AND NONSTRUCTURAL CARBOHYDRATES OF THREE KENTUCKY BLUEGRASSES

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Abstract

Regulated deficit irrigation (RDI) has been assessed in a wide number of field and fruit crops. However, few are the studies dealing with turfgrass. This study was conducted to investigate the morphology, physiology and carbon metabolic responses to regulated deficit irrigation for three Kentucky bluegrass (*Poa pratensis* L.) cultivars. Three Kentucky bluegrass cultivars were grown in PVC (polyvinyl chloride) tubes in a greenhouse and subjected to three soil water treatments in a growth chamber: 1) full irrigation; 2) drought stress, 21 days without water after full irrigation; and 3) drought recovery, stressed plants were re-watered for an additional 21 d. The present study indicated that drought resulted in a decline in turf quality (TQ), leaf relative water content (RWC), and photochemical efficiency (Fv/Fm) and an increase in electrolyte leakage (EL) for the cultivars. The turf quality, RWC, and Fv/Fm of the three Kentucky bluegrass cultivars increased with re-watering. The allocation of ^{14}C increased in the roots of these cultivars during the initial phase of drought stress, where a ^{14}C distribution shift from the roots to the stem and leaves appeared with further drought stress. Moreover, there was a significant accumulation of total nonstructural carbohydrates (TNC) in the leaves and stem. The TNC content in the leaves, stem, and roots did not completely return to the control levels following 21 d of re-watering, which was consistent with the recovery of TQ, RWC, Fv/Fm, and EL. In addition, during the re-watering treatment, the reduction in the TNC content may be due to increases in the demand or usage as a result of a rapid recovery in the growth and physiological activities as shown by increased TQ, RWC, and Fv/Fm and decreased EL. Our results suggested that the changes in the carbon allocation model and the accumulation and storage of TNC, as well as the changes in TQ, RWC, Fv/Fm, and EL, for the three cultivars are an adaptive reaction to drought and re-watering following drought. The accumulation and storage of TNC in plants during severe drought may contribute to the survival of meristematic regions and/or serve as reserves, which contribute to plant re-growth once the drought stress is alleviated. This result suggests that the allocation of carbon assimilates and their accumulation in different organs of turfgrass were a physiological adaptation response to drought and re-watering treatments, thus providing a scientific basis for further investigating the resistance of turfgrass to other adverse environments.

Key words: Drought, Recovery, Nonstructural carbohydrates, ^{14}C allocation.

Introduction

The study of irrigation water saving techniques such as the regulated deficit irrigation (RDI), which can be performed by growers with a minimal or any negative effect on yield, is gaining importance during the last decades due to the future scenario of increasing water scarcity occurring in a large number of areas all over the world (Abraham *et al*., 2004; Zulini *et al*., 2007). Kentucky bluegrass (*Poa pratensis* L.) often experiences long-term drought conditions, which ultimately reduce performance and quality. Therefore, the lack of drought resistance restricts the use of this plant under water deficit conditions. Water is becoming increasingly limited for irrigation. The development of improved water-saving varieties is a high priority in turfgrass breeding programs. Regulated deficit irrigation not only can save water, but also can ensure the plant normal growth quality (Martorell *et al*., 2014). The reactions of the plants to water stress differ significantly at various organizational levels, depending on the intensity and duration of stress as well as on the plant species and its stage of development (Chaves *et al*., 2003). The deleterious effects of drought stress are associated with damage to cell membranes, photosynthesis(Ali *et al*., 2015; Dinler *et al*., 2014; Weng *et al*., 2015), and antioxidant systems in turfgrass (Jiang & Huang, 2000; Abraham *et al*., 2004).

Previous studies have reported that turfgrass species and cultivars varied in their recuperative capability from drought stress alone (Qian & Fry, 1997; Huang *et al*., 1998a). After re-watering following drought stress, the leaf water status and turf quality of drought-tolerant Tall Fescue (*Festuca arundinacea* Schreb.) cultivars recovered to the pre-stress levels, but Fv/Fm did not recover completely, indicating permanent damage of the photosynthetic apparatus in the existing leaf tissues (Huang *et al*., 1998a). These authors also reported that osmotic adjustment contributed to plant re-growth from drought stress following re-watering in warm-season turfgrass species. Regardless of temperature conditions, re-watering was essential for the physiological recovery from the combined drought and heat stress, rapid resumption of Fv/Fm, cell membrane stability, and antioxidant activities were important factors contributing to the recovery of Kentucky bluegrass (Wang & Huang, 2004).

Carbon metabolism is affected by water availability several studies have reported that many species increased the carbon investment in roots to adapt to soil water deficits, as demonstrated by an increased carbon allocation to roots and an increased root-to-shoot biomass ratio (Volaire, 1995; Assuero *et al*., 2002). Huang & Gao (2000), also reported a higher allocation of newly photosynthesized carbon to the roots of drought - tolerant cultivars of tall fescue under soil

drying. Huang & Gao (2000), Huang & Fu (2000) found that the TNC content was either unaffected or increased in Tall Fescue (*Festuca arundinacea* Schreb.) and Kentucky bluegrass (*Poa pratensis* L.) under soil drying conditions. During stress and recovery from stress, the availability and utilization of carbohydrates always change. The TNC content within plant tissues has been used as an indirect measure index to evaluate the physiological response to environmental and cultural factors for turfgrass (Narra *et al*., 2004; DaCosta & Huang, 2006). Under prolonged drought stress, the survival or resistance of perennial grass species may be important for the recovery from the stress when water becomes available.

DaCosta & Huang (2006) reported that carbon allocation to the roots of bentgrass species increased during the early stage of drought stress, exhibiting an adaptive response to short-term drought stress; moreover, carbon allocation and carbohydrate accumulation in the stems increased during prolonged periods of drought stress, which could facilitate the rapid recovery of turf growth and water status following re-watering.

Although there have been several reports on the adverse impact of drought on plant growth and physiological activities, mainly focusing on the relief of drought stress, much less has been published about the recovery of physiological functions following stress relief. For perennial turfgrass, the most important strategy is not the maintenance of growth or production during stress, but the ability to survive and recover rapidly from the drought stress after rainfall or irrigation. Therefore, it is necessary to understand the underlying mechanisms of recovery to improve the survival of cool-season grasses in dry environments, which would enable plant breeders to enhance the selection and screening strategies that are used to identify improved cultivars.

Therefore, the objectives of the study were 1) to examine the physiological factors associated with the persistence and recovery of Kentucky bluegrass cultivars exposed to drought and following re-watering and 2) to determine if the persistence and recovery resilience are associated with differences in physiological factors. We have been carried regulated deficit irrigation (RDI) tests to three Kentucky bluegrasses in greenhouse, aiming to discover the effect of physiological reaction and water-saving, thus providing a scientific basis for RDI in field experiment. In future research, we want to seek the soil water deficit levels when the lawn quality is best, then providing theoretical basis for the scientific management of the lawn. It is important to improve the utilization efficiency of water resources, promote the healthy and stable development of grass industry, maintain the ecological balance, beautify the living environment.

Materials and Methods

Plant material and growth conditions: Three Kentucky bluegrass cultivars were chosen as experimental materials, including "Liberty", "Kerley", and "Merit". Sods (10 cm in diameter and approximately 3 cm in thickness) robust and uniform, were collected from field plots at the YuZhong campus of Lanzhou University, in August 2011. All of the sods were planted in polyvinylchloride (PVC)

tubes (10 cm in diameter, 50 cm high) filled with a mixture of sand and topsoil (1:3,v/v); the soil of the experimental area was the loessal soil form, with a bulk density of 1.3 g·cm^{-3}, organic matter content of 0.737%, 0.0589% total nitrogen, available phosphorus content of 4.77 mg·kg^{-1}, and rapidly available potassium content of 132.95 mg·kg^{-1}.

The plants were grown in a greenhouse under natural light conditions and 20-28°C/ 15-19°C day/ night temperatures and were watered until water ran freely from the bottom of the tubes three times per week. All of the Kentucky bluegrass cultivars were hand clipped weekly at a height of approximately 3 to 4 cm and were fertilized every 2 weeks with controlled-release fertilizer (N-P-K, 16-4-8) to provide nutrients and facilitate plant establishment prior to initiation of the treatments. After 60 d, the plants were then transferred to a growth chamber set at 25/ 16°C day/ night temperatures, 14 h photoperiod, and a photosynthetic photon flux density of 800µmol m^{-2} s^{-1}.

Treatments: The experiment consisted of three soil water content availability treatments: (1) full irrigation, irrigated three times per week with tap water to maintain the soil water content near pot capacity; (2) drought stress, 21 days without water after full irrigation; and (3) drought recovery, maintained well-watered for another 21 d.

Measurements: During the drought stress, the TQ, RWC, EL, Fv/Fm, ^{14}C allocation patterns, and TNC content were determined. The turf quality was measured for percent turf cover and turf color using Sigma Scan Pro software (SPSS, 1998). Here, the percent turf cover and turf color measured by the Digital images technique represented the TQ. The digital images were obtained with a Canon Power Shot S3IS (Canon Inc., Japan) digital camera. Digital images were taken every 5 d for each tube and analyzed for turf density (Richardson, 2001) using a hue range of 45 to 107 and a saturation range of 10 to 100 to isolate the above tube. The digital camera was mounted on a monopod that was designed specifically for this study, when photo was taken, each pot was taken out every time. Each photo was taken 0.8 meters above the turfgrass canopy and between 1000 h and 1100 h. The same size square image was taken of a 10 cm pot.

The leaf water status was determined by measuring the relative water content (RWC, %) calculated as follows (Barrs & Weatherley, 1962):

$$RWC = (FW–DW)/(SW–DW)×100$$

where FW is the leaf fresh weight, DW is the leaf dry weight after drying at 80°C for 2 d, and SW is the leaf turgid weight after being soaked in water for 4 h at room temperature (approximately 20°C). The RWC was measured at 7 d intervals.

The electrolyte leakage of leaves was measured according to the method of Blum and Ebercon (Blum & Ebercon, 1981) and Marcum (Marcum, 1998), with modifications at 7 d intervals. The leaves were excised and cut into 1 cm segments. After being rinsed three times with distilled deionized H$_2$O, the leaves were placed in test tubes containing 20 mL distilled deionized H$_2$O. The test tubes were shaken on a shaker for 17 to 18 h, and the

initial conductivity (C1) was measured (YSI-3100, Guangzhou, China). The leaves then were killed at 120°C for 30 min, and the conductivity of the killed tissue (C2) was measured after the tubes cooled to room temperature. The relative EL was calculated as (C1/C2). The leaf photochemical efficiency was estimated by measuring the chlorophyll fluorescence (Fv/Fm) with a fluorescence induction monitor (Bio Scientific Ltd. Herts, UK). The Fv/Fm was measured after keeping leaves in the dark for at least 30 min measured at 7 d intervals.

The carbon allocation patterns between the shoots and roots were determined using a ^{14}C labeling technique (Huang & Fu, 2000) in three cultivars, demonstrating distinct responses to drought stress. Plants in four containers were labeled in the morning with $^{14}CO_2$ at 0 (pre-stress), 11, and 18 d of drought stress, and 7 and 18 d of re-watering. On these labeling dates, the shoots were enclosed in a transparent plastic film chamber (10cm diameter, 15cm tall), which was fitted tightly to the PVC plant container and exposed for 40 min to 40 μCi$^{14}CO_2$ that was released from $Na_2$$^{14}CO_3$ (6.8μCi mol-1) by reacting with 1N HCl. After the 40 min labeling, excessive $^{14}CO_2$ was absorbed by bubbling the gas through a saturated NaOH solution for 30 min. Three days after labeling, the whole plants (including the shoots and roots) were harvested, and the roots were washed free of soil at 0, 14, and 21 d of drought treatment, and 10 and 21 d of re-watering. The shoots were separated into leaves and stem base components (including basal leaf sheaths, crowns, and lateral stems). The leaf, stem, and root samples were dried in an oven at 85°C for 48 h and then ground with a tissue grinder and stored separately in sealed vials for analyses of ^{14}C activity and carbohydrates content. The ^{14}C activities in the shoots and roots in each cultivar for each treatment were measured with a liquid scintillation analyzer (Beckman LS-6500, Beckman Coulter. Inc., California, US.).

The total nonstructural carbohydrate (TNC) concentrations of the shoots and roots were measured using the method described by DaCosta & Huang (2006).

Statistical analysis: The experiment consisted of two factors (three cultivars and three soil moisture treatments) with four replieations for each treatment in a completely randomized block design. The treatment effects were determined by ANOVA according to the general linear model procedure of the Statistical Analysis System V.8.2 (SAS Institute, Cary, NC). The differences among treatments for each cultivar were determined by the LSD test at the 0.05 probability level.

Results

There were no significant differences for any of the six indices for each cultivar between the pre-stress and control levels; therefore, data for the pre-stress level of each cultivar represents the control level of the respective cultivar. Following re-watering, the TQ for the three cultivars reached different levels, and the recuperative ability of the TQ varied with the cultivar and re-watering time (Fig. 1). Kerley exhibited the highest TQ, Liberty the lowest TQ, and Merit an intermediate TQ during the re-watering period. Kerley recovered up to 84% of the pre-stress TQ level by 21 d of the re-watering treatment; Merit and Liberty recovered up to 86% and 75% of the

TQ pre-stress level, respectively, but none of the cultivars recovered to the pre-stress level.

During the first 7 d of drought stress, the RWC for the three cultivars did not change. After 14 d of drought stress, the RWC for the three cultivars markedly decreased depending on the cultivar, compared to the pre-stress level. At 21d of drought stress, the RWC for Kerley, Merit, and Liberty decreased to 35.5, 47.2, and 24.8%, respectively. Following 7 d of re-watering, the RWC for Merit and Kerley recovered to 57.4, and 46.9%, which was significantly higher than the RWC recovery for Liberty (41.6%) (Fig. 2). At 21 d of re-watering, there were no significant differences in the RWC among the three cultivars. The leaf RWC for the three cultivars fully recovered to the pre-stress levels.

After 7 d of drought stress, the Fv/Fm for the three Kentucky bluegrass cultivars gradually decreased (Fig. 3). Following 14 d drought stress, the Fv/Fm for the three cultivars markedly decreased and the extent of reduction in the Fv/Fm varied with the cultivar and drought duration. At 21 d of drought stress, the Fv/Fm for Merit, Liberty, and Kerley decreased to 0.6, 0.5, and 0.4, respectively. Compared to Merit, the reductions in the Fv/Fm for Liberty and Kerley were more marked but the reduction in the Fv/Fm for Kerley was the most obvious among the three cultivars under drought stress conditions. Following 7 d of re-watering, the Fv/Fm for the three cultivars gradually increased; at 14 d of re-watering, the Fv/Fm for Kerley, Merit, and Liberty recovered to 0.64, 0.71, and 0.61, respectively. At 21d of re-watering, the Fv/Fm for Kerley, Merit, and Liberty did not recover fully, but returned to 0.86, 94, and 85% of the pre-stress level, respectively. The electrolyte leakage for the three Kentucky bluegrass cultivars increased during the drought stress period (Fig. 4).However, the changes in the EL varied with the cultivar and treatment time. The EL for Kerley was significantly higher than that for Liberty and Merit throughout the entire drought stress period; after the drought stress, the EL for Merit was the lowest among the three cultivars.

At 7 d of re-watering, the EL for the three cultivars gradually decreased (Fig. 4). Following 7 d of re-watering, the EL for three cultivars decreased; at 21d of re-watering, the EL for Kerley, Merit, and Liberty was returned to 12.7, 10, and 16.1, respectively, The EL for three cultivars did not completely recover to their pre-stress levels. The carbon allocation among the leaves, stems, and roots for the three cultivars differed with drought stress progression. At 14 d of drought stress, the ^{14}C distribution to the roots of Liberty, Merit, and Kerley was markedly increased and increased from18.3% to 49.8%, 27.6% to 39%, and 25.4% to 41.6%, respectively (Fig. 5-A, B, C). Moreover, the proportion of ^{14}C that was distributed to roots was the greatest, while it was moderate for the stem, and the least for the leaves of the three cultivars at 14 d of drought stress. At 21d of drought stress, the ^{14}C distribution to the roots was markedly decreased to 25.9, 22.6, and 24% for Kerley, Liberty, and Merit, respectively. However, a higher proportion of ^{14}C was distributed to leaves of Kerley, and to the stems of Merit, although there were no significant differences in the proportion of ^{14}C between leaves and stems of Merit.
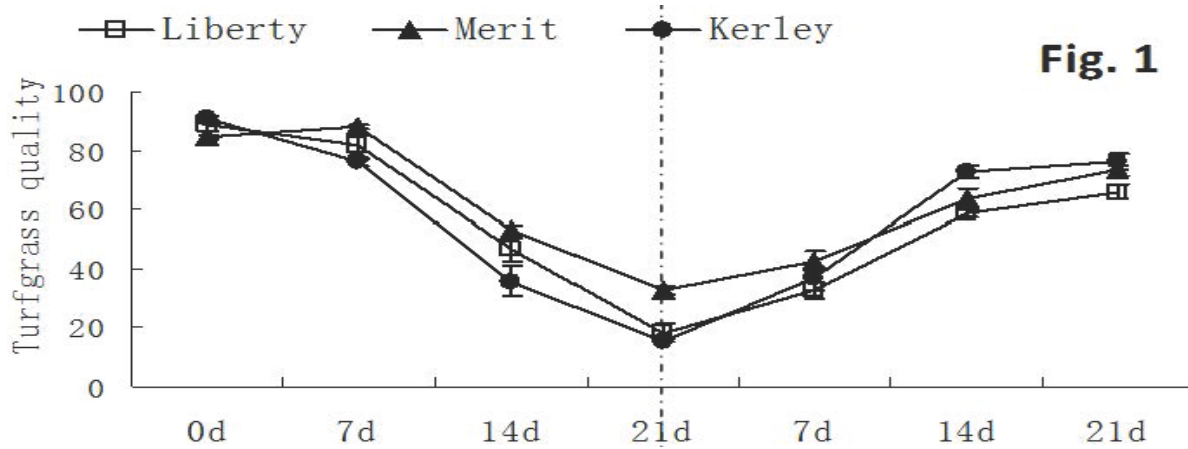
Fig. 1. Changes in turf quality, TQ during drought stress and re-watering for Liberty, Merit, and Kerley. The dashed line separates the drought and recovery treatments and represents the first day of re-watering in which the recovery period was initiated. The vertical bars are standard error values (p<0.05) indicating statistically significant differences for the cultivar comparisons at a given day of treatment.
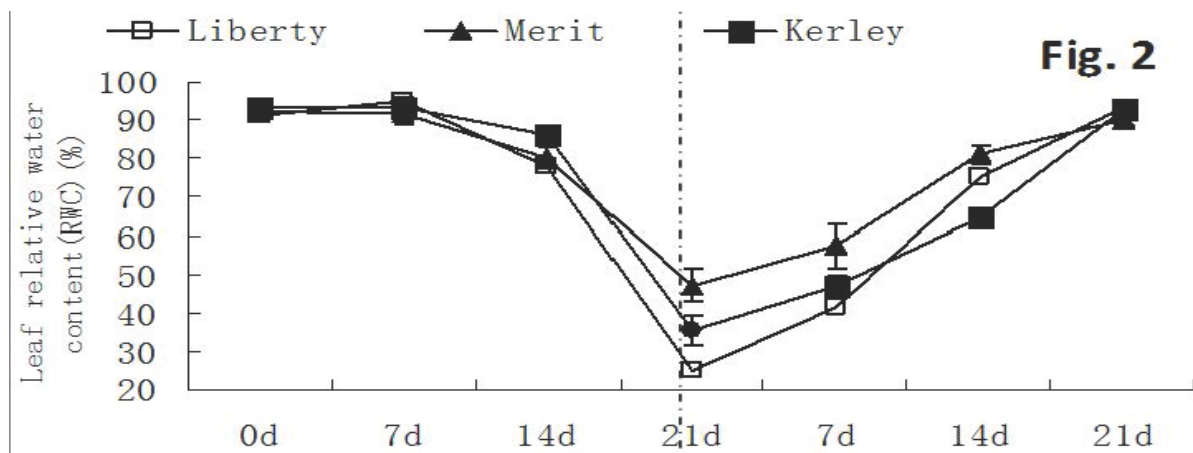

Fig. 2 Changes in the leaf relative water content, RWC during drought stress and re-watering for Liberty, Merit, and Kerley. The dashed line separates the drought and re-watering treatments and represents the first day of re-watering in which the recovery period was initiated. The vertical bars are standard error values (p<0.05) indicating statistically significant differences for the cultivar comparisons at a given day of treatment.

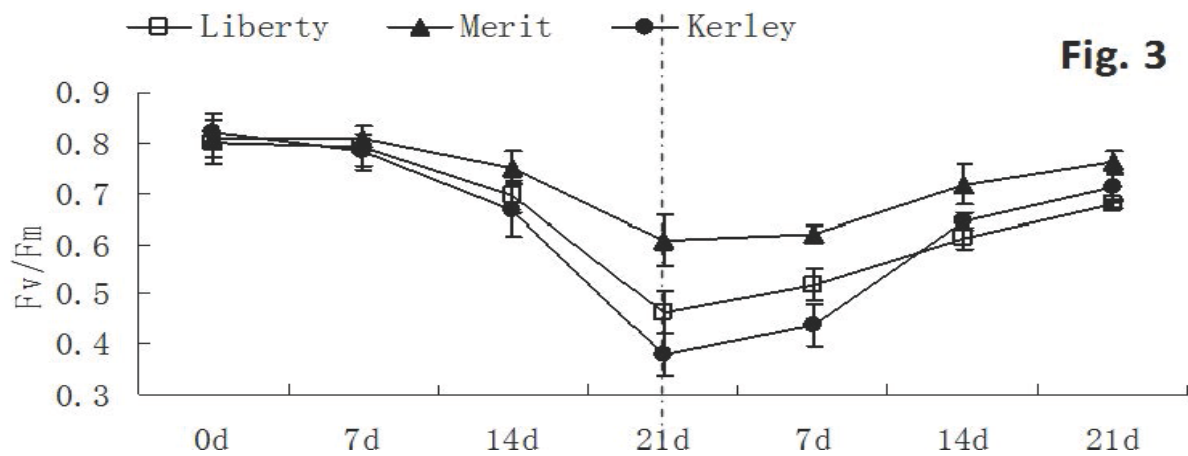Fig. 3. Changes in photochemical efficiency ,Fv/Fm during drought stress and re-watering for Liberty, Merit, and Kerley. The dashed line separates the drought and re-watering treatments and represents the first day of re-watering in which the recovery period was initiated. The vertical bars are standard error values (p<0.05) indicating statistically significant differences for the cultivar comparisons at a given day of treatment.
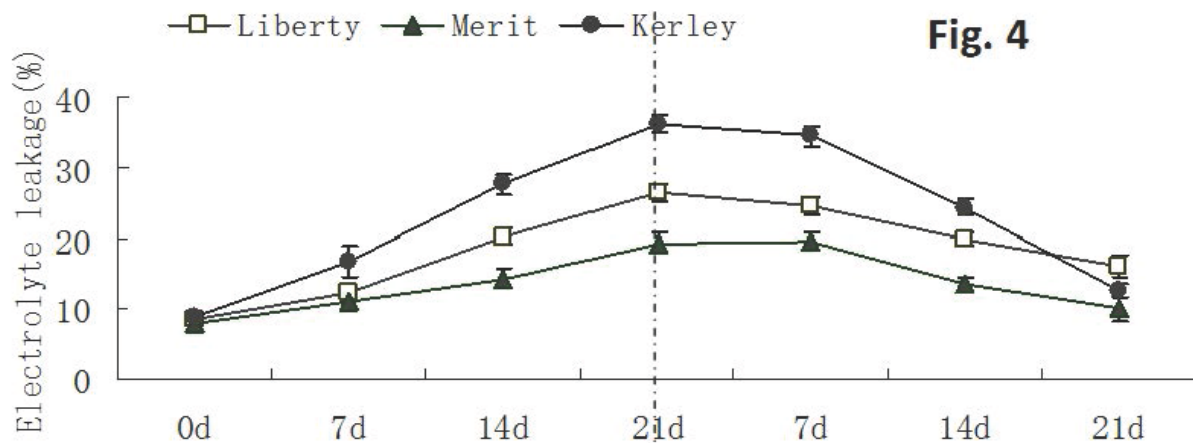
Fig. 4. Changes in electrolyte leakage, EL during drought stress and re-watering for Liberty, Merit, and Kerley. The dashed line separates the drought and re-watering treatments, and represents the first day of re-watering in which the recovery period was initiated. The vertical bars are standard error values (p<0.05) indicating statistically significant differences for the cultivar comparisons at a given day of treatment.
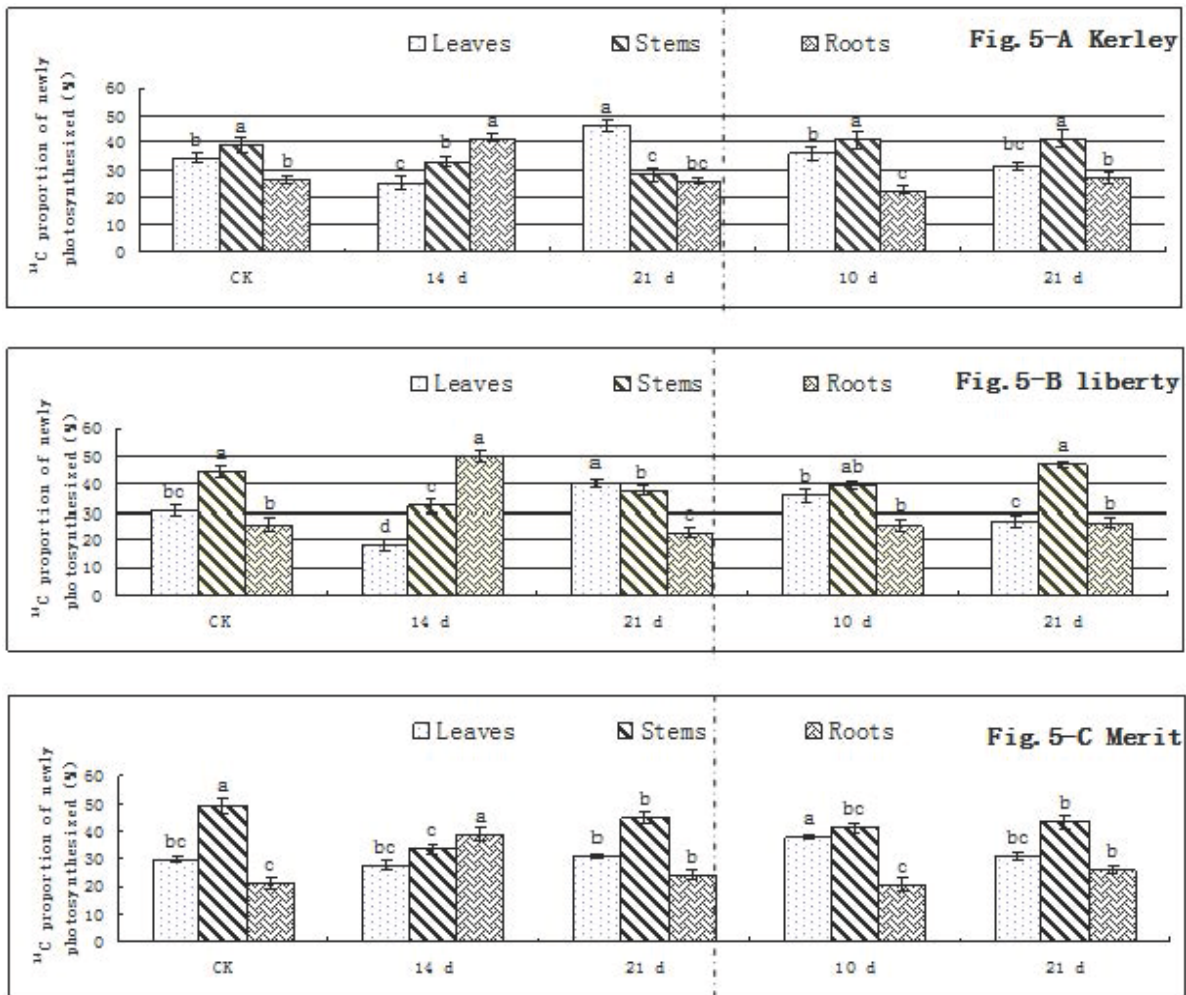

Fig. 5 Proportion of newly photosynthesized 14C allocated to the leaves, stem, and roots during drought stress and re-watering for Liberty, Merit, and Kerley. The data presented are for the assessment of 14C allocation at 0 (pre-stress), 14, and 21d of drought treatment, and 10 and 21 d of re-watering treatment. The values followed by the same lowercase letter for each cultivar indicate no significant differences in the 14C allocation between different plant organs (p<0.05) based on a LSD test. The dashed line separates the drought and re-watering treatments and represents the first day of re-watering in which the recovery period was initiated.

At 10 d of re-watering, the proportion of ^{14}C that was allocated to the leaves significantly decreased but increased in the stems for Kerley; the proportion of ^{14}C allocated to the leaves decreased but significantly increased in the roots for Liberty, compared to that at 21 d of drought stress. In contrast, there was a significant increase in the leaves and a decrease in the roots for Merit. At 21d of re-watering, there was a significant decrease in the leaves and a significant increase in the stem for Kerley and Liberty, compared to that at 21 d drought stress. The general ^{14}C allocation pattern for the three cultivars was stems > leaves > roots during the entire re-watering treatment.

At 14 d of drought stress, there were significant increases in the TNC content in the leaves, stem, and roots for the three cultivars compared to the control levels. Following 14 d of drought stress, TNC content in roots of Kerley and Liberty significantly declined (Fig. 6-A, B, C). The TNC content accumulation model in the leaves, stem, and roots for the three cultivars was leaves > stem > roots during the entire drought stress period. Following 10 d of re-watering, the TNC content in the leaves and stem for the three cultivars significantly decreased compared to the level at 21 d of drought stress; there was a significant decrease in the roots for Merit. At 21 d of re-watering, the TNC content in the leaves, stem, and roots of the three cultivars significantly decreased compared to the level at 21 d of drought stress. The three cultivars showed a TNC content that was greater in the leaves and lower in stem and roots during the entire re-watering treatment.
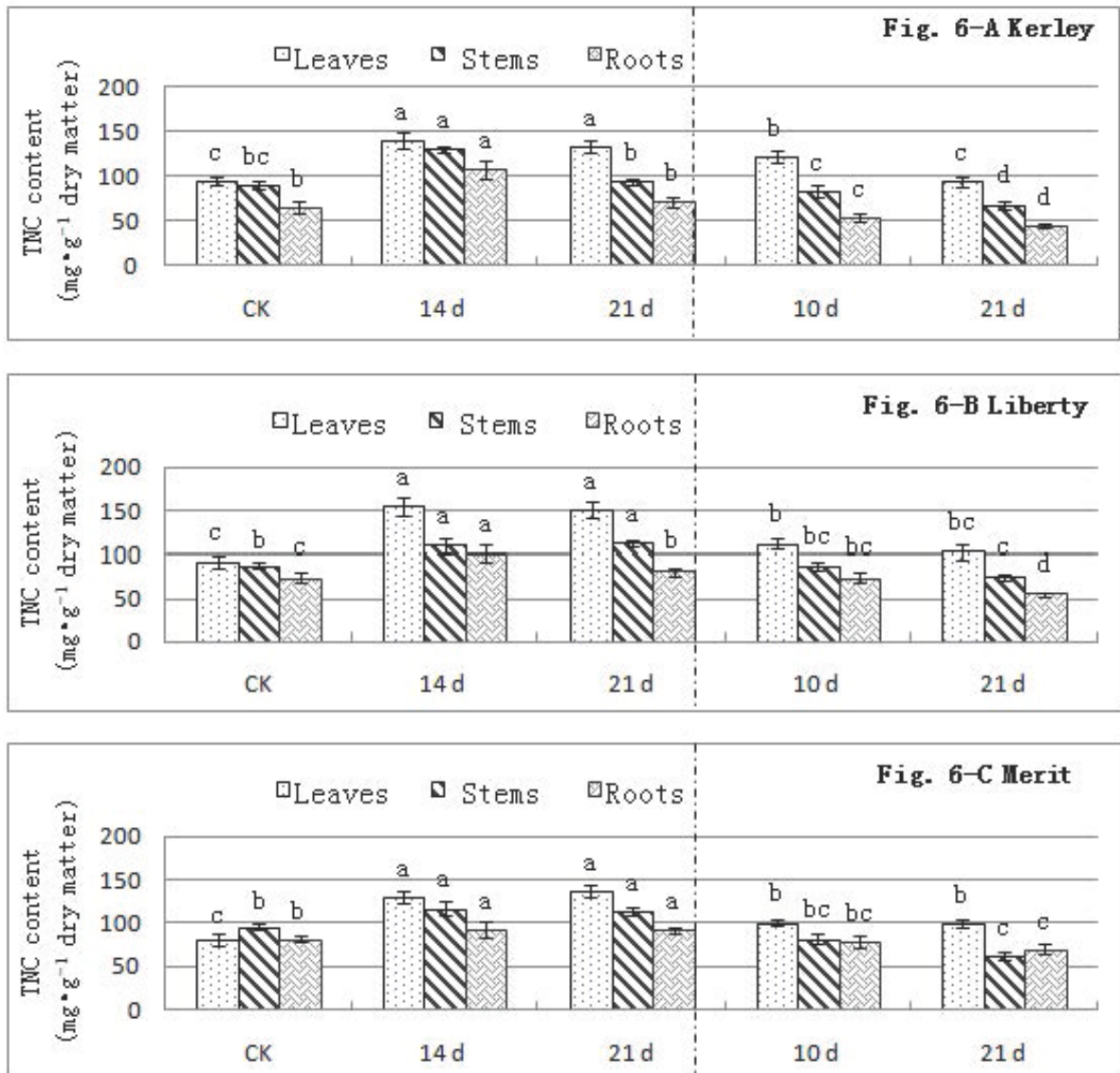

Fig. 6 Changes in the total nonstructural carbohydrate content ,TNC. content in the leaves, stem, and roots during drought stress and re-watering for Liberty, Merit, and Kerley. The data presented are for the assessment of the TNC content at 0 (pre-stress), 14, and 21 d of drought treatment, and 10 and 21 d of re-watering treatment. The values followed by the same lowercase letter for each cultivar indicate no significant differences in the TNC content between different plant organs (p<0.05) based on a LSD test. The dashed line separates the drought and re-watering treatments and represents the first day of re-watering in which the recovery period was initiated.

Discussions

The core content of regulated deficit irrigation research is one of the effect of re-water after drought stress on physiological indexes of plant (Dos *et al.*, 2007). According to the crop water requirement characteristics to carry on the regulated deficit irrigation artificially. Through the soil water management to control water potential of the above-ground plant parts and the growth of the branches. Through the leaf water potential to regulate stomata opening and closing, enhancing plant drought resistance. According to the "root cap balance theory", the RDI makes soil drought, then nutrients back into the root, result in the growth of above-ground plant parts is slow. Performance on the blade, its growth speed is slow, leaf area reducing, plant photosynthetic rate, transpiration rate, nutrient transport are blocked, making the distribution of photosynthetic product in different tissues and organs, regulating the ground and underground dynamic growth.

Drought stress caused significant decreases in the TQ and RWC and increases in the EL for three Kentucky bluegrass cultivars. The changes in the TQ, RWC, and EL for the 3 cultivars were the most pronounced for Kerley, intermediate for Liberty, and least for Merit. In the present study, the dramatic decrease in the TQ under drought stress was closely related to a decrease in the RWC and an increase in the EL during the later periods of stress. The drought environment induces abnormal transpiration water loss, which can also cause rapid cell desiccation and injury, as demonstrated by a decrease in the TQ and RWC and an increase in the EL (Abraham *et al.*, 2004). Drought - tolerant turfgrass are commonly able to maintain high levels of TQ and RWC, but low levels of EL (an indicator of cell membrane stability) during drought stress (Huang & Gao, 1999). These physiological parameters have been widely used as physiological indicators for the selection of drought tolerant plant materials in turfgrass and other species (Jiang & Huang, 2001a). According to changes in TQ, RWC, and EL for the three cultivars in our study, we deduced that Merit was the most tolerant cultivar, Liberty was intermediate, and Kerley was the least.

Following re-watering, the TQ, RWC, and EL for the three cultivars could return to above 75% of the pre-stress level. These results suggest that the three cultivars have certain recovery capabilities once water stress is relieved. Among the three cultivars, the recovery capability in terms of the TQ, RWC, and EL for Kerley during the re-watering treatment was more pronounced than that for Merit and Liberty. Previous studies reported that cultivar differences in resistance to drought stress as well as in the extent of recovery from drought were associated with various morphological, physiological, and biochemical factors (Bonos & Murphy, 1999) and membrane stability (Jiang & Huang, 2001b). The results of the present study agree with previous reports.

Decreases in the Fv/Fm could be related to limited CO_2 diffusion into the intercellular spaces of the leaf as a consequence of reduced stomata opening imposed by the water deficit and/or to impaired metabolism by the direct inhibition of biochemical processes (Colom & Vazzana, 2003). In our study, the decreases in the Fv/Fm for Liberty, and Kerley were higher than that for Merit. Merit

used water more efficiently and maintained a higher TQ and Fv/Fm. The results suggest that the drought stress injured the photosynthetic system, as indicated by the decrease in the Fv/Fm for the three cultivars. Our results are consistent with those of previous reports (Jiang & Haung, 2001b; Colom & Vazzana, 2003; Abraham *et al.*, 2004; Kemin *et al.*, 2007). Following 14 d of re-watering, the Fv/Fm for Merit, Kerley and Liberty recovered to 89%, 78% and 61% of the pre-stress levels, respectively. At 21 d of re-watering, the Fv/Fm for Merit, Kerley, and Liberty returned to 94%, 90%, and 86% of the pre-stress levels respectively. The Fv/Fm for the three cultivars did not recover completely, indicating permanent damage of the photosynthetic apparatus in the existing leaf tissues (Huang *et al.*, 1998b). The ^{14}C radio isotopes techniques may provide insight into the distribution pattern of newly photosynthesized carbon to the shoots and roots, as well as into how the distributing patterns are affected by factors such as water availability. The results from our study indicated that the proportion of newly photosynthesized ^{14}C that is distributed to roots of Liberty, Merit, and Kerley significantly increased during the early stages of drought stress (14 d) prior to severe water deficit and turf damage. Concerning the ^{14}C allocation proportion in different organs, our results show that following 14 d of drought stress, the ^{14}C allocation of the three cultivars shifted toward the leaves and stem; however ,the proportion of ^{14}C that was allocated to the leaves and stem varied with the cultivar. These carbon distribution pattern results are consistent with those from previous studies on root adaptive mechanisms under drought stress for other species (DaCosta & Huang, 2006).

Huang and Gao (2000) reported that soil drying led to an increase in the proportion of ^{14}C distributed to the roots in drought-tolerant cultivars of tall fescue. Da Costa and Huang (2006) also reported that drought resulted in an increasing proportion of ^{14}C distributed to the roots for three bentgrass species. Our results agree with the above reports above, in that a higher investment of carbon to the roots of Merit could contribute to its maintenance of a higher TQ, RWC, and Fv/Fm, and lower EL compared to Liberty and Kerley during prolonged periods of drought.

Following re-watering, the ^{14}C allocation to the leaves of the three cultivars gradually decreased but increased in the stem, and did not change in the roots. Similar to ^{14}C allocation models, during the entire drought stress period, the TNC content in the leaves for the three cultivars was higher than that for the respective control cultivars in our study. The accumulation of carbohydrates in the leaves could be related to a decreasing utilization of carbohydrates due to the inhibition of shoot growth under drought. These results agree with the reports by Volaire & Thomas (1995).

In our study, there were significantly higher stem TNC contents than that in the respective controls for Liberty and Merit during the entire drought stress period; however, no differences in the the stem TNC content appeared at 21 d for drought-stressed and control plants for Kerley. Volaire (1995) reported that a greater accumulation of carbohydrates in the leaf and stem bases was related to an improved recovery following drought stress. Under water

deficit, increases in the TNC content of plants may facilitate osmotic adjustment to sustain turgor and meristem viability or may also serve as reserves for biosynthesis following drought stress (Qian & Fry, 1997). In addition, other product is different drought-tolerant cultivar of White Clover accumulated differeft carbohydrate reserves in the stem, also have different recovery through exogenously applied spermine (Spm) (Li *et al*., 2015). Our results indicate that the greater accumulation of TNC in the stems of Kerley and Merit could be related to the more rapid recovery of TQ, RWC, Fv/Fm, and leaf EL. Following 21 d of re-watering, the TNC content in leaves, stem, and roots of the three cultivars were even lower than even the control levels, which could be related with the recovery in the TQ, RWC, Fv/Fm, and EL. These results suggest that the physiological metabolism of the three cultivars could recover from drought stress following a prolonged period of re-watering. Concerning the TNC content reduction in the leaves, stem, and roots during re-watering, we deduced that it could be associated with increases in the demand or usage due to a rapid recovery in the growth and physiological activities as shown by increased TQ, RWC, and Fv/Fm, and decreased EL. This result Is consistent with that of a previous report (DaCosta & Huang, 2006).

In summary, the three Kentucky bluegrass cultivars exhibited reduced TQ, RWC, and Fv/Fm, and increased EL in response to drought stress. The present study also testified that the distribution of newly photosynthesized carbon and the carbohydrate status in different organs of Liberty, Merit, and Kerley changed with the length of both the drought stress duration period and the re-watering period. The distribution of ^{14}C increased in the roots during the early stage of drought stress, which may serve as a short-term drought adaptive mechanism for the three cultivars. Subsequently, the carbon allocation shifted toward the leaves and stem, where there was a significant accumulation of TNC. During prolonged drought stress, the accumulation and storage of TNC may either contribute to the osmotic adjustment and survival of meristematic regions and/or serve as reserves, thus contributing to plant re-growth once drought stress is relieved. Following prolonged re-watering, the TQ, RWC, EL, and Fv/Fm for the three cultivars could recover to the pre-stress levels. Concerning drought tolerance among the three cultivars, Merit exhibited the highest tolerance, while it was intermediate for Liberty, least for Kerley; however, a higher drought tolerance did not indicate a greater recovery capability as Kerley exhibited a greater recovery capability, and the recovery was intermediate for Merit and least for Liberty. It would be very valuable to breed turfgrass cultivars with high drought survival and recuperative abilities, which are associated with efficient carbon allocation and utilization, to further improve the adaptation of turfgrass to water-limited environments.

Acknowledgements

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