Membrane Fatty Acid Composition and Saturation Levels Associated with Leaf Dehydration Tolerance and Post-drought Rehydration in Kentucky Bluegrass

Lixin Xu[1,2] , Liebao Han[2] and Bingru Huang[1]*
[1]Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ 08901
[2]Turfgrass Institute, Department of Forestry, Beijing Forestry University, Beijing, 100083, China

INTRODUCTION

- For perennial turfgrasses growing in environments with limited rainfall or irrigation, maintaining turf health during drought stress is important. However when drought damage cannot be avoided, quick recovery from drought stress is desirable.
- Cellular membranes are the primary targets of drought stress. Membrane fatty acid composition and saturation level affects membrane properties such as fluidity and stability which may control cell turgor and growth under drought stress. It is reported that higher levels of unsaturated fatty acids were related to better drought tolerance in plants.
- Hypothesis: Changes in membrane fatty acid composition and saturation levels during drought might be important in imparting plant tolerance to drought stress.

OBJECTIVE

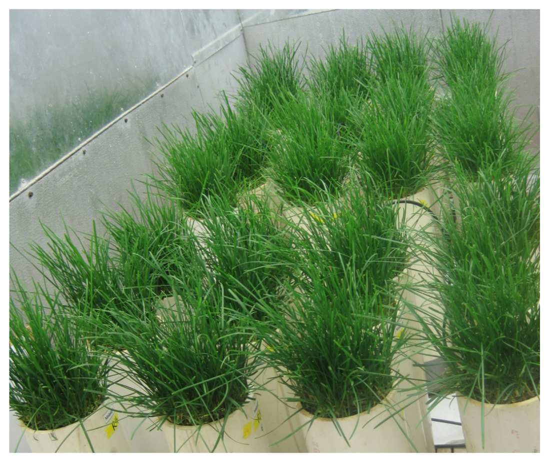
- To determine whether leaf dehydration tolerance and recuperative ability from drought stress was associated with changes in membrane fatty acid composition and saturation in Kentucky bluegrass by comparing the two cultivars contrasting in drought tolerance, 'Midnight' and 'Brilliant'.
- To identify major fatty acids associated with cellular membrane stability and water retention in leaves of Kentucky bluegrass exposed to drought stress.

MATERIALS AND METHODS

PLANT MATERIALS:
- Drought-tolerant 'Midnight'
- Drought-sensitive 'Brilliant'

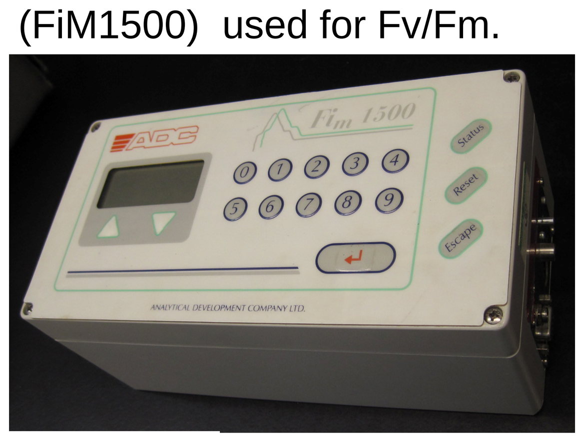
GROWING CONDITIONS:
- Growing medium - sand and soil mixture (1:1, v/v) contained in polyvinyl chloride tubes (12-cm diameter, 60-cm height).
- Growth chamber conditions: 22/15 C , 14 hours of photoperiod, relative humidity of 60%, and photosynthetic photon flux density of 600 μmol m^{-2} s^{-1}.

Plants in growth chamber



TREATMENT AND EXPERIMENTAL DESIGN:
- Well-watered control treatment: plants were watered every day to keep soil volumetric water content (SWC) at field capacity (SWC = 27%)
- Drought stress: irrigation was withheld for 15 d until soil moisture dropped to 4%.
- Re-watering: Drought-stressed plants were re-watered daily for 6 d to maintain soil water content at approximately 60% of the well-watered control level (SWC = 17%).

Fluorescence induction monitor (FiM1500) used for Fv/Fm.



PHYSIOLOGICAL MEASUREMENTS:
- Turf Quality (TQ) - rated visually on a scale from 1 to 9, where 1 = completely desiccated and brown turf and 9 = turgid and green turf.
- Relative water content (RWC) - determined from fresh weight (FW), dry weight (DW) and turgid weight (TW) using the formula RWC (%) = (FW-DW)/ (TW-DW) 100.
- Photochemical efficiency (chlorophyll fluorescence, Fv/Fm) by fluorescence induction monitor (FiM1500).
- Soil volumetric water content (SWC) by time domain reflectometry (Trase TDR).
- Membrane stability expressed as electrolyte leakage (EL) – % conductivity of incubation solution with fresh leaves (Ci) over incubation solution with dead leaf tissues (Cmax). EL = (Ci/Cmax

Trase TDR used for SWC

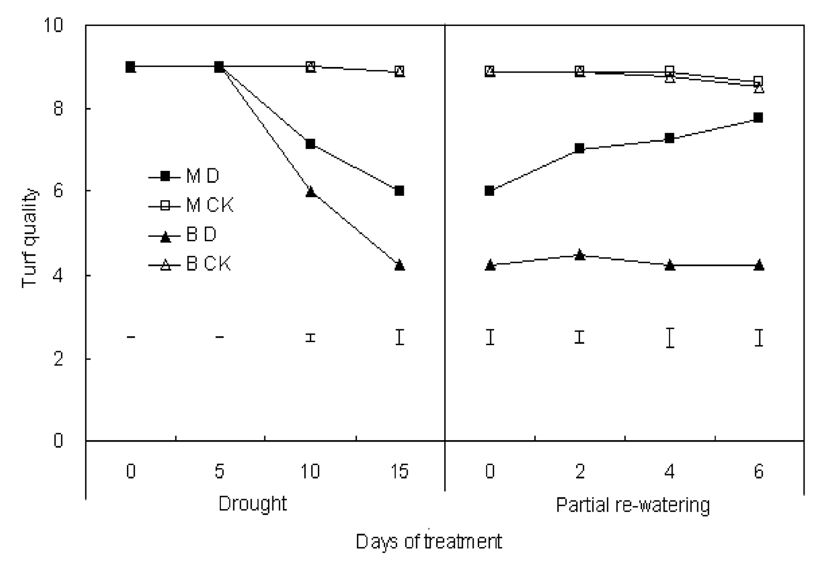
FATTY ACID ANALYSIS:
- Fatty acids were extracted from leaf tissue and identified with a HP GC-MS (Hewlett Packard). Unsaturation level of all fatty acids was estimated using double bind index (DBI): DBI = 0 x ([16:0] + [18:0]) + 1 x ([16:1] + [18:1]) + 2 x ([16:2] + [18:2]) + 3 x [18:3].

Square brackets indicate the percentage of the total fatty acid content which was made up by each fatty acid species.

RESULTS AND DISCUSSION

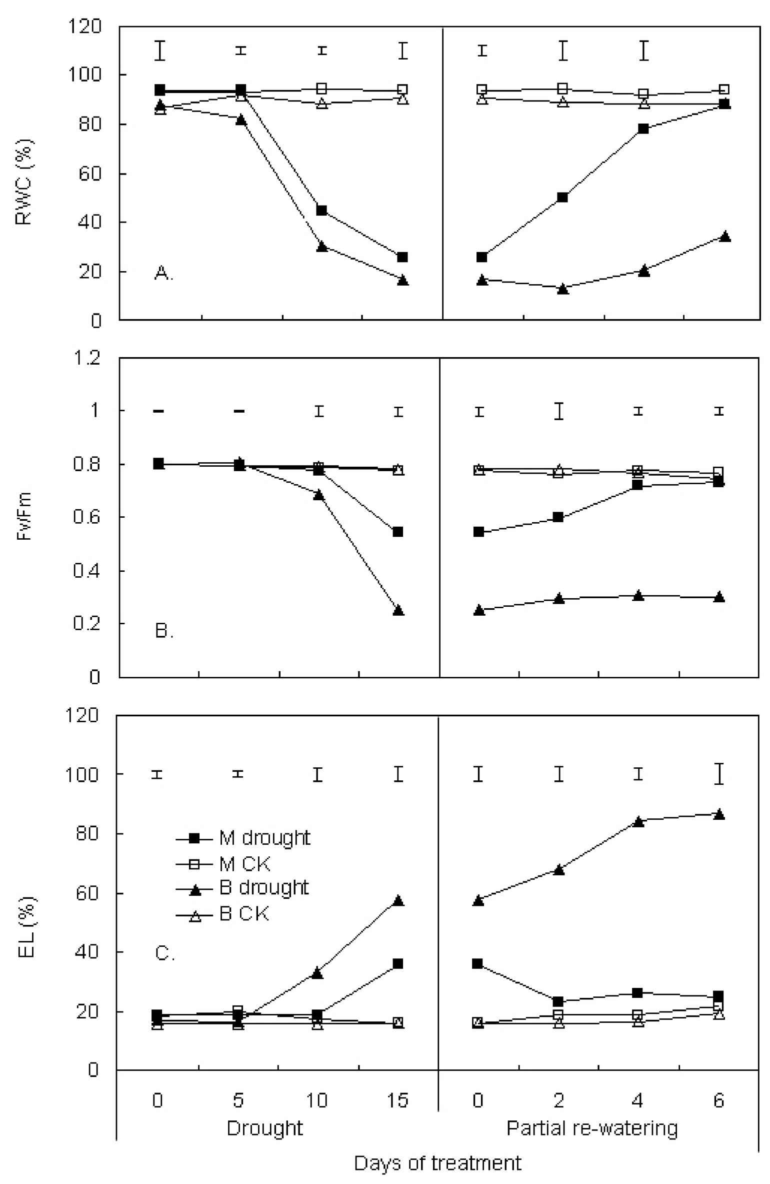
In all the figures, MD = 'Midnight' stress, MCK = 'Midnight' control, BD = 'Brilliant' stress, BCK = 'Brilliant' control. Vertical bars indicate LSD values (P < 0.05) for treatment comparison at a given day of treatment.

Fig. . Turf quality changes in two Kentucky bluegrass cultivars



- At 15 d drought, TQ of 'Brilliant' declined below the minimum acceptable level (6.0), but 'Midnight' maintained TQ at the acceptable level.
- TQ in 'Midnight' was significantly higher than that in 'Brilliant' under drought stress and during re-watering.

Fig. 2. Changes in RWC, Fv/Fm, EL during drought and re-watering for 'Brilliant' and 'Midnight'.



- 'Midnight' maintained higher level of RWC, Fv/Fm and lower EL than 'Brilliant' and recovered quickly upon re-watering.
- 'Brilliant' suffered severe desiccation when leaf RWC dropped to below 20% at 15 d of drought, and were unable to fully recover upon re-watering.

- Superior drought tolerance and recuperative ability in 'Midnight' was associated with the maintenance of higher cellular hydration, photochemical activities, and membrane stability.

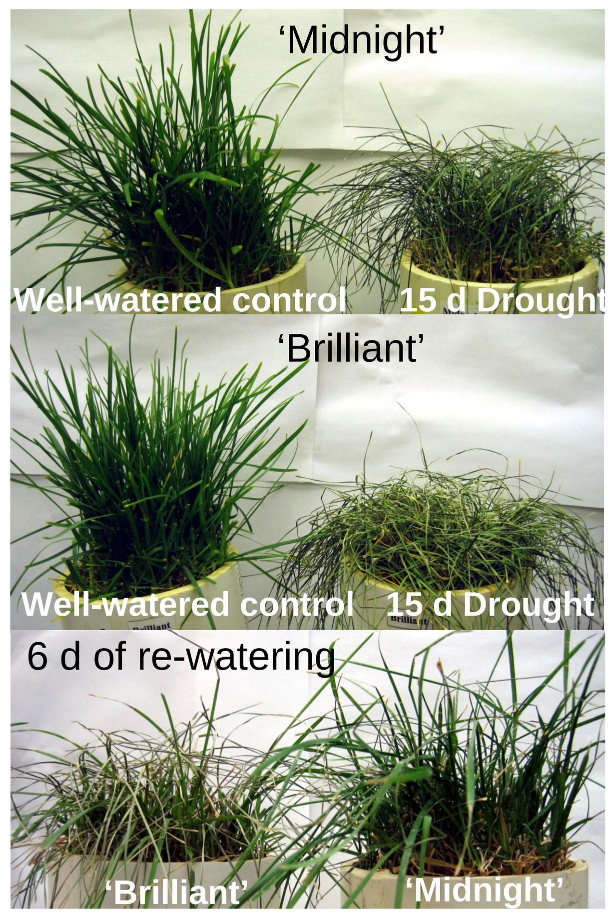
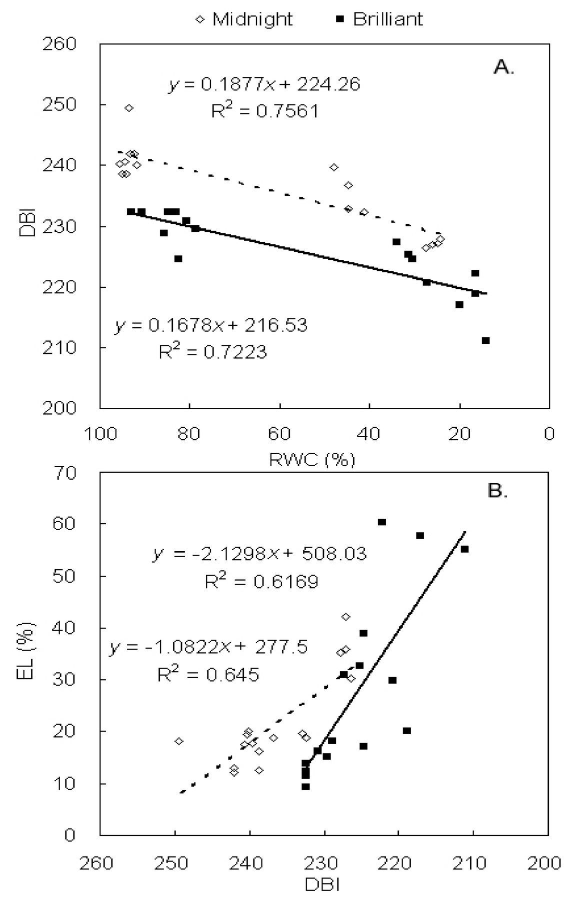


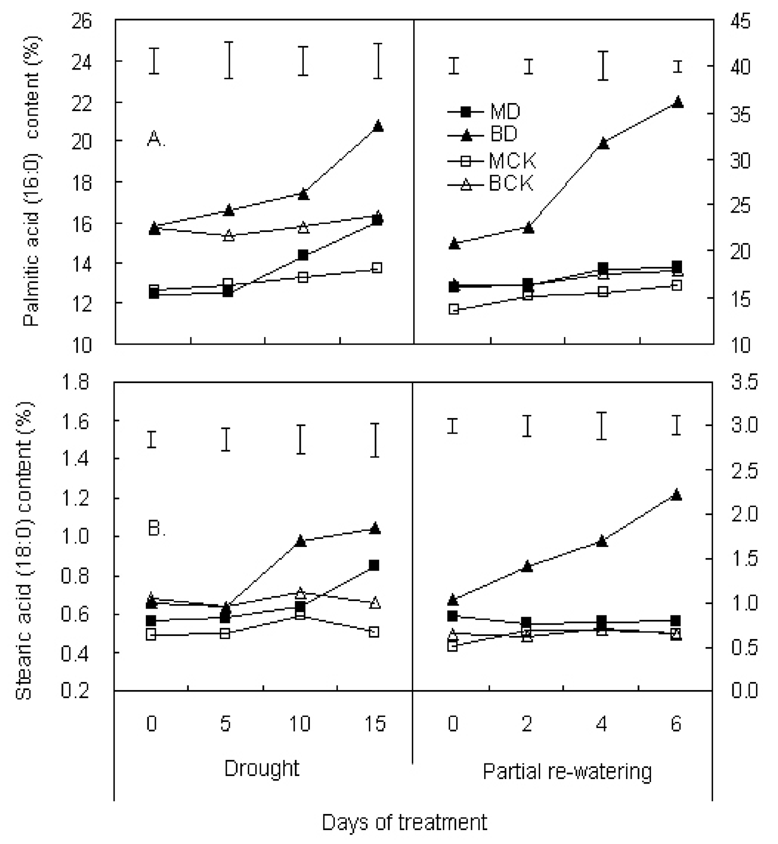
Fig. 3. Correlation changes of RWC and DBI, EL and DBI in drought-tolerant 'Midnight' and drought-sensitive 'Brilliant'.



- Double bond index (DBI) decreased with the decline in RWC during drought stress in both cultivars, but at the same level of water deficit or RWC, 'Midnight' had higher DBI than 'Brilliant'.
- Electrolyte leakage (EL) was negatively correlated with DBI. In addition, EL changed more rapidly with changing DBI in 'Brilliant' than in 'Midnight'.

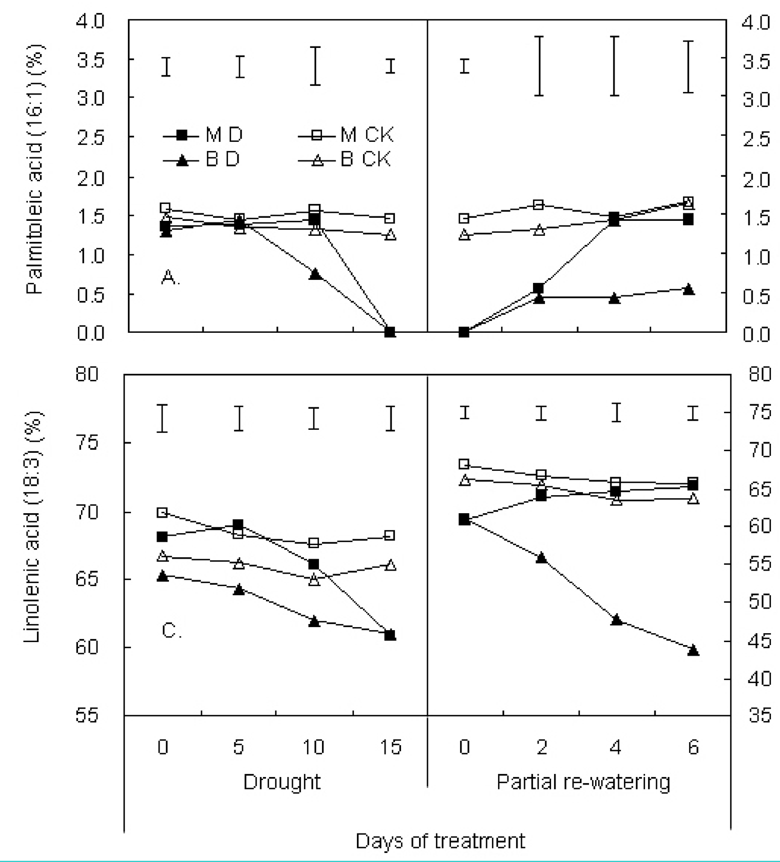
- Fatty acids became more saturated during leaf dehydration under drought stress, which lead to increased electrolyte leakage or lower cellular membrane stability.
- Drought-tolerant 'Midnight' was able to maintain higher level of fatty acid unsaturation at the same level of leaf water deficit than 'Brilliant', which could account for the greater cell membrane stability.

Fig. 4. Composition changes of saturated fatty acids (16:0 and 18:0) in 'Midnight' and 'Brilliant' during drought stress and partial re-watering.



- The content of palmitic acid (16:0) and stearic acid (18:0) increased during drought, and was significantly higher in 'Brilliant' than in 'Midnight'.
- The level of both fatty acids decreased to the well-watered control level during re-watering in 'Midnight', but did not 'in Brilliant'.

Fig. 5. Composition changes of unsaturated fatty acids (16:1 and 18:3) in 'Midnight' and 'Brilliant' during drought stress and partial re-watering.



- The content of unsaturated fatty acids (palmitoleic acid 16:1 and linolenic acid 18:3) decreased with drought stress, but was higher in 'Midnight' than 'Brilliant' at 10 d of drought.
- Unsaturated fatty acid content recovered to the control level for 'Midnight' but did not for 'Brilliant' following re-watering.

- Lower content of saturated fatty acids (16:0 and 18:0) or higher content of unsaturated fatty acids (16:1 and 18:3) was associated with better drought tolerance and recovery in 'Midnight'.

CONCLUSIONS

- Drought caused decline in the content of unsaturated fatty acids and increase in saturated fatty acids, leading to high saturation level of membrane fatty acids, which could contribute to the lower cell membrane stability, photochemical activities, and leaf dehydration.
- Leaf dehydration tolerance and post-drought recovery in Kentucky bluegrass was associated with their ability to maintain relative higher composition of unsaturated fatty acids and lower saturated fatty acids composition, particularly palmitic palmitic acid (16:0) and stearic acid (18:0). These fatty acids could be used as biomarkers to select for drought-tolerant grass germplasm.