

TALL FESCUE PERFORMANCE AND PROTEIN
ALTERATION DURING DROUGHT STRESS

By

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Beijing, China

2009

Bachelor of Science in Agriculture

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East Lansing, MI, USA

2009

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2011

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ACKNOWLEDGMENTS

I especially want to thank my major advisor, Assistant Professor Dr. Justin Q. Moss, for his encouragement, patience, excellent guidance and complete support during my study and research at Oklahoma State University (OSU). I feel very lucky and proud to have such a great advisor to guide me as a graduate student.

I would also like to give the equal thanks to Dr. Neils O. Maness and Dr. Yanqi Wu for providing me with good advice, proper guidance and help at anytime during my research as my committee members. I especially thank Dr. Guolong Zhang in the OSU Animal Science Department for teaching me useful laboratory techniques and for his generosity in letting me use his laboratory. I also want to express high gratitude to Dr. Kemin Su for working out the laboratory techniques with me and help me with my laboratory protocols. I couldn't have completed my research successfully without Dr. Zhang and Dr. Su's help.

I want to thank Dr. Mike Richardson at the University of Arkansas for supplying the tall fescue seeds for this project. I am very grateful for the help from the entire staff at the OSU Turfgrass Research Center. I also want to express my gratitude to the OSU Department of Horticulture and Landscape Architecture and the Department Head, Dr. Dale M. Maronek, for providing support to me. My deepest appreciation goes to my family, where I obtained sincere love and support throughout my entire life.

TABLE OF CONTENTS

Chapter	Page
I. Introduction.....	1
Review of Literature.....	4
Goals and Objectives.....	12
Literature Cited.....	13
II. Response of tall fescue to drought stress.....	24
Abstract.....	24
Introduction.....	26
Materials and Methods.....	29
Results and Discussion.....	35
Conclusion.....	41
Literature Cited.....	42
Figures and Tables.....	52

LIST OF TABLES

Table	Page
Table 1. Test of fixed effects of volumetric soil water content (VSWC), leaf relative water content (RWC), turf quality (TQ), and electrolyte leakage (EL) of ‘2 nd Millennium’, ‘Rebel Exeda’ and ‘Kentucky 31’ tall fescue at 0 d (A), 7 d (B) and 11 d (C) following initiation of treatments.....	58
Table 2. Root length density (RLD), dry root weight (DRW), average root diameter (ARD), and root surface area (RSA) in different soil depths of ‘2 nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue under well-watered conditions in the growth chamber at Oklahoma state University, Stillwater, OK.	60
Table 3. Turf quality of ‘2 nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue at 0 d, 7 d, 14 d, 21 d and 28 d after rewatering.	62

LIST OF FIGURES

Figure	Page
Figure 1. Volumetric soil water content (VSWC) of ‘2 nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue in the 0-20cm profile under (A) well-watered and (B) drought conditions.....	52
Figure 2. Leaf relative water content (RWC) of ‘2 nd Millennium’ (2M), ‘Rebel Exeda (RE)’ and ‘Kentucky 31’ (K31) tall fescue under (A) well-watered and (B) drought conditions	53
Figure 3. Turf quality (TQ) of ‘2 nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue under (A) well-watered and (B) drought conditions ...	54
Figure 4. Electrolyte leakage (EL) of ‘2 nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue under (A) well-watered and (B) drought conditions.....	55
Figure 5. SDS-PAGE profiles of soluble protein from tall fescue leaves of ‘2 nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) under drought stress	56
Figure 6. Immunoblots of dehydrin protein expression of ‘2 nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue under drought stress	57

CHAPTER I

Introduction

Drought is considered a major challenge for turfgrass management in the United States. Cool-season grasses are commonly under drought stress during summer in the U.S. transition zone. Turfgrasses can survive drought stress through both morphological and physiological adaptations. The primary drought resistance mechanisms encompass both drought avoidance and drought tolerance. Drought avoidance is the ability to avoid tissue damage by postponing dehydration during drought condition, while drought tolerance is the ability to tolerate dehydration during drought stress and recover after experiencing symptoms of drought stress (Jones et al., 1981). Drought tolerance is an extremely important factor for turfgrass selection in areas with inadequate rainfall and/or areas prone to prolonged drought periods (Turgeon, 2008).

Tall fescue (*Festuca arundinacea* Schreb.) is native to Europe and was introduced to the United States in the 1800's (Hoveland, 2009). It soon became the most important pasture/forage grass in the United States, covering approximately 15 million ha (Buckner et al., 1997). It is used as both turf- and forage-type grass in the United States, and

researchers have developed improved tall fescue cultivars in recent years (Kelly et al., 2009). The use of tall fescue as a turfgrass started in the 1960's and developed slowly mainly because of its coarse texture (Murray, 1979). Since the initial use of tall fescue for turfgrass purposes in the United States, many improved turf-type tall fescues have been developed and evaluated for improved turfgrass quality (TQ) (Thomas et al., 2009). After several decades of turf-type tall fescue development, it is currently a popular and widely used turfgrass species in the U.S. northern, transition and mid-south zones (Thomas et al., 2009).

Turfgrasses are categorized as either cool-season (C_3) or warm-season (C_4) species (Hull, 1992). Warm-season turfgrasses are categorized as C_4 plants due to their photosynthetic pathway termed the photosynthetic dicarboxylic acid cycle, where the first stable metabolite is oxaloacetate, a four carbon product (Hull, 1992). Bermudagrass [*Cynodon dactylon* (L.) Pers], a C_4 grass, is the most widely used turfgrass in Oklahoma. Cool-season turfgrasses are known as C_3 plants due to their photosynthetic pathway called the Calvin cycle, where the first stable metabolite is PGA (3-phosphoglyceric acid), a three carbon product (Hull, 1992). Cool-season turfgrass species are best adapted to cool environments and perform best at temperatures ranging from 18 °C to 24 °C for shoot growth and 10 °C to 18 °C for root growth (Beard, 1973). Generally speaking, cool-season turfgrass have better cold tolerance and shade tolerance, but lower drought resistance than warm-season turfgrasses (Turgeon, 2008). The general drought tolerance ratings for cool-season turfgrasses are: fine fescues (*Festuca* spp.) > tall fescue > Kentucky bluegrass (*Poa pratensis* L.) > perennial ryegrass (*Lolium perenne* L.) > colonial bentgrass (*Agrostis capillaris* L.) > creeping bentgrass (*Agrostis stolonifera* L.) >

rough bluegrass (*Poa trivialis* L.) (Turgeon, 2008). Although fine fescues have better drought tolerance than tall fescue, tall fescue has better heat tolerance than fine fescues. Tall fescue is the most commonly used cool-season turfgrass in Oklahoma due to their superior drought, heat, and disease performance compared to other cool-season grasses. Tall fescue is often used on shady turfgrass sites in Oklahoma due to its superior shade tolerance compared to bermudagrass.

Exposure to drought stress in the environment could lead to plant changes in protein expression, synthesis, and accumulation (Ramagopal, 1987; Chen and Tabaeizadeh, 1992; Cheng et al., 1993). For example, drought-stress treatments changed the accumulation of dehydrin-like proteins in developing soybean [*Glycine max* (L.) Merr.] seeds (Samarah, 2006). Dehydrin-like proteins of 28- and 32-kDa were detected in drought-stressed developing soybean seeds but not in seeds from the well-watered plants. In mature seeds, dehydrin-like proteins (28-, 32-, and 34-kDa) were detected in seeds from drought-stressed plants and the well-watered plants. For the materials in the field, dehydrin-like proteins accumulated similarly under irrigated and non-irrigated conditions. Accumulation of dehydrin proteins under physiological stress were also detected in turfgrasses such as zoysiagrass (*Zoysia* spp.), bermudagrass, and tall fescue (Patton, 2007; Hu et al., 2010; Jiang and Huang, 2002). However, there is only one study about protein alteration in drought stressed tall fescue. The 23- and 27-kDa dehydrin polypeptides were detected at 10 d in drought-stressed only and drought stressed with ABA treatment plants of 'Southeast' and 'Rebel Jr.' tall fescue (Jiang and Huang, 2002). Further study is needed to discover the relationship between the change of dehydrin proteins and the drought tolerance for both drought tolerant and drought sensitive tall fescue cultivars.

Review of Literature

Physiological response of cool-season turfgrass to drought stress

The true measure of drought resistance in plants is the ability of the plant to survive under drought stress without permanent injury (Maximov, 1929). Several physiological parameters can indicate whether a turfgrass species is drought resistant or susceptible. Generally speaking, drought resistant turfgrasses can sustain relatively higher TQ, leaf relative water content (RWC), photosynthetic rate, and lower electrolyte leakage (EL) than drought susceptible turfgrasses. The EL is lower in more drought resistant turfgrasses primarily through enhanced cell membrane stability (Huang and Gao, 1999; Qian and Fry, 1997). For example, based upon RWC, photochemical efficiency (Fv/Fm), and EL, 33 genotypes of Kentucky bluegrass (*Poa pratensis* L.), Texas bluegrass (*Poa arachnifera* Torr.) and their hybrids were grouped into low, moderate, or high drought resistant categories (Abraham et al., 2004). All groups had reduced RWC and photochemical efficiency (Fv/Fm) with drought stress, but the high drought resistant group showed higher RWC and Fv/Fm compared to the low drought resistant group. In addition, the high drought resistant group showed lower EL than the other groups, suggesting these plants were better able to maintain membrane stability.

A study in Griffin, GA on seven species of turfgrasses indicated that leaf wilting and leaf firing were two important symptoms of drought stress (Carrow, 1996b). Turfgrasses varied in drought resistance as determined by leaf wilting and leaf firing during drought treatment. Drought resistance rankings based on this experiment were:

very high for ‘Tifway’ bermudagrass [*C. dactylon* (L.) Pers. x *C. transvaalensis* Burtt. Davy] and common bermudagrass, high for ‘Raleigh’ St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze] and common centipede [*Eremochloa ophiuroides* (Munro) Hack.], medium-high for ‘Rebel II’ tall fescue, medium for ‘Kentucky-31’ tall fescue, and medium-low for ‘Meyer’ zoysiagrass (Carrow, 1996b). Similarly, deep rooting (20 – 60 cm deep) measurements in late summer were: ‘Tifway’ bermudagrass > ‘Rebel II’ tall fescue = common bermudagrass > ‘Kentucky 31’ tall rescue > ‘Raleigh’ St. Augustinegrass > common centipedegrass > ‘Meyer’ zoysiagrass.

In a study of bentgrass (*Agrostis* spp.) species under 18 d drought stress, results indicated that initial drought stress responses included increased carbon partitioning and longer term drought stress resulted in carbon partitioning and carbohydrate accumulation in stems (DaCosta and Huang, 2006). The increase in carbohydrate accumulation during prolonged periods of drought stress could help the plant to better recover upon re-watering (DaCosta and Huang, 2006). Drought or heat stress alone and the combined effects of drought and heat caused significant declines in TQ, RWC, and leaf chlorophyll content in both tall fescue and Kentucky bluegrass. The detrimental effects were more severe for the combined drought plus heat stress than either stress alone. Prolonged drought, heat, and the combined stresses could lead to loss of chlorophyll and lipid peroxidation, resulting in further turf quality decline (Jiang and Huang, 2001). Kentucky bluegrass plants were capable of adapting to localized soil drying by maintaining TQ, photosynthetic rate, RWC, and root growth by using water in the deeper soil profile (DaCosta et al., 2004). Another study evaluated the effects of high temperature and drought on the physiology and growth of three turfgrasses: ‘Apollo’ Kentucky bluegrass,

‘Dynasty’ tall fescue, and ‘Thermal Blue’, a hybrid bluegrass. The combination of high temperature and drought resulted in rapid decline of visual quality and dry matter production in all species, but ‘Thermal Blue’ hybrid bluegrass performed better than ‘Apollo’ Kentucky bluegrass and ‘Dynasty’ tall fescue. Results of the study indicated greater heat resistance, but not drought resistance, in ‘Thermal Blue’ hybrid bluegrass than in ‘Apollo’ Kentucky bluegrass or ‘Dynasty’ tall fescue (Su et al., 2007).

Tall fescue response to drought stress

Turf-type tall fescue is very popular species in the northern, transition and mid-south zones in U.S. (Thomas et al., 2009). In order to provide green color and promote growth, N is applied at 50 to 200 kg ha⁻¹ yr⁻¹ according to the desired lawn appearance and management. As a cool season grass, tall fescue has comparatively deep roots, so it may need less frequent irrigation to maintain its quality (Walker et al., 2007).

Richardson (1993) demonstrated that photosynthesis of tall fescue decreased due to the increasing water stress induced by drought stress. As water stress increased, tall fescue leaf elongation rate declined both in dark period and light period, but the rate decreased slower in the dark period than it did in the light period due to the water loss resulting from open stomata (Wolf and Parrish, 1982; Spollen and Nelson, 1994). With the drought stress, the dry matter content of the leaf base in tall fescue increased 23%, mainly because of increased water-soluble carbohydrate near the ligules and the increased water-soluble, carbohydrate-free dry matter at distal positions. Also, sucrose and hexose increased 258% and 187% in the leaf base, respectively (Spollen and Nelson, 1994).

Huang and Fry (1998), Huang et al. (1998), Carrow (1996a), and White et al. (1993) found that forage-type tall fescue cultivars were more drought resistant than turf-type cultivars. Grasses having deeper roots can maintain longer period of drought stress than the one with shallow roots (Watschke and Schmidt, 1992). Root depth and length of tall fescue are greater in sand than in silt loam. Proper root development is essential for establishment of tall fescues in lower water holding capacity soils thus enabling them to extract water and nutrients from deeper within the soil profile (Brar, 1995).

Tall fescue cultivars vary in drought resistance (White et al., 1993; Carrow 1996a; Huang and Gao, 1999). Huang and Gao (1999) found that the variation among different cultivars was associated with their different responses in photosynthetic capacity and water relations. For the different cultivars, the dwarf-type tall fescues exhibited lower drought resistance compared to 'Kentucky 31' (White et al., 1993; Carrow, 1996a; Huang and Fry, 1998; Huang et al., 1998; Huang and Gao, 1999; Huang and Gao, 2000). 'Bonsai', 'Phoenix', and 'Rebel Jr.' showed lower net photosynthesis under drought stress compared to 'Kentucky 31' (Huang and Gao, 1999; Huang and Gao, 2000).

Du and Wang (2008) indicated that heat stress was more harmful to photochemical reactions and cell membrane stability than drought stress for both tall fescue and zoysiagrass. Compared to zoysiagrass, tall fescue was more sensitive to both heat and drought stress, particularly heat stress. Three parameters changed during 30 days of heat or drought stress: TQ and photochemical efficiency (Fv/Fm) declined while electrolyte leakage (EL) increased.

Carrow (1996a) showed the rankings for tall fescue drought resistance among selected cultivars was: 'Rebel II' > 'Rebel Jr.', 'Kentucky 31', GA-5-EF, and GA-5-EI >

'Bonsai' based on the decline in TQ, leaf firing, and/or wilt expression under drought stress. Two essential parameters contribute to drought resistance of tall fescue, one is high root length density in the deeper root zone and the other is the ability to maintain evapotranspiration (ET) as the soil dries. Drought is one of the main reasons for the root death in the field (Smucker et al., 1991). Huang and Gao (2000) found that severe leakage of organic solutes from roots in drying soil gives evidence that root death of tall fescue cultivars during drought stress may correlate with root desiccation.

Deep roots are essentially related to drought resistance (Taylor, 1983; Hays et al., 1991; Marcum et al., 1995). Plants may avoid drought damage by producing an extensive root system to extract water from deep soil (Hurd, 1974). Progressed root development and maintenance of roots are important aspects for drought avoidance (Beard, 1989). Root physiological characteristics benefit to enhance root growth, root activity and the water uptake from lower soil during drought stress. Tall fescue has the better ability to avoid drought than Kentucky Bluegrass because it can maintain green leaf color longer by developing a deeper, more extensive root system (Ervin and Koski, 1998). Tall fescue is able to extract more water from deep soil for continued transpiration, keeping a lower canopy temperature than Kentucky bluegrass when surface soil moisture becomes limited (Ervin and Koski, 1998).

High root/shoot ratio selection can improve the drought performance of turf-type tall fescues. Tall fescues were selected for high root/shoot ratio from greenhouse studies and then planted in field trials. The selection with higher root/shoot ratios generally performed better in the field compared to lower root/shoot ratio selections and were the first to recover from the drought stress after re-watering (Karcher et al., 2008). Carrow

and Duncan (2003) showed that breeding efforts can also improve the drought tolerance of tall fescue cultivars. Root death of tall fescue cultivars due to drought stress is associated with water loss in roots proofed by severe leakage of organic solutes from roots in drying soil, but the carbohydrate supply to roots didn't contribute to root death during drought stress (Huang and Gao, 2000).

Plant dehydrin proteins response to environmental stress

Exposure to drought stress in the environment could lead to the changes in plant protein expression, synthesis, and accumulation (Ramagopal, 1987; Chen and Tabaeizadeh, 1992; Cheng et al., 1993). Late embryogenesis abundant (LEA) proteins are produced during seed development, and their expression is related to the onset of drought stress (Close, 1996), but cold or osmotic stress also contribute to the induction of their synthesis (Welin et al., 1994). Dehydrin proteins are synthesized in response to dehydrative stress and belong to the Group II LEA or LEA-D11 family (Close and Chandler, 1990; Leprince et al., 1992; Dure, 1993). Dehydrins have been the most commonly observed proteins among ones that accumulate in plants during the late stages of embryogenesis or in response to low temperature stress, ABA application, and dehydrative stress such as drought, salinity, or extracellular freezing (Close 1997). Dehydrin proteins are hydrophilic, heat-stable, free of cysteine and tryptophan and rich in lysine (Close et al., 1989; Mundy and Chua, 1988; Vilardell et al., 1990; Close, 1996; Godoy et al., 1996). The dehydrins may help maintain physiological integrity of cells and protect other proteins (Bray, 1993; Close et al., 1993). In the 1990's, researchers

hypothesized that dehydrins were surfactants having the ability to preserve integrity of structure by inhibiting the coagulation of a range of macromolecules (Close, 1997). Currently, the function of dehydrins is not completely known. Dehydrins most likely have disordered structure (Lisse et al., 1996; Soulages et al., 2003; Mouillon et al., 2006). However, all dehydrins seem to contain a highly conserved sequence segment, known as the K-seg, and may or may not include other conserved sequences called the S-seg, the Y-seg (Close, 1996) and the ChP-1 (Mouillon et al., 2006). Since dehydrins accumulate at very high concentrations inside cells (Bartels and Salamini, 2001), they are unlikely to be signal molecules or conventional enzymes. The general role of dehydrins is that they may stabilize membranes, act as chaperones, or buffer the altered solvent properties inside water-stressed cells by other means (Close, 1996; Garay-Arroyo et al., 2000; Boudet et al., 2006).

Patton et al. (2007) indicated higher soluble protein concentrations were detected among genotypes of zoysiagrasses due to cold acclimation (CA) than the non-acclimated grasses. Dehydrin polypeptides (23- and 25-kDa) increased during CA, and the abundance of the 23-kDa dehydrin polypeptide was positively associated with genetic variation in freezing tolerance. The results suggested that dehydrins can accumulate during zoysiagrass cold acclimation, while only the 23-kDa dehydrin contributes to improving freeze tolerance.

Low molecular weight proteins were detected in fluorographs of two-dimensional gels when relative water content of needles of *Pinus ponderosa* var *scopulorum* fell below 70%. The protein synthesis induced by water stress in drought-tolerant seedlings may help seedlings to resist the effects of cellular dehydration (Vance, 1990). Certain

dehydrins such as the 25 kDa dehydrin could be induced in bermudagrasses by cold acclimation (CA). Proper dosages (50 to 150 mM) of exogenous ABA may induce endogenous ABA and other defense responses to improve bermudagrass freezing tolerance, similar to those occurring during CA. Bermudagrass persistence in the transition zone could be improved by the use of cultivars with rapid accumulation of ABA and dehydrins during CA or in response to exogenous ABA (Zhang et al., 2008).

Protein alteration in tall fescue in response to drought stress

There are few studies of protein alteration in tall fescue in response to drought stress. The 23- and 27-kDa dehydrin polypeptides were detected at 10 d in drought-stressed and ABA-treated plants in tall fescue 'Southeast' and 'Rebel Jr.', which could protect the plants from the dehydration damage, but the levels are higher in the drought-stressed plants without ABA (Jiang and Huang, 2002). A cytosolic-heat shock protein (HSC 70) was present in those two cultivars in drought-stressed, ABA treated and well-watered plants of both cultivars, while its abundance is higher in drought-stressed and ABA-treated plants (Jiang and Huang, 2002).

Goals and Objectives

The goals of this research project were to understand the physiological response during drought stress in diverse cultivars of tall fescue and to study protein alteration of drought stressed tall fescue plants.

The objectives of this research were to:

1. Evaluate the physiological performance of different tall fescue cultivars during drought stress.
2. Measure tall fescue root characteristics under well-watered condition to see whether it contributes to drought avoidance.
3. Investigate the response of dehydrin proteins to drought stress in tall fescue during different periods of drought.

Research hypotheses:

1. 2nd Millennium and Rebel Exeda will have the best and worst physiological performance, respectively.
2. 2nd Millennium will have the most extensive root system among the cultivars studied.
3. Dehydrin protein expression will be observed in immunoblotting analysis of drought stressed grasses and expression will increase with prolonged drought stress.

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CHAPTER II

Response of tall fescue to drought stress

Abstract

Cool-season turfgrasses are commonly under drought stress during the summer in the United States and often require supplemental irrigation to maintain acceptable turfgrass quality. A study was conducted at Oklahoma State University to evaluate the performance and protein alteration of tall fescue during drought stress. Mature tall fescue plants of three diverse cultivars ('Kentucky 31', '2nd Millennium' and 'Rebel Exeda') were subjected to two treatments: well-water and drought condition. Volumetric soil water content (VSWC), leaf relative water content (RWC), turf quality (TQ) and electrolyte leakage (EL) were measured at 0, 7, and 11 d following treatments. In well-watered plants, root characteristics were measured at the end of the study period. Protein analysis was conducted to detect for changes of dehydrin protein expression. The VSWC was maintained at the same level for each treatment for all three cultivars throughout the study. There were no differences in RWC among the three cultivars under well-watered conditions throughout the study. Tall fescue RWC of all three cultivars declined after initiation of the drought treatment. Under drought stress, TQ decreased for all three cultivars as drought stress increased but 'Kentucky 31' had higher TQ compared to '2nd

Millennium' and 'Rebel Exeda' at 7d of drought treatment. After 11 d of drought stress, there were no differences in TQ among the three cultivars and the average TQ rating was 2. The EL results suggested that the three cultivars had similar membrane stability under drought stress throughout the study period. Root length density, dry root weight, and root surface area were highest in '2nd Millennium' in the 0-40 cm profile suggesting that '2nd Millennium' may have greater ability to avoid drought under field conditions. Drought stress induced expression of dehydrin proteins, but there were no differences in soluble proteins or dehydrin protein expression among the three cultivars. Although dehydrin polypeptides were observed in many studies and assumed to contribute to drought tolerance, the results of this study did not indicate a relationship between protein changes and drought tolerance in diverse tall fescue cultivars.

Key words: drought stress, turf quality, leaf relative water content, volumetric soil water content, electrolyte leakage, root characteristics, dehydrin protein expression.

Introduction

Cool-season turfgrasses are commonly under drought stress during the summer in the United States and often require supplemental irrigation to maintain acceptable turfgrass quality (TQ). Water is a limited resource and the competition for urban water resources is increasing due to increasing human population and urban sprawl (Richie et al., 2002). Therefore, selection and use of more drought resistant cultivars of turfgrasses is critical for urban landscape water conservation.

The primary drought resistance mechanisms encompass both drought avoidance and drought tolerance. Drought avoidance is the ability to avoid tissue damage by postponing dehydration during drought condition, while drought tolerance is the ability to tolerate dehydration during drought stress and recover after experiencing symptoms of drought stress (Jones et al., 1981). Turfgrasses may possess high drought avoidance primarily by a deep, extensive, viable root system and/or by morphological or physiological features to reduce evapotranspiration (ET) losses (Levitt, 1980). Several physiological parameters can indicate whether a turfgrass species is drought resistant or susceptible. Generally speaking, drought resistant turfgrasses can sustain relatively higher TQ, leaf relative water content (RWC), photosynthetic rate, and lower electrolyte leakage (EL) than drought susceptible turfgrasses. The EL is lower in more drought resistant turfgrasses primarily through enhanced cell membrane stability (Huang and Gao, 1999; Qian and Fry, 1997).

Turf-type tall fescue is very popular species in the northern, transition and mid-south zones in U.S. (Thomas et al., 2009). Turf-type tall fescues are allohexaploid with

42 chromosomes ($2n=6x=42$) and each plant in a seeded tall fescue population represents a unique genotype. Among cool season grasses, tall fescue has comparatively deep roots, so it may need less frequent irrigation to maintain its quality (Walker et al., 2007). For example, tall fescue had 3 to 12 times greater root length in deep soil profiles (60 – 80 cm) compared to Kentucky bluegrass and hybrid bluegrass (Su et al., 2008). Selection of tall fescue genotypes with high root/shoot ratios is also important (Bonos et al., 2004). Tall fescue genotypes selected for higher root/shoot ratios generally performed better in the field compared to lower root/shoot ratio selections and were the first to recover from the drought stress after re-watering (Karcher et al., 2008).

Deep roots are essentially related to drought resistance (Taylor, 1983; Hays et al., 1991; Marcum et al., 1995). Plants may avoid drought damage by producing an extensive root system to extract water from deep soil (Hurd, 1974). Progressed root development and maintenance of roots are important aspects for drought avoidance (Beard, 1989). Root physiological characteristics benefit to enhance root growth, root activity and the water uptake from lower soil during drought stress. Tall fescue has the better ability to avoid drought than Kentucky Bluegrass because it can maintain green color longer by developing a deeper, more extensive root system (Ervin and Koski, 1998). Tall fescue is able to extract more water from deep soil for continued transpiration, keeping a lower canopy temperature than Kentucky bluegrass when surface soil moisture becomes limited (Ervin and Koski, 1998). Tall fescue has better drought resistance than other cool season grass, such as perennial ryegrass (Sheffer et al., 1987).

Tall fescue cultivars vary in drought resistance (White et al., 1993; Carrow 1996a; Huang and Gao, 1999). Huang and Gao (1999) found that the variation among different

cultivars was associated with their different responses in photosynthetic capacity and water relations. For the different cultivars, the dwarf-type tall fescues exhibited lower drought resistance compared to ‘Kentucky 31’ (White et al., 1993; Carrow, 1996a; Huang and Fry, 1998; Huang et al., 1998; Huang and Gao, 1999; Huang and Gao, 2000). A study in Arkansas compared 42 cultivars of tall fescue, Kentucky bluegrass, and hybrid bluegrass for the number of days needed to for green turf coverage to reach 50% decline after withholding irrigation (Karcher et al., 2007 and 2008). Among tall fescue cultivars, 2nd Millennium’ and ‘Rebel Exeda’ had relatively good and bad performance, respectively, while ‘Kentucky 31’ performed worse than ‘2nd Millennium’ but better than ‘Rebel Exeda’ (Karcher, et al. 2007 and 2008)

Exposure to drought stress in the environment could lead to plant changes in protein expression, synthesis, and accumulation (Ramagopal, 1987; Chen and Tabaeizadeh, 1992; Cheng et al., 1993). Dehydrins have been the most commonly observed proteins among ones that accumulate in plants during the late stages of embryogenesis or in response to low temperature stress, ABA application, and dehydrative stress such as drought, salinity, or extracellular freezing (Close 1997). Dehydrin proteins are hydrophilic, heat-stable, free of cysteine and tryptophan and rich in lysine (Close et al., 1989; Mundy and Chua, 1988; Vilardell et al., 1990; Close, 1996; Godoy et al., 1996). The dehydrins may help maintain physiological integrity of cells and protect other proteins (Bray, 1993; Close et al., 1993). In the 1990’s, researchers hypothesized that dehydrins were surfactants having the ability to preserve integrity of structure by inhibiting the coagulation of a range of macromolecules (Close, 1997).

Jiang and Huang (2002), Panta et al. (2001), Patton et al. (2007) and Zhang et al. (2008) showed that low temperature, ABA application and other stresses can also induce the accumulation of dehydrin proteins. There are several studies about dehydrin proteins response to different stresses in various plant species, such as wheat, soybean, zoysiagrass, bermudagrass (Lopez, et al., 2003; Samarah, 2006; Patton, 2007; Hu et al., 2010) and many other species (Arora et al., 1998; Close et al., 1993; Han et al., 1997; Mohammadkhani and Heidari, 2008), but there is only one study related to tall fescue. The 23- and 27-kDa dehydrin polypeptides were detected at 10 d in drought-stressed and ABA-treated plants in tall fescue ‘Southeast’ and ‘Rebel Jr.’, which could protect the plants from the dehydration damage. Dehydrin levels were higher in the drought-stressed tall fescue plants without ABA, but there were no differences between the cultivars studied (Jiang and Huang, 2002).

The objectives of this research were to: 1) measure physiological parameters and evaluate the performance of diverse tall fescue cultivars subjected to drought stress; 2) evaluate the root growth characteristics of diverse tall fescue cultivars under well-water condition; and 3) to detect for differences in dehydrin protein expression among diverse tall fescues cultivars subjected to drought stress.

Materials and Methods

Plant materials and growth conditions

This study was conducted from 12 Aug. 2010 to 10 Jan. 2011 at the Oklahoma State University (OSU) CERL (Controlled Environment Research Laboratory) in Stillwater, OK. Three diverse tall fescue cultivars were used including a standard cultivar ‘Kentucky 31’, a good field drought performer ‘2nd Millennium’, and poor drought performer ‘Rebel Exeda’ (Karcher et al., 2007; Karcher et al., 2008). Pure live seeds of each cultivar were planted at a rate of 39 kg m⁻² in lysimeters made of polyvinylchloride (PVC) tubes, 5.08 cm in diameter and 45 cm in height, with holes in bottom of PVC tubes for drainage. Lysimeters were filled with mixture of top soil and sand (1:1 v/v) and established in a greenhouse with average temperature of 25/14 °C (day/night), photosynthetically active radiation (*PAR*) of 300 μmol m⁻² s⁻¹ during the daytime for 4 months, then transferred to a growth chamber with the 20/15 °C day/night air temperature, a 14 hour photoperiod with *PAR* of 450 μmol m⁻² s⁻¹, and 75% relative humidity. Plants were fertilized by a soluble fertilizer (20N–8.7P–16.6K) at a weekly N rate of 61 kg ha⁻¹ and were mowed twice a week at 6.5 cm height of cut.

Treatment and measurement

Treatment for whole-plant experiment

The experiment consisted of two treatments, well-watered control and drought stress. Four replications of each cultivar for each treatment were placed in the growth chamber randomly and lysimeter location was changed every 48 h to minimize the effect of any variations in growth chamber microenvironment. Before treatments started, all lysimeters were watered and maintained under well-watered conditions by the method of Bremer (2003) with modifications. Lysimeters were irrigated, allowed water to drain

through the bottom hole until the free drainage ceased followed by sealing and weighing the lysimeters. Lysimeters were weighed every three days and any water loss was determined to be from evapotranspiration (ET). Lysimeters were watered every 3 d according to ET losses. After treatments started, the lysimeters under well-watered condition still used this method, but for the drought-stress plants, the stress was imposed by withholding irrigation for 11 d. Lysimeters for all treatments were not mowed and were not fertilized during the 11 d drought treatment period. After the 11 d drought treatment, drought stressed plants were re-watered to observe recovery for 4 weeks.

Measurements of Physiological Parameters

Several physiological parameters were measured to evaluate the differences in drought stress among three cultivars, including VSWC, RWC, TQ, and EL. Volumetric soil water content was measured at 0, 7, and 11 d after treatment initiation by time domain reflectometry (TDR) (HydroSense System, Campbell Scientific Inc., Salt Lake City, Utah) from 0-20 cm profile inside the lysimeters.

To measure RWC, five fully expanded leaves were detached from the plants and the fresh weight was weighed immediately after collecting the leaves. The leaves were placed in small brown bottles filled with 20ml of distilled deionized water and stored for 24 hours at 4 °C before determining the turgid weight (TW). The leaves were placed in an oven at 85 °C for 3 days to determine dry weight (DW). The equation $RWC = (FW - DW) / (TW - DW) \times 100$ was used and RWC was measured at 0, 7, and 11 d after treatment initiation.

Turfgrass visual quality was evaluated based on plant color, density, texture and uniformity on a scale of 1-9 (1 = poorest TQ, 9 = highest TQ, 6 = minimally acceptable TQ) (Emmons, 2000). Turf quality ratings were recorded at 0, 7, and 11 d after treatment initiation. The EL was measured by the method of Blum and Ebercon (1981) and Marcum (1998) to determine the cell membrane stability. Five living leaves about the same age were excised from each plant and immediately cut into 2cm segments. After rinsing three times with distilled deionized water, leaf segments were placed in a test tube filled with 20 ml distilled deionized water. Test tubes were agitated in an incubating shaker (Model Classic C24, New Brunswick Scientific, NJ) to shake at 120 rpm for 24 hours to dissolve the electrolytes leaking from cells due to the drought stress. The solution conductivity (C1) was measured with a conductivity meter (Accumet AP75 Portable Waterproof Conductivity TDS Meter, Fisher Scientific, Singapore). Then the leaf tissues were killed in autoclave at 140 °C for 20 minutes to destroy the cell membranes. After being shaken on the shaker for 24 hours at room temperature to extract all the electrolytes from the cells, conductivity of the solution containing killed tissue (C2) was measured again. The relative EL was calculated using the equation $EL = C1/C2 \times 100$. EL was measured at 0, 7 and 11 d after the treatments initiated.

Measurement of root characteristics

Four lysimeters for each cultivar under well-watered condition were cut into three sections (0-15cm, 15-30cm and 30-40cm) and each section of root core was taken out of lysimeters. Root samples of each section were washed clean and stained with Methyl blue (5 g L⁻¹ water) to enhance the detection of fine roots. Total root length, average root

diameter (ARD), and root surface area (RSA) for each section were measured optically with a computer scanner and WinRhizo software (Regent Instruments Inc., Quebec City, Canada). The root samples were placed in an oven at 85 °C for three days to obtain dry weights. Root length density (RLD) was calculated by using the root length divided by the volume of soil inside each section.

Protein extraction and quantification

Leaf samples were collected at 0, 7, and 11 d after treatments began for protein analysis. Total soluble protein was extracted from leaf samples by using the method described by Jacobs et al. (2001) and Damerval et al. (1986) with modifications. Briefly, 0.15 g leaf tissue was grounded to powder using mortar and pestle in liquid nitrogen. Proteins were extracted in 10% w/v trichloroacetic acid and 0.07% v/v 2-mercaptoethanol in cold (-20 °C) acetone solvent, and samples were centrifuged at 16000 x g for 15 min at 4 °C before supernatant was collected. Proteins were quantified by the Bradford assay (Bradford, 1976). Briefly, Bovine serum albumin was used as standard and a mixture of 1 µl protein sample with 999 µl dye reagent was used to measure absorbance at 595 nm at 20 minutes by using a spectrophotometer (GE Healthcare, Piscataway, NJ). The total soluble protein concentration for each sample was obtained by the BSA standard linear equation.

SDS-PAGE

The protein samples were diluted with sample buffer 1:1 (v/v) and pre-heated at 100 °C before an equal amount protein (30 µg) was loaded in each lane in 1 mm thick

gels separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 5% stacking gel and 12% running gel. The SDS-PAGE ran at 60 v for 30 minutes, then 120 v for 75 minutes in Bio-Rad Mini-Protean Tetra Cell (Bio-Rad Laboratories, Hercules, CA). The stacking gel was then cut off and the running gel was washed three times by the distilled deionized water before staining with coomassie blue.

Immunoblotting analysis

The SDS-PAGE procedure was repeated, but the running gel was not stained with coomassie blue. The separated polypeptides on the unstained running gel were transferred onto a polyvinylidene fluoride (PVDF) membrane with an electrophoresis transfer unit Bio-Rad Mini-Protean Tetra Cell (Bio-Rad Laboratories, Hercules, CA) by using transfer buffer (25mM Tris base, 192 mM glycine, 10% (v/v) methanol, pH 8.3) at 100 v for 1 h. The gel membrane was incubated with blocking buffer (5% dry skim milk in TTBS solution) at 4 °C over night. Then the membrane was probed with 1:250 dilution of rabbit anti-dehydrin polyclonal antibody (PLA-100, Enzo life sciences International Inc. Plymouth Meeting, PA) for 1 h. After being washed three times with TTBS, goat anti-rabbit AP secondary antibody (Bio-Rad Laboratories, Hercules, CA) was diluted in TTBS (dilution 1:3000) to incubate the membranes for 1 hour. After washing three times with TTBS then one time with TTBS, a representative blotting image was detected by gently shaking the membrane immersed in color development solution (mixture of 0.2 ml AP color reagent A, 0.2 ml color reagent B, and 20 ml 1x color development buffer, Bio-Rad Laboratories, Hercules, CA) at room temperature on a shaker table at 30 rpm (Midwest Scientific, St Louis, MO).

Statistical analysis

Data for VSWC, RWC, TQ, and EL were analyzed by the mixed procedure of the Statistics Analysis System (SAS) (SAS Institute Inc., Cary, NC). Effects of drought stress treatment was analyzed by comparing responses with the well-watered control on any given day after treatment initiation (days of treatment) for TQ, VSWC, RWC and EL. SAS PDIFF option (P=0.05) was used to detect the differences among treatment means. For root characteristics under well-watered condition and turf quality ratings during recovery from drought stress, analysis of variance was performed using the GLM procedure of SAS at the 0.05 probability level. When the requirements for ANOVA were met, mean separation tests were performed using the least significant difference (LSD) test at the 0.05 probability level.

Results and Discussion

Volumetric soil water content

There were no differences in VSWC between treatments or among cultivars at the beginning of the study (Table 1). There were no differences in VSWC among the cultivars on any rating date for the well watered lysimeters (Figure 1). As drought stress increased, VSWC decreased, but no differences were detected among cultivars (Figure 1). Average VSWC declined to $0.08 \text{ m}^3 \text{ m}^{-3}$ at 7d of drought treatment and to $0.04 \text{ m}^3 \text{ m}^{-3}$ after 11 d of drought treatment and no differences were detected among the three cultivars (Figure 1). These data show that VSWC was maintained at the same level for each treatment for all three cultivars throughout the study.

Leaf relative water content

There were no differences in RWC between treatments or among cultivars at the beginning of the study (Table 1). There were no differences in RWC among the three cultivars under well-watered conditions throughout the study (Figure 2), but tall fescue RWC declined after initiation of the drought treatment (Figure 2). Although RWC declined after 7 and 11 d of drought stress, there were no RWC differences detected among cultivars.

Turfgrass quality

At the beginning of the study, there was a cultivar difference in TQ, but there was no difference between treatments (Table 1). ‘Kentucky 31’ had lower TQ compared to both ‘2nd Millennium’ and ‘Rebel Exeda’ due to its light green genetic color and coarse leaf texture (Figure 3). Under drought stress, TQ decreased for all three cultivars as drought stress increased but ‘Kentucky 31’ had higher TQ compared to ‘2nd Millennium’ and ‘Rebel Exeda’ at 7d of drought treatment (Figure 3). After 11 of drought stress, there were no differences in TQ among the three cultivars and the average TQ rating was 2.

These results contradict a field study by Karcher et al. (2007 and 2008) where ‘2nd Millennium’ had the higher TQ compared to ‘Kentucky 31’ and ‘Rebel Exeda’ during drought stress. The Karcher et al. (2007 and 2008) field study was conducted on native soil which allowed root growth deep into the soil profile, but this experiment was restricted by the limited soil volume of the tall fescue lysimeters. The medium in the lysimeters of this study was 40 cm deep but the tall fescue roots can grow as deep as

80cm or more into native soil profiles. Therefore, the differences in TQ ratings in this study compared to the work of Karcher et al. (2007 and 2008) was likely due to root growth restriction. This experiment may have more comparison to tall fescues grown in shallow soils or in poor soil condition such as home lawns, in which grasses grow in soils that are often disturbed and/or compacted during the home construction process (Hamilton and Waddington, 1999).

Electrolyte leakage

There were no treatment differences in EL at the beginning of the study, but there were treatment differences at 7 and 11 d after treatments (Table 1). The EL of well watered plants did not differ among cultivars at 7 or 11 d following treatment (Figure 4). The EL increased with increasing drought stress, but there were no EL differences among cultivars at 7 or 11 d of drought stress (Figure 4). The results suggested that the three cultivars have similar membrane stability under drought stress.

Root characteristics

Root length density, DRW, and RSA were highest in '2nd Millennium' in the 0-40 cm profile (Table 2). No significant differences were detected in ARD among cultivars in each section (0-15 cm, 15-30 cm and 30-45 cm) (Table 2). In the top 15 cm profile, '2nd Millennium' and 'Rebel Exeda' had greater DRW than 'Kentucky 31' and '2nd Millennium' had higher RSA than both 'Rebel Exeda' and 'Kentucky 31' (Table 2). The majority of tall fescue roots existed in the upper soil profile for all cultivars. In the deepest profile (30-40cm), there was no differences in RLD among the three cultivars at

the 0.05 significance level, but '2nd Millennium' had greater RLD compared to the other two cultivars at the 0.10 significance level with a *p* value of 0.08.

Most turfgrass roots grow in the upper 30 cm soil profile (Beard, 1976). The results indicated '2nd Millennium' had the most extensive root system than other two cultivars in the entire depth in this study, and it exhibited greater total root mass and RLD compared to both 'Rebel Exeda' and 'Kentucky 31'. Tall fescue can possess better drought avoidance than other cool-season species by development of roots deep into the soil profile and higher RLD (White et al. 1993). Greater root systems in the deep soil may contribute to the drought avoidance during drought stress even if these roots only present a small percentage of the total root system (Garrot and Mancino, 1994; Carrow 1996b). Huang et al. (1997) showed that root characteristics were associated with greater drought resistance when the surface soil was drying in the drought condition, and the drought stress can enhance the water uptake from deeper soil profile. Presumably, '2nd Millennium' had greatest and most extensive roots among cultivars in the deeper soil (deeper than 40cm in the field), and it may be able to extract water from deeper soil than the other two cultivars (Karcher et al., 2007 and 2008). Therefore, further study is needed to determine if deep root systems are related to drought avoidance for these three tall fescue cultivars. This may be the reason that '2nd Millennium' maintained green color longer than 'Rebel Exeda' and '2nd Millennium' during the drought stress in the field (Karcher et al. 2007 and 2008).

Recovery after drought stress

After the termination of drought treatment, the drought stressed plants were re-watered for 4 weeks. All the grasses were dormant at the end of the drought treatment. During recovery, there were no cultivar differences in TQ ratings at any rating date. The average TQ rating for all cultivars was 2 at one week after recovery and was 5 at four weeks after recovery. Karcher et al. (2008) showed that the most drought tolerant cultivars were the first to green up during the recovery after drought stress. In this study, there was no difference in green up among cultivars during recovery from drought stress. Smucker et al. (1991) reported that drought is one of the main reasons for the roots death in the field. Deans (1979) and Persson (1979) also claimed that drought stress was considered as the primary cause of root death in the field, especially in the surface soil. This statement is especially the case when the most of roots grow in the surface soil, which is common for the turfgrasses (Hays et al., 1991; Marcum et al., 1995; Carrow, 1996b; Huang et al., 1997). The availability of water at root surfaces and the distribution of root system are considered two important factors for water absorption. Our study was a lysimeter study with limited soil depth, so the recovered grasses had less ability to absorb water than the grasses in the field because drought stress damaged the surface root system. Based on this recovery experiment, TQ was not different among the cultivars during recovery from drought stress which was likely due to loss of roots during drought stress and root growth restriction caused by the limitation of the lysimeter soil depth.

Dehydrin protein expression

The SDS-PAGE analysis of soluble protein from tall fescue leaves did not reveal differences between well-watered plants and drought stressed plants (Figure 5). No

dehydrins were detected in well-watered tall fescue leaves but the immunoblots indicated dehydrin polypeptides of 15-, 19-, 23- and 35-kDa were detected in drought stressed tall fescue leaves at 7d of drought treatment (Figure 6). Regardless of treatment, no genotypic differences were observed for the level of dehydrins expression among three cultivars. The levels of 19-, 23- and 35-kDa dehydrin polypeptides were strongly accumulated in drought stressed plants at 11d drought treatment, but there were no differences of 15-kDa dehydrin expression between 7 d and 11 d drought stressed plants. The 35-kDa dehydrin was slightly accumulated at 7 d of drought and increased during after 11 d of drought.

The SDS-PAGE gel indicated that there was no difference in total soluble proteins between well-watered and drought stressed plants. So the alteration of the low molecular weight dehydrin polypeptides didn't change much in volume, but they did accumulate in drought stressed plants, and they were induced more as the drought stress became more severe. Although dehydrin polypeptides were observed in many studies and assumed to contribute to drought tolerance (Arora et al. 1998; Bewley et al. 1983; Riccardi et al. 1998; Hu et al. 2010), these results did not indicate a relationship between protein changes and drought tolerance, similar to the results obtained by Perez-Molphe-Balch et al. (1996) and Jiang and Huang (2002).

In this lysimeter study, drought-induced dehydrin proteins were present ranging from 19 to 35kDa; the expression of 19- and 23-kDa was especially great during the drought stress. The intensity of dehydrin proteins increased with progressive water deficit as the RWC dropped after drought treatment. The RWC was about 15% for all three cultivars at 11d of drought treatment, and the dehydrin proteins expressed the highest level. The results suggested the dehydrin proteins were induced strongly by drought, and

they largely accumulated as the drought was severe. Jiang and Huang (2002) also found the 23-and 27-kDa dehydrin protein accumulated in response to drought stress in leaves of tall fescue ‘Southeast ‘and ‘Rebel Jr.’. Hu et al. detected 19-kDa and other low molecular weight dehydrin polypeptides in 8 bermudagrass genotypes of drought stress. Cellier et al. (1998), Han and Kermode (1996) and Volaire (2002) all reported that dehydrin proteins may protect grass cells from further damage by continuous drought stress, but it is still necessary to discover the specific function of dehydrin proteins during drought stress in the further studies.

Conclusion

Based on the results of this study, the hypothesis that ‘2nd Millennium’ and ‘Rebel Exeda’ had the best and worst drought tolerance, respectively, is rejected. The hypothesis that ‘2nd Millennium’ will have the most extensive root system among the cultivars studies is confirmed. Lastly, the hypothesis that dehydrin expression will be observed in the tall fescue cultivars and expression will increase with prolonged drought stress is confirmed. According to the results of this research, there were no differences in drought tolerance among ‘2nd Millennium’, ‘Rebel Exeda’, and ‘Kentucky 31’ tall fescue, but ‘2nd Millennium’ may have improved drought avoidance compared to ‘Rebel Exeda’ and ‘Kentucky 31’ via increased RLD and root mass in the deeper soil profile.

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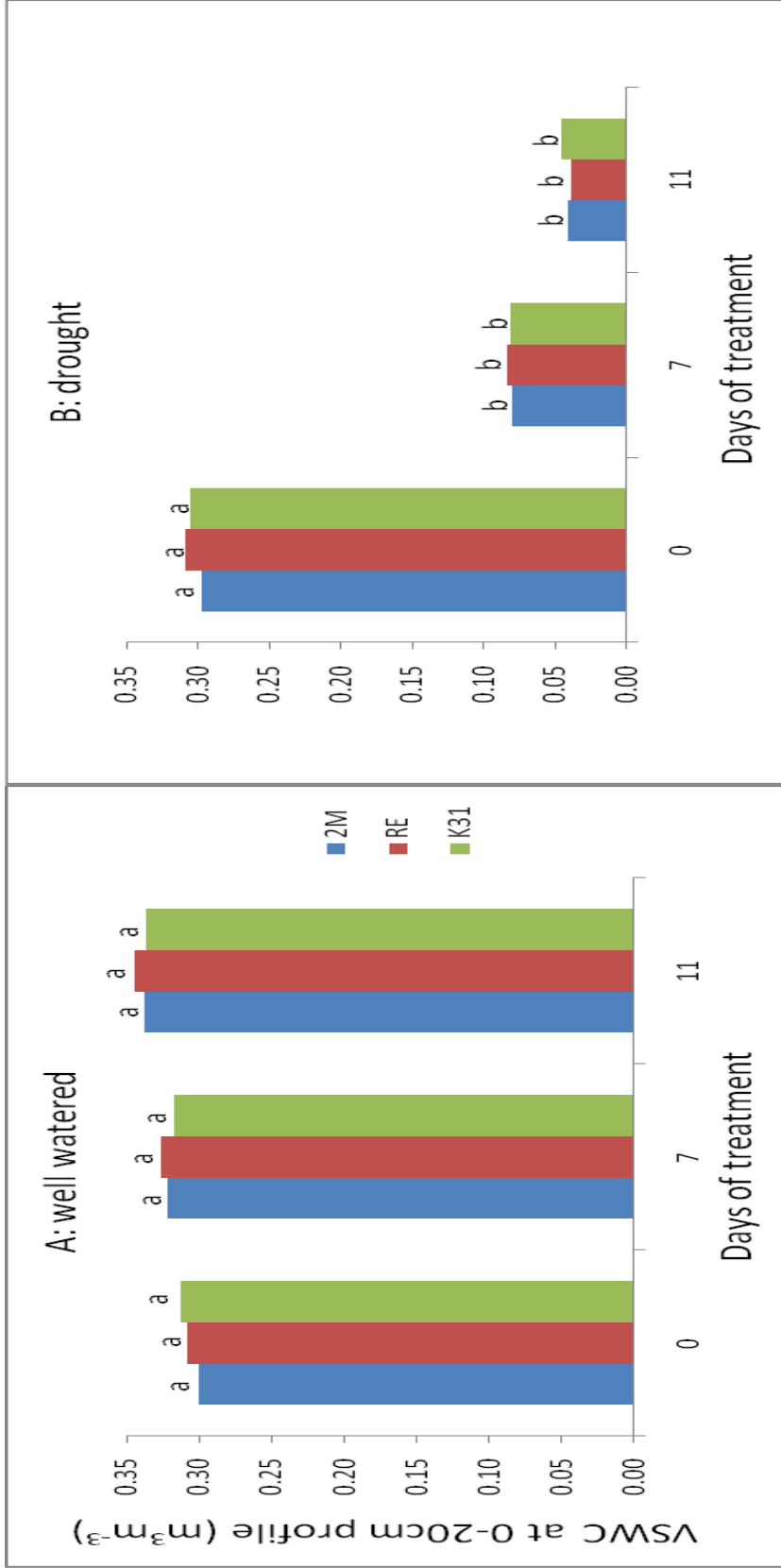


Figure 1. Volumetric soil water content (VSWC) of ‘2nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) in the 0-20cm profile under (A) well-watered and (B) drought conditions in the growth chamber. Means followed with the same letter on a given day after treatment initiation (days of treatment) are not significant different (P=0.05).

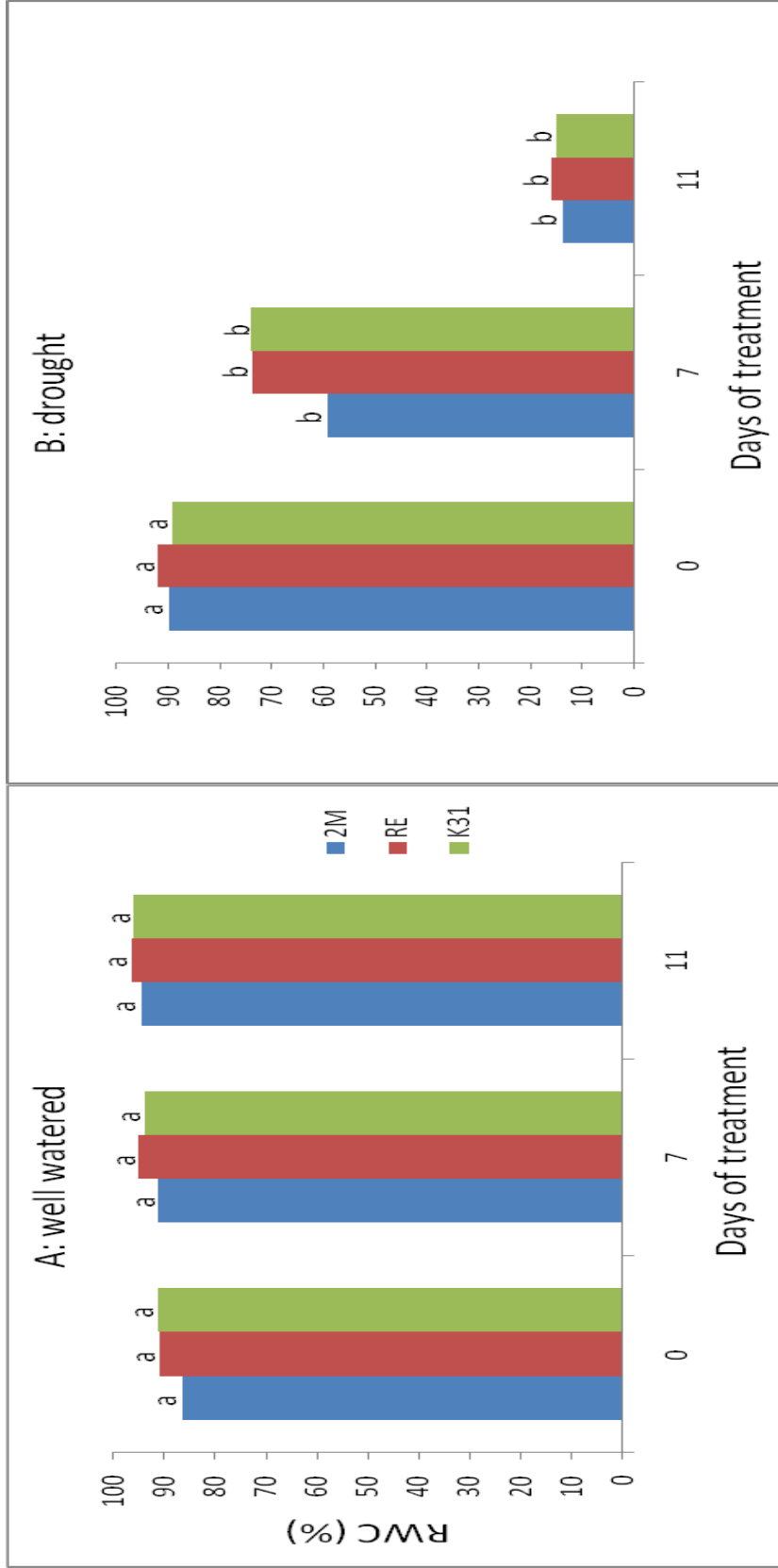


Figure 2. Leaf relative water content (RWC) of ‘2nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) under (A) well-watered and (B) drought conditions in the growth chamber. Means followed with the same letter on a given day after treatment initiation (days of treatment) are not significant different (P=0.05).

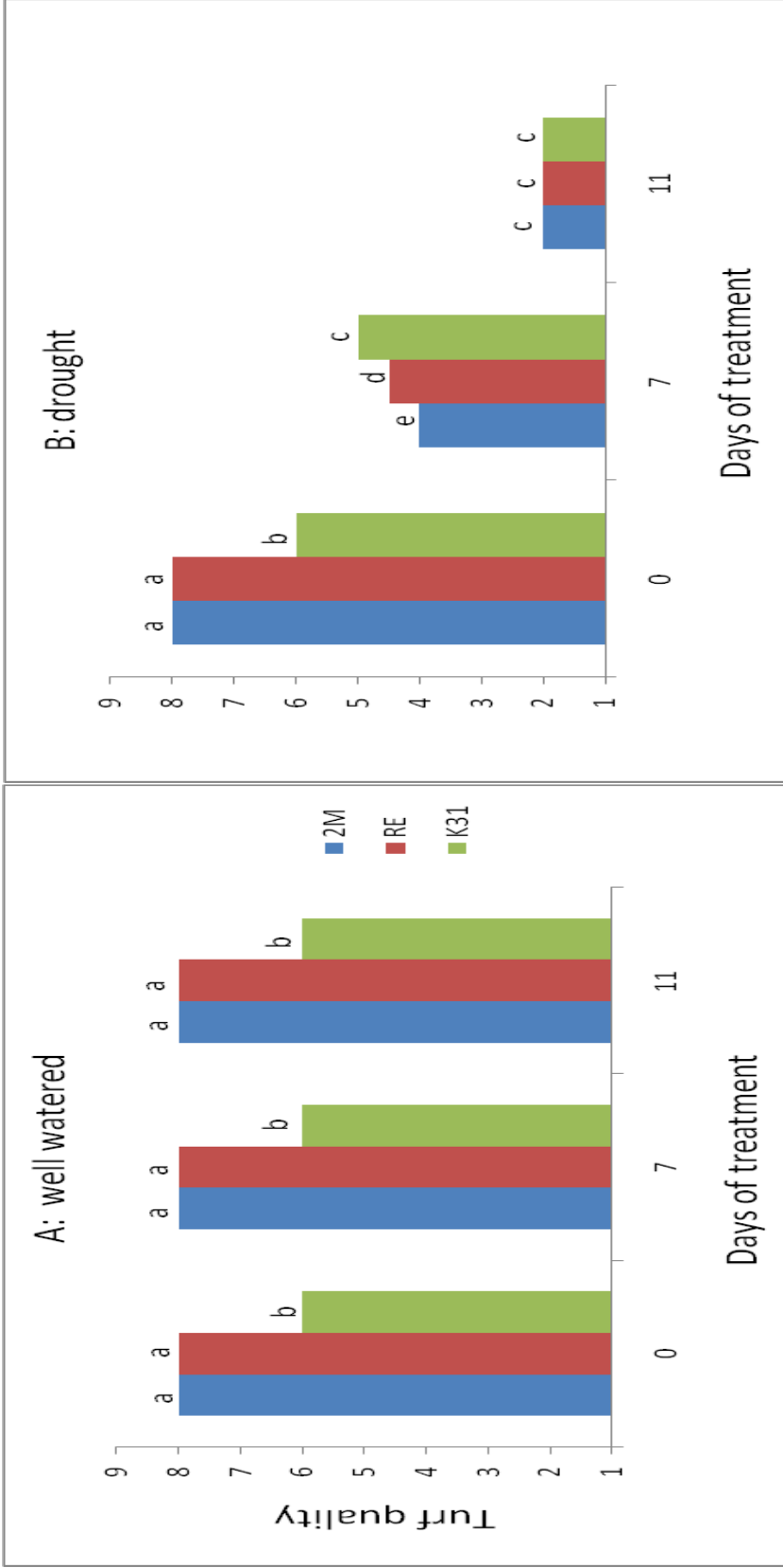


Figure 3. Turf quality of ‘2nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) under (A) well-watered and (B) drought conditions in the growth chamber. Means followed with the same letter on a given day after treatment initiation (days of treatment) are not significant different (P=0.05).

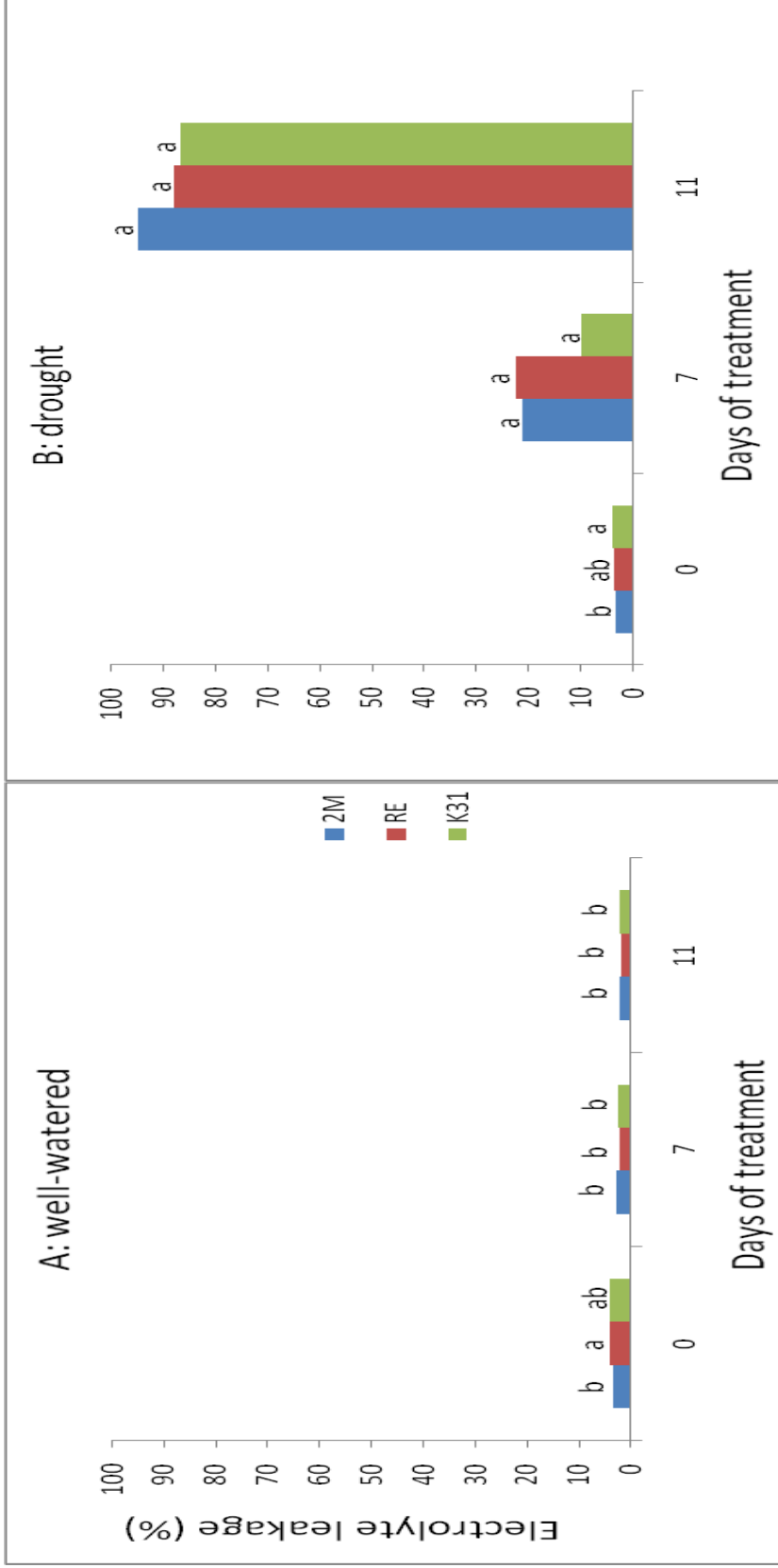


Figure 4. Electrolyte leakage (EL) of ‘2nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) under (A) well-watered and (B) drought conditions in the growth chamber. Means followed with the same letter on a given day after treatment initiation (days of treatment) are not significantly different (P=0.05).

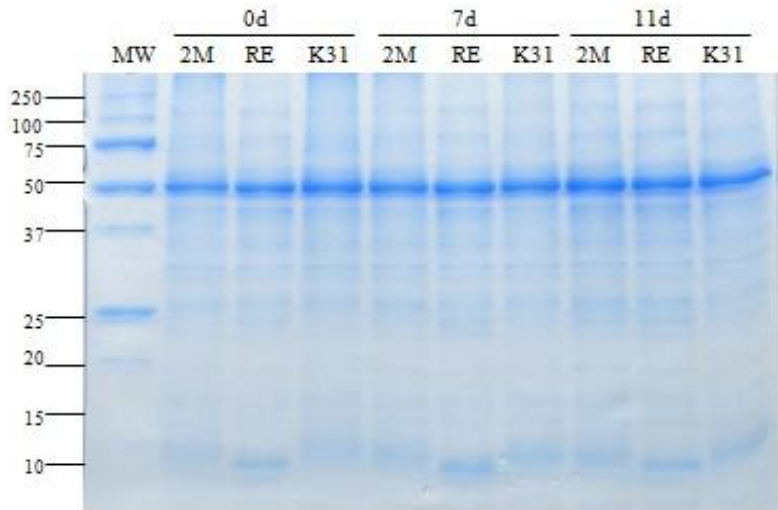


Figure 5. SDS-PAGE profiles of soluble protein from tall fescue leaves of ‘2nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue under drought stress in the growth chamber. From left to right: Marker; Lane 1, 2M well watered; Lane 2, RE well-watered; Lane 3, K31 well-watered; Lane 4, 2M 7 d of drought stress; Lane 5, RE 7 d of drought stress; Lane 6, K31 7 d of drought stress; Lane 7, 2M 11 d of drought stress; Lane 8, RE 11 d of drought stress; Lane 9, K31 11 d of drought stress. Equal amounts of proteins (30 μ g) were loaded in each lane.

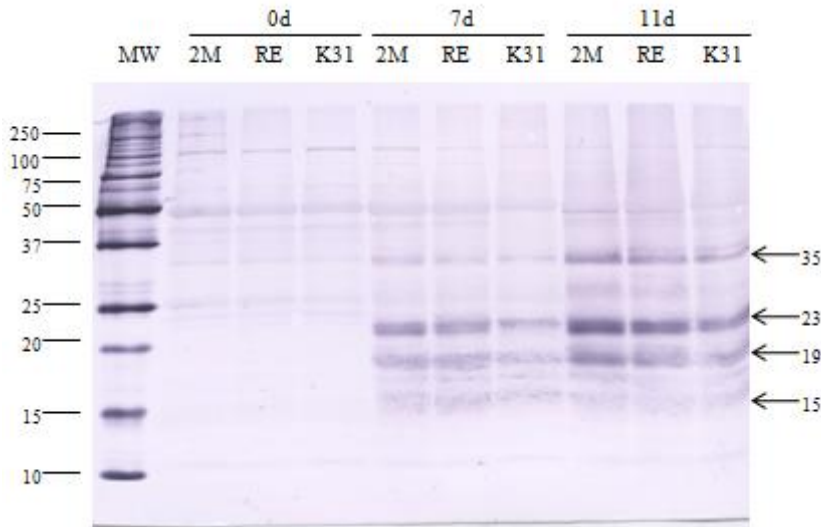


Figure 6. Immunoblots of dehydrin protein expression of ‘2nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue under drought stress in the growth chamber. From left to right: Marker; Lane1, 2M well watered; Lane 2, RE well-watered; Lane 3, K31 well-watered; Lane 4, 2M 7 d of drought stress; Lane 5, RE 7 d of drought stress; Lane 6, K31 7 d of drought stress; Lane 7, 2M 11 d of drought stress; Lane 8, RE 11 d of drought stress; Lane 9, K31 11 d of drought stress. Equal amounts of proteins (30µg) were loaded in each lane. Arrows indicated dehydrin proteins change in response to drought stress.

Table 1. Test of fixed effects of volumetric soil water content (VSWC), leaf relative water content (RWC), turf quality (TQ), and electrolyte leakage (EL) of ‘2nd Millennium’, ‘Rebel Exeda’ and ‘Kentucky 31’ tall fescue at 0 d (A), 7 d (B) and 11 d (C) of initiation of treatments.

Table 1A.

0d	Source	Num DF	Den DF	F Value	Pr>F
VSWC	Treatment (T)	1	18	1.74	0.2032
	Cultivar (C)	2	18	3.42	0.0551
	T×C	1	18	0.91	0.4214
RWC	Treatment (T)	1	18	0.25	0.6251
	Cultivar (C)	2	18	1.79	0.1946
	T×C	1	18	1.24	0.3131
TQ	Treatment (T)	1	18	0.00	1.0000
	Cultivar (C)	2	18	3.6E+14	<0.0001
	T×C	1	18	0.00	1.0000
EL	Treatment (T)	1	18	2.66	0.1204
	Cultivar (C)	2	18	6.67	0.0068
	T×C	1	18	1.95	0.1718

Table 1B.

7d	Source	Num DF	Den DF	F Value	Pr>F
VSWC	Treatment (T)	1	18	2628.13	<0.0001
	Cultivar (C)	2	18	0.5	0.6147
	T×C	1	18	0.13	0.8833
RWC	Treatment (T)	1	18	35.02	<0.0001
	Cultivar (C)	2	18	2.09	0.1530
	T×C	1	18	0.87	0.4362
TQ	Treatment (T)	1	18	507.00	<0.0001
	Cultivar (C)	2	18	3.00	0.0751
	T×C	1	18	39.00	<0.0001
EL	Treatment (T)	1	18	17.35	0.0006
	Cultivar (C)	2	18	1.23	0.3146
	T×C	1	18	1.23	0.3169

Table 1C.

11d	Source	Num DF	Den DF	F Value	Pr>F
VSWC	Treatment (T)	1	18	0.14	<0.0001
	Cultivar (C)	2	18	2666.50	0.8662
	T×C	1	18	1.01	0.3826
RWC	Treatment (T)	1	18	7640.17	<0.0001
	Cultivar (C)	2	18	1.51	0.2474
	T×C	1	18	0.04	0.9561
TQ	Treatment (T)	1	18	2.47E+17	<0.0001
	Cultivar (C)	2	18	0.00	1.0000
	T×C	1	18	3.86E+15	<0.0001
EL	Treatment (T)	1	18	4051.36	<0.0001
	Cultivar (C)	2	18	3.38	0.0567
	T×C	1	18	2.99	0.0758

Table 2. Root length density (RLD)^z, dry root weight (DRW)^y, average root diameter (ARD)^x, and root surface area (RSA)^w in different soil depths of ‘2nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue under well-watered conditions in the growth chamber at Oklahoma state University, Stillwater, OK.

Soil depth (cm)	Cultivar	RLD (cm cm ⁻³)	DRW (g)	ARD (mm)	RSA (cm ²)
0-15	2M	20.45 a ^v	2.410 a	0.33 a	2563 a
	RE	18.65 a	2.174 a	0.33 a	2330 b
	K31	18.11 a	1.743 b	0.30 a	2098 c
	LSD (0.05) ^u	NS	0.2503	NS	139
15-30	2M	5.87 a	0.403 a	0.30 a	675 a
	RE	5.20 a	0.370 a	0.30 a	599 a
	K31	5.94 a	0.370 a	0.28 a	644 a
	LSD (0.05)	NS	NS	NS	NS
30-40	2M	7.20 a	0.199 a	0.26 a	482 a
	RE	4.92 a	0.184 a	0.30 a	370 a
	K31	5.75 a	0.167 a	0.27 a	391 a
	LSD (0.05)	NS	NS	NS	NS
0-40	2M	11.67 a	3.012 a	0.31 a	3720 a
(Total)	RE	10.17 b	2.729 b	0.32 a	3299 b
	K31	10.46 b	2.279 c	0.29 a	3132 b
	LSD (0.05)	0.79	0.2832	NS	202

^z RLD was calculated by using the root length divided by the volume of soil inside each section.

^y DRW was measure after the samples were placed in an oven at 85 °C for three days.

^xARD and ^wRSA were measured by an image analysis system called WinRHIZO (Regent Instruments Inc., Quebec City, Canada).

^vMeans followed by the same letter within a specific soil depth and a column indicated no significant differences ($P=0.05$) were detected.

^uLSD (0.05) = Least significant difference values at the 0.05 significance level where NS = no significant difference.

Table 3. Turf quality of ‘2nd Millennium’ (2M), ‘Rebel Exeda’ (RE) and ‘Kentucky 31’ (K31) tall fescue at 0 d, 7 d, 14 d, 21 d and 28 d after initiation of rewatering.

Cultivar	0d	7d	14d	21d	28d
2M	2	2	3	4	5
RE	2	2	3	4	5
K31	2	2	3	4	5
LSD (0.05) ^z	NS	NS	NS	NS	NS

^zLSD (0.05) = Least significant difference values at the 0.05 significance level where NS

= no significant difference.

VITA

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Scope and Method of Study:

Cool-season turfgrasses are commonly under drought stress during the summer in the United States and often require supplemental irrigation to maintain acceptable turfgrass quality. A study was conducted at Oklahoma State University to evaluate the performance and protein alteration of tall fescue during drought stress. Three diverse cultivars of mature tall fescue ('Kentucky 31', '2nd Millennium' and 'Rebel Exeda') were subjected to two treatments: well-water condition and drought. Volumetric soil water content (VSWC), leaf relative water content (RWC), turf quality (TQ) and electrolyte leakage (EL) were measured at 0, 7, and 11 d following treatments. In well-watered plants, root characteristics were measured at the end of the study period. Protein analysis was conducted to detect for changes of dehydrin protein expression.

Findings and Conclusions:

The VSWC was maintained at the same level for each treatment for all three cultivars throughout the study. There were no differences in RWC among the three cultivars under well-watered conditions throughout the study, but tall fescue RWC declined after initiation of the drought treatment. Under drought stress, TQ decreased for all three cultivars as drought stress increased but 'Kentucky 31' had higher TQ compared to '2nd Millennium' and 'Rebel Exeda' at 7d of drought treatment. After 11 of drought stress, there were no differences in TQ among the three cultivars and the average TQ rating was 2. The EL results suggested that the three cultivars had similar membrane stability under drought stress throughout the study period. Root length density, DRW, and RSA were highest in '2nd Millennium' in the 0-40 cm profile suggesting that '2nd Millennium' may have greater ability to avoid drought under field conditions. Drought stress induced expression of dehydrin proteins, but there were no differences in soluble proteins or dehydrin protein expression among the three cultivars. Although dehydrin polypeptides were observed in many studies and assumed to contribute to drought tolerance, the results of this study did not indicate a relationship between protein changes and drought tolerance in diverse tall fescue cultivars.

ADVISER'S APPROVAL: Dr. Justin Quetone Moss
