CHARACTERIZING THE COLD HARDINESS AND DROUGHT RESPONSE OF NEWLY DEVELOPED BERMUDAGRASS GENOTYPES

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Abstract: Bermudagrasses are often subjected to various abiotic stresses affecting their aesthetic quality, and functionality. Abiotic stresses, such as drought, and freezing are some of the common detrimental factors for bermudagrass growth in the transition zone. Utilizing freeze tolerant bermudagrass in golf courses or athletic facilities would decrease the cost associated with the re-establishment of turf lost to winter injury Therefore, developing bermudagrasses with better freeze or drought resistance is the priority of any bermudagrass germplasm improvement program. This study has evaluated the freeze and drought response of newly developed bermudagrass genotypes. The lethal temperature to kill 50% of the population (LT_{50}) for the selected new genotypes was lower than that of industry standards 'Champion Dwarf' and 'Tifway' indicating higher freeze tolerance. Tahoma 31 consistently had the lowest LT_{50} value in this study. This research will help in prescreening genotypes prior to field evaluation and identifying genotypes with superior freeze tolerance to serve in future breeding efforts. In the drought response study, experimental genotypes from the University of Georgia had turf quality above 6 when subjected to 60 and 49 days of drought stress in 2017 and 2018, respectively. The superior performance of these genotypes could be due to their ability to extract water from the deeper soil profile during drought stress. However, when grown in a short 17 cm pot under controlled environment conditions, these genotypes dropped below the minimum acceptable turf quality within 6 and 9 days of drought stress in 2019 and 2020, respectively. The inconsistency in the performance of the genotypes in the restricted and unrestricted soil highlights the importance of soil depth to maintain turfgrasses without depending on supplemental irrigation successfully. The high correlation between the qualitative and quantitative measurements of drought response indicates their usefulness as relative drought resistance measurements. The wide genetic variations in bermudagrass response to drought and freeze stress, as discussed in this study, will serve as a valuable source for the advancement of bermudagrass breeding.

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CHAPTER I

A REVIEW OF LITERATURE

A Review of Bermudagrass

Cynodon L. C. Rich., a genus of the tribe Cynodonteae, subfamily Choridoideae, and family Gramineae (Poaceae), consists of nine species and ten varieties (Harlan et al. 1970a; de Wet and Harlan, 1970). The primary center of origin for bermudagrasses (*Cynodon* spp.) is southeastern Africa (Forbes and Burton, 1963). The biosystematic and molecular phylogenetic research in *Cynodon* species revealed that the secondary centers of origin were South Africa, India, Afghanistan, China, and Australia (Harlan and de Wet, 1969). Bermudagrasses are some of the most important, widely adapted warm-season turfgrasses. They are commonly used on home lawns, athletic fields, golf courses, and other utility turf areas. Bermudagrasses are known by different names in different regions, including ‘couch' or ‘green couch' in Australia, ‘dhoub' in India and Bangladesh and ‘quick' and ‘kweek' in South Africa (Hurcombe, 1948; Kneebone, 1966, Skerman and Riveros, 1990). The common name, bermudagrass, is regarded to be its American name and is speculated to be introduced from the West Indies (Kneebone, 1966). The introduction to the North and South America is speculated to be around the 1600s due to the unintended trans-atlantic human movement via ships of the Spanish conquistadors (Wu, 2011).

The *Cynodon* taxa, which are valued for use as turfgrasses are common bermudagrass (*Cynodon dactylon* [L.] Pers. var. *dactylon*) and African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) (Taliaferro et al., 2004) and their interspecific hybrids (*C. dactylon* X *C. transvaalensis*). *C. dactylon* often referred to as the "cosmopolitan weed of the world," has its origin in Africa and Seleucid Empire which includes portions of what is now West Pakistan, Afghanistan, Uzbekistan, Turkmenistan, Iran, Iraq, Syria, and a large portion of Turkey (Way, 2014; Harlan et al., 1970a). The cultural significance of *C. dactylon* dates to 1500 BCE, especially in South Asia. Multiple references in the ancient Vedic texts of Hinduism points to this (Kneebone, 1966; Way, 2014). *C. dactylon* is propagated through stolons, shoots, rhizomes, and seed. The seed generation is highly outcrossed due to cross-pollination and a high degree of self-incompatibility (Burton and Hart, 1967). Seed propagated varieties usually possess an upright growth habit and medium to coarse leaf texture due to increased internode length **(**Cattani et al., 1996)**.** *C. transvaalensis* is a species, probably introduced from South Africa to the United States by humans, with minimal dispersal and naturalization (Beard, 2013). *C. transvaalensis* is diploid in chromosome number. It has small, erect leaves, short stature, fine texture, and yellow-green color (Hanna, 1986; Harlan et al., 1966, 1970a, 1970b; Juska and Hanson, 1964). It is vegetatively propagated by sprigs, stolons, or sod. The interspecific hybrids of bermudagrass often form vigorous and aggressive turf, with many having high shoot density, as well as improved turf quality and color, and fine-textured leaves (Beard, 1973).

The breeding program initiated by Dr. Glenn Burton in 1946 was the first documented bermudagrass breeding program (Taliaferro, 2003). The scientific procedure of breeding triploid interspecific turf bermudagrass varieties between *C. dactylon* and *C. transvaalensis* was created by the same breeding program. The interspecific hybrids rarely produce viable seeds, and they must be propagated vegetatively by sprigs, stolons, or sod. Bermudagrasses are best adapted to moderately well-drained, fertile, loamy soil; however, these grow well over a wide range of edaphic conditions (Casler and Duncan, 2003). They tolerate the often hot and humid summers of the United States transition zone; however, they enter a dormancy period with the onset of winter. Bermudagrasses are regarded as having excellent tolerance to heat and drought, but low tolerance to freezing temperature (Beard, 1973).

Oklahoma State University (OSU) has been actively engaged in bermudagrass breeding since the mid-1980s (Taliaferro et al., 2004). The major goal of the breeding program is to develop high quality bermudagrasses with enhanced abiotic tolerance (mainly cold and drought), sod strength, and tolerance to spring dead (Taliaferro et al., 2004). Some of the bermudagrasses with high quality and improved cold hardiness developed by OSU that are commercially available are ‘Patriot,’ ‘Yukon,’ ‘Riviera,’ ‘Latitude 36,’ ‘NorthBridge’ (Wu and Martin, 2015), and ‘Tahoma 31^{TM}’ (United States Patent PP31695). Latitude 36 is a vegetatively propagated F1 hybrid that was produced by crossing *C. dactylon* accession 'A 12198' (2n=4X=36) and C. *transvaalensis* OSU selection '2747' (2n=2X=18) (Wu et al., 2014). Tahoma 31 (‘OKC 1131’) is a new clonally propagated F1 hybrid from a cross of C *dactylon* var. *dactylon* accession ‘A12268’ (2n=4x=36) x C. *transvaalensis* OSU selection ‘2747’ (2n=2x=18). Researchers and plant breeders from the University of Georgia (UGA) have also made significant advances that resulted in improved bermudagrass genotypes. ‘Tifway’ bermudagrass, a triploid (2n=3X=27) hybrid, is a vegetatively propagated variety selected and released from the Tifton breeding program that was headed at that time by Dr. Glenn Burton (Alderson and Sharp, 1994 and Wu et al., 2011). ‘Tif'uf ®’ (DT-1) is an interspecific triploid (2n = 3x =27) hybrid of C. *transvaalensis* and C. *dactylon* co-released from UGA and the U.S. Department of Agriculture–Agricultural Research Service in 2014 (Schwartz et al., 2018). ‘Celebration’ bermudagrass was originally developed by Rod Riley, a turfgrass breeder in Australia, and was released in the U.S. in 1999. Fine-textured, ultradwarf interspecific triploid ($2n = 3x = 27$) hybrid bermudagrass [*C. transvaalensis* ($2n = 2x = 18$) × *C. dactylon* var. *dactylon* ($2n = 4x = 36$)] is widely used on golf putting greens (Lyman et al., 2007). The most commonly used ultradwarf bermudagrasses include ‘Champion Dwarf’ ‘TifEagle,’ and ‘Mini Verde’. Champion is a mutation selected from a Tifdwarf putting green in Texas (Brown et al., 1997). TifEagle bermudagrass is an artificially induced mutation from ‘Tifway II’ in Georgia (Hanna and Elsner, 1999). , but molecular marker data indicated it is a mutation of Tifdwarf or Tifgreen (Wang et al., 2010). Mini Verde is a natural mutation from Tifdwarf bermudagrass selected in Arizona (Kaerwer and Kaerwer, 2001).

Freezing Process in Plants

Plant growth and development are strongly correlated to environmental conditions. Every plant has a specific temperature range for optimum growth and is sensitive to changes in temperature. Temperatures that are too high or too low will result in abnormal development and reduced yield. Low_temperature stress is divided into chilling and freezing stresses. Chilling stress is defined as the temperature below optimum that affects plant activity (Levitt, 1980). It is difficult to establish a specific temperature below which chilling stress occurs as different plants have different temperature ranges for this stress (Levitt, 1980). However, freezing stress occurs when the temperature drops below zero, which can cause devastating damages to plants because the formation of ice crystals ruptures the cell, impairing plant growth, development, and productivity. Earth has only one-third of its total land area that is used for agricultural needs (Ramankutty et al., 2008), and only 25% of the land is completely free of frost (Hoffman, 1963; Sakai and Larcher, 1987). Consequently, it is critical to understand the magnitude of freezing resistance and also understand the physiological and morphological changes that occur within the plant.

Ice Nucleation

Ice melts at 0 °C; however, the temperature at which water freezes have not been clearly established (Ashworth, 1992; Wisniewski et al., 2009). A stable ice nucleus is formed when water molecules come together either spontaneously (homogeneous nucleation), or with the help of moisture or another substance (heterogeneous nucleation) (Pearce, 2001; Wisniewski and Fuller, 1999). Pure water has been shown to freeze at very low temperatures as low as -40 °C due to homogenous nucleation temperature. However, pure water could also freeze at much warmer temperature due to heterogeneous nucleation (Wisniewski et al., 2009). Two types of heterogeneous nucleators are associated with plant freezing. Extrinsic nucleators such as ice-nucleation active (INA) bacteria and intrinsic nucleators such as natural components of the plants (organelles, proteins, and other dissolved compounds in the cytosol). These nucleators act as a template for water molecules to take on a crystalline arrangement, and it continues to grow by freezing the surrounding water molecule (Wisniewski and Fuller 1999).

Freezing at the Cellular Level

A plant cell is divided into the symplastic (also known as cytoplasmic) compartment on the inner side of the plasma membrane and the apoplastic (also known as extracellular or cell wall) compartment on the outer side. The ice formation starts in the apoplast because the solute content of the apoplastic fluid is less, hence the freezing point is higher than that of the symplast (Srivastava, 2002). The vapor pressure of ice formed in the apoplast is lower than the cell water in the symplast, causing the water to diffuse through the plasma membrane to the apoplast leading to cell dehydration (Sakai and Larcher,1987). The migrated water also freezes to form ice crystals leading to cell contraction. The mechanical strain on the cell wall and plasma membrane makes it difficult for the cell to regain its shape upon thawing, resulting in cell rupture (expansion-induced lysis) (Uemura and Steponkus, 1997). The plasma membrane is considered to be the primary site of freezing injury due to membrane destabilization and freeze induced dehydration (Levitt, 1980; Steponkus et al., 1984). A loss of semi-permeability of the plasma membrane was reported in winter wheat when exposed to freezing temperature (Chen and Gusta, 1978). The increased diffusion of manganese ions into the cell in this study indicated the loss of semi-permeability of the plasma membrane during freeze stress.

Cold Acclimation

A significant level of winter-hardiness can be achieved by exposing plants to low nonfreezing temperatures, and this process is known as cold acclimation (Levitt, 1980). Plants undergo various physiological changes while acclimating to lower temperatures (Guy, 1990). During this period, the plant cell undergoes various modifications, including cell membrane alteration, an adjustment in cell metabolism, an increase in solute concentration, extensive reprogramming of gene expression, upregulation of proteins, etc. (Ruelland et al., 2009). Dowget and Steponkus (1984) studied the behavior of the plasma membrane of isolated protoplasts of 'Puma' rye (*Secale cereal*e L.) during a freeze-thaw cycle. In this study, nonacclimated protoplasts, when exposed to freezing temperatures, showed the appearance of vesicles inside the protoplast (endocytotic vesicles), causing a reduction in the surface area. Once thawed, these cells were unable to hold water from the melting ice leading to cell rupture. On the contrary, the cold acclimated protoplast, when exposed to freezing temperatures, showed no change in the surface area due to exocytotic vesicle formation instead of endocytotic vesicles. Therefore, upon thawing, the cell could regain its original shape and resist mechanical failure.

The differential behavior of the plasma membrane during freeze stress is due to its lipid composition. The fluidity of the plant membrane is essential to maintain the function of a plant. The increased fluidity of the lipid bilayer is due to the increase in unsaturated fatty acid (FA) residues (Voet and Voet, 1990). The artificial enrichment of nonacclimated protoplast of rye with mono- or di-unsaturated species of phosphatidylcholine resulted in the formation of exocytotic extrusions instead of exocytotic vesicles (Steponkus et al., 1988).

Solute Accumulation During Cold Acclimation

According to Raoult's law, the freezing point of a solution depends on the concentration of solutes in it. Depression in freezing point owing to solute accumulation increased freeze tolerance of cold-acclimated plants. However, if the temperature declines rapidly, the concentration of cell solutes cannot keep pace with the thermodynamic effect of the drop in temperature, eventually leading to lethal freezing of the cell (Olien, 1981). Koster and Lynch (1992) confirmed an increase in the intracellular osmotic potential due to the accumulation of glycinebetaine, proline, sucrose, and raffinose in ‘Puma’ rye when subjected to 2°C for six weeks. They observed that higher accumulation of solutes took place 2 weeks after acclimation.

Carbohydrates also accumulate when plants are exposed to low temperatures as a consequence of decreased translocation (West 1973). Trunova and Tumanov (1963) incubated wheat seedlings in sucrose solution and another set of wheat seedlings in water in the dark at 2°C. An increase in freeze tolerance was observed for wheat seedlings immersed in sucrose solution when compared to the set immersed in water. This increase in freeze tolerance was associated with the rise in the concentration of monosaccharides and sucrose in the seedling.

A rise in cellular proline concentrations during acclimation helped in osmotic adjustment during freezing stress, reducing the possibility of cytoplasm dehydration, extracellular ice formation, and cell rupture (Rossi, 1997). Dehydrins, one of the most common proteins, have been reported to accumulate during dehydrative stress caused by drought, freeze, and salinity stress (Close, 1996). The increased level of dehydrin has been correlated with freeze tolerance in barley and zoysiagrass (Zhu et al., 2000; Patton et al., 2007).

Freezing in Turfgrasses

The number of carbons in the first stable compound formed after carbon fixation during photosynthesis, classify plants into C3 plants, C4 plants, and Crassulacean acid metabolism cycle (CAM). The C3 species make up to approximately 85 % of all higher plant species, C4 species account for about 5 %, and CAM species make up the remaining 10 %. (Yamori et al, 2013). The C4 plants are adapted to high light intensities, high temperatures, and dryness and have higher photosynthetic efficiency (Long, 1999). C3 plants gain an advantage over C4 plants at low temperatures via protection from freezing injury (Osborne, 2008). The C4 pathway of photosynthesis is rare in plant species, but it is common in turfgrasses. Turfgrasses are divided into cool-season and warm-season turfgrasses. Cool-season turfgrasses thrive at a temperature from 18-24°C and follow the C3 photosynthetic pathway and hence are more tolerant to freezing temperatures (Turgeon, 2006). Warm-season grasses contain the C4 photosynthetic pathway, and are adapted to subtropical and tropical regions and have an optimum growing temperature around 25 to 35°C (Turgeon, 1996). Cool-season turfgrasses such as annual bluegrass store carbohydrates in the form of water-soluble fructans (Dionne et al., 2001b). Warm-season turfgrass store carbohydrates in the form of water-insoluble starch (Moore and Hatfield, 1994). Consequently, unlike starch, water-soluble fructans could help in freezing point depression. During fall, accumulation of nonstructural carbohydrates in organs such as tiller bases, crowns, stolons, and rhizomes has been reported to improve freeze tolerance in winter in annual bluegrass (*Poa annua* L.)

(Dionne et al., 2001b). A decline in the concentration of all of these soluble carbohydrates have been observed from February to April to encourage spring green-up in saltgrass (*Distichlis spicata* var. *stricta* (L.) Greene) (Shahba et al., 2003).

The survival of turfgrass during freezing temperatures depends on the magnitude of injury within the crown (Dipaola and Beard, 1992). Turfgrass freezing occurs at 0° C (32 °F) and colder temperatures. The injury caused is due to the formation of ice crystals, especially in the crown, the regenerative region of the plant (Beard, 1973). Leaves tend to freeze earlier than crowns or roots due to air temperatures being lower than soil temperature (Pearce and Fuller, 2001). Maintaining the integrity of the turfgrass crown is essential for freezing stress survival since leaf, root, and lateral shoot regeneration occurs from the turfgrass crown during spring (Beard, 1973). The grass may not recover if too many cells of the crown are injured due to freezing stress (Beard, 1973).

Numerous studies have been conducted to understand the freeze tolerance of turfgrasses in the field and through artificial freezing in a controlled environment. The ability of the turfgrass species to survive the severities of winter has been measured in the past using the percentage of winterkill post green-up in spring (Anderson et al., 1988). Winterkill is a general term used to define turf loss during the winter (Beard, 1973). The controlled environment studies assessing the lethal temperature required to kill 50% of the population (LT_{50}) showed significant correlations to spring green-up and winterkill estimated in the field (Patton and Reicher, 2007; Dunne et al., 2019). While field evaluations can provide plant breeders with the most accurate assessment of winter survivability, environmental conditions are often unpredictable and difficult to replicate (Anderson and Taliaferro 2002). Electrolyte leakage (EL) is a rapid indicator of plant stress and has been used to estimate LT_{50} in turfgrasses (Cardona et al., 1997; Fry et al., 1993). However, the accuracy of this method is still questionable. The EL produced higher LT_{50} values for nonacclimated seashore paspalum (*Paspalum vaginatum* Swartz) in comparison to whole plant survival (Cardona et al., 1997) and lower LT_{50} values for centipedegrass (*Eremochloa ophiuroides* (Munro) Hack) when compared to field regrowth method (Fry et al., 1993). Therefore, the artificial freezing test with a controlled rate of cooling has been successfully conducted in the past to evaluate the freeze tolerance of plants. Most of the artificial freezing studies determined the LT_{50} value based on the whole-plant survival following exposure to freezing temperature (Anderson et al.,1993, 2002; Patton et al., 2007). Intra and interspecies variation in freeze tolerance has been reported in such controlled environment tests for various turfgrass species (Anderson et al., 1993; Patton et a., 2007).

Freeze Tolerance of Cool-Season Turfgrasses

The LT_{50} values of the crown for several cool-season turfgrasses were determined by Gusta et al. (1980). Creeping bentgrass (*Agrostis stolonifera* L) had the lowest LT_{50} value (-35°C), followed by bromegrass *(Bromus* spp.), Kentucky bluegrasses (*Poa pratensis* L.), red fescue (*Festuca rubra*), alkaligrass *(Puccinellia* spp.), hard fescue *(Festuca longifolia* Thuill.), and perennial ryegrass (*Lolium perenne L.*). The freeze tolerance and carbohydrate levels of velvet bentgrass (*A. canina* L.) and creeping bentgrass was determined after being subjected to four acclimation treatments (i. nonacclimated, ii. acclimation at 2°C for two weeks, iii. 2°C for four weeks, and iv. 2°C for four weeks plus subzero acclimation at −2°C for two weeks) (Espevig et al., 2011). The freeze tolerance was evaluated using whole plant survival, EL, and 2,3,5-triphenyl tetrazolium chloride (TTC) reduction tests. Plant tillers from each acclimation treatment were exposed to -4, −6, −9, −12, −15, −18, and −21°C for 2 hours (h). Each acclimation treatments improved freeze tolerance in both the grasses. However, there was no significant difference in the LT_{50} of both grasses among acclimation treatments. The LT_{50} determined through TTC, and EL slightly underestimated freezing tolerance when compared to whole plan survival. They concluded that the higher LT_{50} values determined by TTC could be because crowns make up a small portion of the plant when compared to surrounding nonmeristematic tissues, and much of the nonmeristematic tissue was injured by frost was unable to recover. A study was conducted to compare the freeze tolerance among 13 commercially available perennial ryegrass cultivars (Goslee et al., 2017). The cultivars were established from seeds and were maintained at a temperature of 21/13°C day/night for 14 days. This step was followed by a cold acclimation period of 14 days at a temperature of 4/2°C day/night. Plants were exposed to −10, −15, or −20°C for 1 h. The regrowth was evaluated after 36 days. The LT_{50} values ranged from −12.9 ('Bargala') to −20.8°C ('Mara').

Freeze Tolerance in Warm-Season Turfgrasses

Numerous studies have been conducted to evaluate the freeze tolerance of warm-season turfgrasses due to their susceptibility to winterkill. Patton et al. (2007) conducted a study to determine the freeze tolerance of 35 and 13 zoysiagrass (*Zoysia* spp.) genotypes in the field and controlled environment conditions, respectively. The commercially available genotypes with the least winter injury in the field were 'Meyer', 'Chinese Common,' and 'Zenith.' The genotypes with the most winter injury were 'Victoria,' 'DeAnza,' 'Diamond,' and 'Empress.' Similar results were obtained in the controlled environment freeze test with the LT_{50} values ranging from −8.4°C (Diamond) to −11.5°C (Meyer and Zenith). The LT_{50} values obtained in controlled environment testing and winterkill data had a correlation coefficient of 0.48, indicating growth chamber-based procedures produced results reflective of that expected in the field (Patton et al., 2007).

Hinton et al. (2012) determined the LT_{50} values of zoysiagrass cultivars in a controlled environment that were naturally cold-acclimated in a field setting. Plugs of nine cultivars were extracted from the field post-fall acclimation and also post spring green-up to test their ability to withstand late freeze. The plugs were exposed to temperatures from -6 to -14℃. The LT_{50} values ranged from -9.8 to -10.2℃ and -5.6 to -9.8℃ for Z. *japonica* and Z. *matrella* cultivars, respectively, for the winter trials. The LT_{50} values of all the cultivars were higher for the plugs collected after spring green-up, suggesting that late freeze could cause greater damage to zoysiagrass cultivars.

Cold hardiness is a major limiting factor in the widespread use of St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) as turf, especially in the transition zone. A study was conducted to evaluate the freeze tolerance of three St Augustinegrass genotypes, 'Floratam,' 'Raleigh,' and 'FX-332' by collecting stolons from the field between October and March (Maier et al.,1994). The stolons were maintained in a growth chamber at 1℃ overnight. The following day, chamber temperature was reduced at the rate 2°C h^{-1} (–2 to –8°C). Raleigh had the highest survival percentage for stolons collected on all dates. The stolons collected in December and January had a superior survival rate for all three cultivars due to the natural acclimation in the field during this period. The water content of Raleigh stolons collected between January and March had a negative correlation (r = –0.80) with winter survival. This negative correlation was attributed to the crystallization of water within the crown, ultimately causing cells to rupture, and potentially killing the plant. In another study, nine genotypes of St Augustinegrasses were evaluated for freeze tolerance to identify the ideal temperature for screening St. Augustinegrass and to identify a reliable method to assess survival and recovery post freezing (Kimball et al., 2017). The genotypes were exposed to four target temperatures (−3 °C, −4 °C, −5 °C, and −6 °C). Results indicated that −4 °C was the most suitable temperatures for evaluating freeze survival in St. Augustinegrass as -5 and −6 °C had a low average regrowth rating of 0.4 and 0%, respectively.

The relative freezing tolerance and seasonal changes in freezing tolerance level of six buffalograss (*Buchloe dactyloides* (Nutt.) Engelm**.)** were studied by Qian et al. (2001). The buffalograss cultivars were established in the field, and stolons were collected at monthly intervals from September through April and from October through May the following year. These stolons were then subjected to freezing. All cultivars had similar high LT_{50} values in September due to the lack of acclimation. However, all the cultivars exhibited a decrease in LT_{50} with an increase in the period of acclimation in the field. The ability to acclimate and the level of freeze tolerance were significantly different among the cultivars.

Bermudagrass Cold Hardiness

Developing bermudagrasses with enhanced winter survival is the priority of any bermudagrass germplasm improvement program. Many studies have been conducted to understand the freeze tolerance of bermudagrass in a controlled environment by estimating the LT_{50} values. Anderson et al. (1993) studied the freeze tolerance of vegetative cultivars, which were clonally propagated in cone-tainers. These cone-tainers were placed in a greenhouse for about six weeks and were transferred to a plant growth chamber for cold acclimation at 8/2°C day/night temperature for four weeks. The cone-tainers were then moved to a freezer overnight and equilibrated at -3°C. The freezer was programmed to lower the temperature at the rate of 1°C h^{-1}. At every 1°C interval, plants were removed and thawed at 4°C. These cone-tainers were returned to the greenhouse to initiate recovery. At the end of four weeks, these were evaluated for regrowth to determine the LT_{50} values of each cultivar. The results of this study showed significant differences in the LT_{50} values among bermudagrass cultivars, with values ranging from -7.7 (‘Tifgreen’) to -9.6°C (‘Midiron’). Anderson et al. (2002) conducted multiple freeze tolerance evaluations on bermudagrass cultivars based on their intended use (vegetatively propagated fairway and putting greens and seeded bermudagrasses). The clonal bermudagrass ‘GN-1’ (-5.9°C) was most susceptible to freezing stress and ‘Quickstand’ (-8.0°C), and ‘Midlawn’ (-8.4°C) were the most tolerant in the fairway types. Among the seed-propagated bermudagrasses, ‘Arizona Common’ (-5.6°C) was found to be the least tolerant and ‘Guymon’ (-7.4°C), and ‘Yukon’ (-7.6°C) had superior freeze tolerance with lower LT_{50} values. Among the putting green types, ‘Champion’ (-4.8°C), ‘Floradwarf’ (-4.9°C), and ‘MS Supreme’ (-5.2°C) were more susceptible to freezing. ‘Tifdwarf’ (-6.6°C), ‘TifEagle’ (-6.0°C), and ‘Tifgreen’ (-6.5°C) were found to be hardier in this group.

The magnitude of freeze damage in bermudagrass not only depends on the drop of temperature but also on the duration of exposure to freezing temperatures (Anderson et al., 2003). The results of this study indicated that bermudagrasses exhibited a decrease in the survival percentage with an increase in the duration of exposure to freezing temperatures. Recently a multiyear field and laboratory study was conducted to screen African and common bermudagrass germplasm collection for freeze tolerance (Dunne et al., 2019). The laboratory testing was done by performing independent experiments for four different freezing temperatures (−4, −5, −6, and −7°C). The spring green-up and winter survival of the standards used in the field testing ranked in the order of 'Patriot' > 'Tifsport' > Quickstand > 'Tifway.' Some of the genotypes had higher spring green-up and winter survival and lower LT_{50} values than the top-performing industry standard Patriot, suggesting that these could be used as additional sources of cold hardiness in bermudagrass breeding. The controlled environment testing showed significant correlations to spring green-up (-0.26), and winterkill (-0.24) estimated in the field.

Due to the winterkill susceptibility of bermudagrasses, many golf courses having bermudagrass putting greens have been advised to install covers whenever the temperature is forecasted to reach -4°C (25 °F) or lower (O'Brien and Hartwiger, 2013). However, if the threshold temperature to covered the greens can be decreased below -4℃ without an increase in winter injury would help in reducing labor costs associated with covering and uncovering greens. Therefore, recently a study was conducted at University of Arkansas to test the effect of four low-temperature thresholds (-15, -7.8, -5.6, and -4℃) used for applying covers on winterkill and spring green-up of the three ultradwarf bermudagrass genotypes, (Champion, TifEagle and MiniVerde) (DeBoer et al., 2019). There were no drastic differences in winter survival and spring recovery for all the cover treatment indicating the possibility to reduce the recommended low-temperature threshold for covering greens.

Physiological basis for differences in freeze tolerance in bermudagrass

All the above-mentioned studies had a cold acclimation period before the initiation of freeze test to mimic the fall acclimation found in nature. Cold acclimated bermudagrasses exhibited better freeze tolerance with lower LT_{50} values than the ones which were nonacclimated (Gatschet et al., in 1994). Gatschet et al. (1994) conducted an experiment to characterize alteration in protein synthesis in the bermudagrass cultivars Midiron and Tifgreen associated with cold acclimation. The cultivars were cold acclimated by transferring these to a plant growth chamber maintained at a temperature of 8/2°C day/night temperature for 28 days with a photosynthetic photon flux of $\approx 300 \mu mol \cdot m^{-2 \cdot} s^{-1}$ during 10-h photoperiods and another set was nonacclimated and was maintained at 28/24°C day/night temperature. The LT_{50} values for Midiron and Tifgreen, after cold acclimation, were lowered by 5°C. The LT_{50} values of acclimated plants decreased from –6.5 to –11.3°C for Midiron and –3.6 to – 8.5°C for Tifgreen, respectively. Midiron was also found to have higher levels of intermediate molecular weight (MW) (32 to 37 kDa) and low-MW (20 to 26 kDa) cold-regulated proteins (COR) proteins than Tifgreen. The results of this study indicated that cold acclimation improved freeze tolerance, and the superior performance of Midiron was due to the increased levels of COR proteins.

Zhang et al. (2006) tested changes in the levels of carbohydrates, proteins, antioxidant enzymes, and EL during cold acclimation of two bermudagrass cultivars, Riviera, and Princess 77. After 21 days of acclimation, these cultivars were transferred to a freezer, which was programmed to cool at the rate of – 2°C every 2 h. The results of this study revealed that cold acclimation induced accumulation of sugars and proline in both cultivars, and decreased EL. Riviera had lower LT_{50} value than Princess 77 in the acclimated and nonacclimated sets. This could be due to an increase in total nonstructural carbohydrates and protein observed in Riviera.

A decrease in photochemical efficiency and higher nitrogen metabolites (proline, dehydrin, and amino acid) accumulation during cold acclimation was observed in freeze tolerant bermudagrass cultivars, Patriot and Riviera (Zhang et al., 2011). In this study, the cultivars with higher levels of nitrogen (N) metabolites exhibited quicker green-up when compared to those with lower levels of N metabolites. The photochemical efficiency improved as the N metabolites decreased at higher temperatures. The decline in N metabolites at higher temperatures was attributed to the increased mobilization of N for promoting new growth. The study conducted by Dunn and Nelson (1974) consisted of three bermudagrass cultivars ‘Midway,' ‘Westwood' (Missouri selection), and ‘U-3’. The sprigs of each cultivar, collected from the field, were subjected to freezing. These sprigs were then planted to record the regrowth. U3 was the least cold hardy when compared to Midway and Westwood. The higher freeze tolerance of Midway bermudagrass was attributed to the presence of more rhizome and stolon tissue. Stolons of both cultivars had higher starch content in November than in rhizomes suggesting that stolons are the more important storage organs of carbohydrate during the fall hardening period. A four-fold increase of unsaturated FA: saturated FA ratio was observed during cold acclimation in cold tolerant Midiron indicating a possibility of specific desaturase enzymes that help in controlling membrane lipid/fatty acid composition in response to low temperature and ultimately help in avoiding the winter injury (Samala et al., 1998).

Drought Stress in Plants

Water scarcity is now a reality and not a warning as it is increasingly prevalent all around the globe. The World Economic Forum has ranked water crisis as one of the top three global risks in terms of impact since 2012. The Comprehensive Assessment of Water Management in Agriculture (CA, 2007) categorizes water scarcity into two forms: physical and economic water scarcity. Physical water scarcity is the inadequacy of natural water resources to meet the demands of the growing population. Economic scarcity is the poor management of available water resources that hinders access to these resources. The most significant impact of water scarcity has been on the agriculture sector as it accounts for 70% of global freshwater withdrawals (FAO Water Reports, 2012). Water stress is a common abiotic stress that plants encounter. Water stress could arise from insufficient or excessive water. However, most terrestrial plants experience insufficient water condition termed as water deficit stress or drought (Levitt, 1980). Drought is meteorologically defined as a period without significant rain (Turner, 1979) or as a condition which plants experience after a prolonged period of water deprivation that causes depletion of moisture in the root zone (Youngner, 1985). The factors that affect the drought response of plants are drought severity, drought length, soil physicochemical conditions, and plant vigor (Hossain et al., 2016). Drought affects physiological processes such as photosynthesis through pathway regulation by stomatal closure leading to decreased flow of CO_2 into the plant (Chaves, 2002). Loss of cell turgor has been the most common indicator of plant drought stress, having impacts on cellular structural integrity, metabolism, and whole-plant performance (Hopkins and Huner, 2008).

Components of drought resistance

The ability of a plant to survive periods of water deficit stress is called drought resistance (Levitt, 1980). Plants respond to drought conditions by undergoing physiological and morphological modifications to adapt. Therefore, drought resistance has three major components; tolerance, avoidance, and escape (Levitt, 1980). These components may or may not occur together in the same plant, and the relative importance of each mechanism depends on drought duration, drought severity, and the plant (Levitt, 1980; Huang et al., 2014). However, understanding these concepts and the mechanisms adopted by plants during drought stress help in mitigating the harmful effects of drought stress.

Drought avoidance

A plant can avoid drought stress by undergoing specific morphological and anatomical modifications. Drought avoidance in plants is achieved by maintaining high tissue water potential by increased stomatal and cuticular resistance, and changes in leaf area and anatomy, (Oosterhuis et al., 1991 Matthews, 1986). There are two distinct kinds of drought avoiders, water savers and water spenders (Levitt, 1980). The savers possess adaptive traits to minimize water loss by reducing transpiration, transpiration area, and radiation absorption under drought stress. The reduction in transpiration is due to the closure of stomata. The mechanism of drought-induced stomatal closure was attributed to an increase in the endogenous level of abscisic acid (ABA) in water-stressed tissue

(Beardsell and Cohen, 1975). On the contrary, water spenders optimize water by achieving higher tissue water status by maintaining water uptake through increased rooting. Hydraulic conductance of such plants increases due to extended root growth and root density (Plaeg and Aspinall, 1981). This trait is essential for plants in environments where surface soil water is limited, and water is stored in deeper soil profiles (Carrow, 1996b).

Drought tolerance

The mechanism of drought tolerance involves the maintenance of turgor pressure, which could be achieved by osmotic adjustments (involving inorganic ions, carbohydrates, and organic acids), and increasing membrane stability (Levitt, 1980). The accumulated solutes help in lowering the osmotic potential, thus increasing the water uptake by roots. Such solutes are called compatible solutes because they do not have any negative effects on enzymes and other macromolecules at high concentrations (Plaeg and Aspinall, 1981). Once drought stress ends, the compatible solutes remobilize for plant regrowth (Plaeg and Aspinall, 1981).

Drought escape

Plants complete their life cycle before severe drought through increased metabolic activity and rapid growth (Beard, 1973). Such plants do not experience drought stress, as they modify their vegetative and reproductive growth according to the availability of water. These plants show rapid plant growth but produce a minimal number of seeds and do not undergo any morphological, physiological, or biochemical modifications (Basu et al., 2016). Annual grasses complete their life cycle before the onset of drought, whereas perennial grasses become dormant during drought periods (Huang, 2008).

Drought Resistance in Turfgrasses

The movement of water from the roots into the turfgrass plant occurs through the symplastic pathway (the inner side of the plasma membrane), or the apoplastic pathway (outer side of the plasma membrane) or a combination of the two (Taiz and Zeiger, 1998). Turfgrasses contain 75 to 85% water by weight (Beard, 1966) and begin to wilt with a 10% decrease in water content (Beard, 1973). According to Beard (1973), turfgrasses survive drought stress by escape, dormancy, increasing water absorption through extensive rooting, increasing stomatal resistance, or physiological changes to avoid dehydration. These mechanisms mentioned above fall into the three major components of drought resistance mentioned earlier in the literature review. Drought avoidance and tolerance mechanisms are essential for turfgrasses because the maintenance of green cover is the most desirable trait (Beard, 1989).

Warm-season turfgrasses have superior drought survival and enhanced osmotic adjustments when compared to cool-season grass (Qian and Fry, 1997). However, tall fescue (*Festuca arundinacea* Schreb.), a cool-season grass, is better able to avoid drought through the extensive rooting system when compared to other cool-season turfgrasses such as perennial ryegrass or Kentucky bluegrass (Sheffer et al., 1987; Qian and Fry, 1996a).

Drought resistance research in turfgrasses has been conducted by withholding irrigation or excluding rainfall from field plots or in controlled environmental conditions

(Colmer and Barton, 2017). Whole-plant responses of turfgrasses to drought stress are useful in identifying drought resistant species and cultivars (Kopp and Jiang, 2013). Rainout shelters have proven to be a successful means to identify superior turfgrass cultivars under drought stress in the field by protecting the plots from undesired precipitation during the drought period (Colmer and Barton, 2017). Dry-down experiments have also been conducted in pots or deeper lysimeters under controlled environment conditions. Leaf firing (LF), visual turf quality (TQ), evapotranspiration (ET) rate, and ability to recover after drought stress are general criteria for evaluating drought resistance in turfgrass research (Beard, 1989; Beard and Sifers, 1997; Carrow, 1996). Leaf firing manifests in the form of leaf chlorosis starting at the leaf tips and margins and progressing down the leaf, turning it into a brown color, indicating the death of the leaf (Carrow, 1996). Photosynthetic, stomatal conductance, osmotic adjustments, leaf water potential, EL, and altered antioxidant are some of the physiological and biochemical responses evaluated during drought stress in turfgrasses (Kopp and Jiang, 2013). Some of the alternative techniques for assessing drought responses are digital image analysis (DIA) and the normalized difference vegetation index (NDVI). In the recent years, NDVI has been used to measure the response of turfgrasses to drought stress due to its strong correlation with visual ratings such as LF and TQ (Sonmez et al., 2008, Poudel, 2015).

Drought resistance in cool-season turfgrasses

Richardson et al. (2009) conducted a study to assess the drought resistance of nine Kentucky bluegrass and 18 hybrid bluegrass (HBG) cultivars (*P. pratensis* × *P. arachniferaa*, *P. pratensis* × *P. angustifolia*, *P. pratensis* × *P. nemoralis*, and *P. pratensis*

× *P. densa*) in the field. The drought response was evaluated weekly using DIA (Karcher and Richardson, 2013) until all plots reached 25% green turf cover, and then the drought recovery was initiated. The commercially-available Kentucky bluegrass cultivars, 'Mallard,' 'Bluestone,' and 'Arrow' were the top-performing cultivars in both the years by taking a longer time to reach 50% green turf cover during the drought stress. Among the HBG cultivars, most of the *P. pratensis* × *P. arachnifera* hybrids did not perform well in both years. However, one hybrid between P. *arachnifera* and P. *angustifolia* (103-509) did have excellent drought resistance performance, suggesting that improvements in drought resistance can be made via hybridization. The days to reach 50% green cover in all entries ranged from 4.4 to 10.9 days in 2006 and from 4.2 to 31.1 days in 2007. Cultivars that had the best drought resistance during dry-down were also the quickest to recover following drought in this study.

A similar study was conducted by Goldsby et al. (2015) to evaluate percent green coverage, using DIA, of 28 entries of Kentucky bluegrass and two HBG when subjected to prolonged drought and recovery in the field under an automated rainout shelter. By the end of the 82-day dry-down period in 2010, entries reached a green cover percentage of 0 to 3%. In 2011, the entries reached 7 to 27% green cover at the end of the 62-day drought period. The entry 'Apollo' was the top-performing cultivar, which was quick to recover and showed a slower decrease in green cover percentage. The slow recovery of all entries in the 82-day, when compared to the 62-day dry-down period, indicated that the duration of drought stress had a significant impact on its speed of recovery. The slow recovery in 2010 led to a faster decline in green percentage during the drought stress in 2011 for the cultivars 'Bartitia,' 'Nu Destiny,' 'Park,' 'Kenblue,' and 'Envicta,' A study was conducted to evaluate the performance differences between a drought resistant ('Mallard') and drought sensitive ('Snap') Kentucky bluegrass cultivars when subjected to variable amounts of deficit irrigation (Sandor et al., 2019). A 90- day deficit-irrigation treatments that replaced either 100, 80, 60, 50, or 40% of crop evapotranspiration estimate (ET_c) was implemented. The percentage of green turf coverage was measured using DIA. Mallard and Snap maintained a green percentage above 65% and 30%, respectively, from June to September in both years at 100% ET_c. Snap consistently had a lower green cover percent than Mallard at all irrigation treatments. Acceptable green turf coverage was observed for Mallard irrigated at 60% $ET_{c,}$ indicating that a drought resistant cultivar such as Mallard could maintain acceptable turf coverage when irrigated below 100% ETc.

McCann et al. (2008) compared the drought resistance of six creeping bentgrass cultivars (Penn A-4,' 'Independence,' 'Declaration,' 'L-93,' 'Penncross,' and 'Putterand') and determined the drought tolerance and avoidance characteristics of these cultivars. The top-performing cultivars (Penn A-4,' Independence, and L-93) had higher water use efficiency, root viability, root elongation, or root production.

The root anatomical, physiological, and morphological responses of two tall fescue cultivars 'Kentucky-31' (forage-type), and 'MIC18' (dwarf, turf-type) were examined under well-watered and drought conditions (Huang and Fry, 1998). The worst performing cultivar was MIC18, which also had the shortest root length, lowest root dry weight, smallest root to shoot ratio, and highest electrolyte leakage when compared to Kentucky-31. More extensive root hairs were observed in Kentucky-31, and MIC18 exposed to drought stress. They reported that the increase in root hair development under moderate stress might be an adaptive strategy in tall fescue cultivars to obtain water by increasing the contact area between the root and the surrounding soil.

Seven tall fescue genotypes were subjected to 12 days of drought stress under controlled environment conditions (Sun et al., 2013). Drought-resistant genotypes had lower root EL in upper and lower soil profiles than drought sensitive genotypes during drought stress. Root EL and root water content were positively and negatively correlated, respectively, to canopy temperature differential (canopy temperature minus air temperature (CTD). Higher root water content and viability (decreased root EL) enabled plant roots to effectively extract water from drying soil to sustain stomatal opening and transpiration as well as photosynthesis, thus increasing net photosynthesis and stomatal conductance and lowering canopy temperature. The study concluded that cultivars with higher stomatal aperture, root length, root weight, and root water content, and lesser EL, and lower CTD were able to maintain higher TQ during drought stress.

Drought resistance in warm-season turfgrasses

Carrow (1996) used leaf firing data to describe the relative drought resistance of seven of the most commonly used turfgrasses in the humid region of Southeastern USA as being “very high” (‘Tifway’ and common bermudagrass); “high” (‘Raleigh’ St. Augustinegrass, common centipedegrass); “medium-high” (‘Rebel H’ tall fescue); “medium” (‘Kentucky-31’ tall fescue), and “medium-low” (‘Meyer’ zoysiagrass (*Zoysia japonica* Steud)).

Huang et al. (1997a) conducted a study to understand the shoot response of seven warm-season turfgrasses (common bermudagrass, centipedegrass, seashore paspalum), and zoysiagrass) to surface soil drying. The 60 cm long pots were divided into sections, and four treatments were employed 1) well-watered, 2) upper 20-cm soil drying, 3) upper 40 cm soil drying, 4) rewatered. ‘Emerald’ zoysiagrass was described as the worst performing turfgrass in the upper 0-20 cm drying due to reduced shoot growth, chlorophyll content, relative water content, and increase canopy temperature when compared to well-watered control. When the upper 40 cm soil dried, Emerald zoysiagrass and common bermudagrass were most affected, and ‘TifBlair’ and ‘PI 509018’ were least affected by drought stress. The superior drought resistance of PI 509018 and TifBlair was attributed to increased root growth and rapid root water uptake at deeper soil layers, lesser root EL, and rapid root regeneration after rewatering (Huang et al., 1997b). The ranking of relative drought resistance in the 0- to 40-cm drying regime was: Paspalum PI 509018 = TifBlair centipedegrass > ‘AP14’ = ‘PI 299042’ > ‘Adalaydm’> Common bermudagrass =Emerald zoysiagrass. The multiple regression analysis conducted by Huang et al. (1997b) indicated that reduction in shoot growth was associated more with root viability than to root length, suggesting that root viability is an important factor in determining drought resistance.

The LF and canopy temperatures of three species (bermudagrass, St. Augustinegrass, and zoysiagrass), when subjected to a 60-day drought, were measured under a rainout shelter (Steinke et al., 2009). Canopy temperature was measured every 7 to 13 days. The overall LF severity in both the years was bermudagrass < St. Augustinegrass < zoysiagrass. The highest canopy temperature and fastest and greatest LF in zoysiagrass were attributed to the dense growth habit, which resisted the air movement through the turf canopy and could have created a dormant boundary layer above the zoysiagrass canopy. A strong inverse relationship for correlation coefficients between LF and canopy temperatures indicated that canopy temperatures would begin to increase at the onset of LF. Canopy temperature differential was found to be higher in drought susceptible and lower in drought resistant bermudagrass ecotypes from Australia (Zhou et al., 2014).

Severmutlu et al. (2011) conducted a study to evaluate the drought resistance of eight bermudagrass, two zoysiagrass, five buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.**)** cultivars, and one cultivar each of tall fescue, bahiagrass (*Paspalum notatum* Flugge), centipedegrass, and seashore paspalum. The study was conducted at two sites (sandy soil or silty clay loam) in Turkey by imposing 90-day drought stress and 60-day recovery period. The grasses in sandy soil experienced a faster decline in quality and an increase in LF. The percentage of LF for all warm-season grasses was below 20% at 15 days after drought stress at the site with silty clay loam soil. The top-performing species were bermudagrass, bahiagrass, and buffalograss. The worst performing species were centipedegrass and zoysiagrass because of a high rate of LF. Since this study was conducted in an unrestricted soil profile, the cultivars that showed superior drought performance could be due to drought avoidance mechanism. The order of species for shoot recovery from high to low in this study was bermudagrass = buffalograss ≥ bahiagrass ≥ seashore paspalum > centipedegrass > zoysiagrass > tall fescue.

The transpiration response, canopy wilting, LF, and spectral reflectance response of three warm-season turfgrasses, zoysiagrass, bahiagrass and St. Augustinesgrass to gradually drying soil was measured in controlled condition (Cathey et al., 2011). The gradual drying treatments were based on the normalized transpiration ratio (NTR), which is the transpiration ratio of drying to well-watered plants. The study consisted of five treatments (well-watered, water stress to a 0.3 NTR, water stress to a 0.1 NTR, and water stress at 0.1 NTR for three days, and water stress at 0.1 NTR for seven days). Zoysiagrass was not severely wilted until 0.1 NTR and showed only slight firing even when the drought stress was prolonged to seven days at 0.1 NTR. Zoysiagrass had a lower water use rate than the other grasses and had quicker recovery potential after a period of severe drought. The authors stated that the drought stress in this study was imposed on a restricted root zone (31 cm deep pots), which may not be the case in the field because zoysiagrasses tend to have the shallowest root zone than the other two grasses in this study. Therefore, the delayed LF of zoysiagrass was associated with the extra time used by these grasses to reach the stress thresholds, due to lower water use rate. This extra time would have allowed additional time for these grasses to acclimate to drought stress. The delayed LF in zoysiagrass could also be due to osmotic adjustments, which was previously reported in zoysiagrasses (Qian and Fry, 1997).

Turfgrasses can accumulate solutes in response to drought stress (Beard, 1973). Osmotic adjustment is a result of metabolic processes initiated by stress, which generates a more negative leaf water potential to facilitate water movement into the leaf to increase leaf turgor (Kopp and Jiang, 2013). A positive correlation between osmotic adjustment during drought stress and recovery was reported in turfgrasses grown in 27 cm deep pots (Qian and Fry, 1997). The osmotic adjustment helped in maintaining turgor and photosynthesis during the stress and also aided in faster recovery. The osmotic adjustment was in the order ’Prairie’ buffalograss > Meyer zoysiagrass > ‘Midlawn’ bermudagrass > ‘Mustang’ tall rescue. The higher osmotic adjustment and a faster recovery in Meyer despite shallow rooting (Carrow, 1996) suggest that it may possess the drought tolerance mechanism. The inverse seems to be true in the case of tall fescue as it has shown to have deeper rooting but lower osmotic adjustment.

Poudel (2015) performed four separate studies in the greenhouse to evaluate the drought response of 13 bermudagrass, 13 zoysiagrass, 12 St. Augustinegrass, and seven seashore paspalum genotypes in 45 cm long pots. No grasses survived beyond 28 days of drought stress suggesting that none of the cultivars were able to avoid drought by extending the roots due to the limited soil depth. At seven days after drought stress, the mean TQ of all bermudagrass, seashore paspalum, zoysiagrass and, St. Augustinegrass genotypes ranged from 5.2 – 6.8, 5.8-4.7, 5.2-3.8, and 6.5-5, respectively. The experimental genotypes ‘OKC 1302’ (bermudagrass) and ‘UGP 10’ (seashore paspalum) performed better than the rest of the entries in each species. Even though not quantified in this study, the superior performance of these genotypes was attributed to the drought tolerance mechanism as these genotypes were grown in shallow pots. None of the experimental genotypes of zoysiagrass and St. Augustinegrass performed better than their respective commercial cultivars. The parameter, TQ, LF, NDVI, and DIA, were highly correlated to each other.

Past Drought Research in Bermudagrass

Previous studies have documented the superior drought tolerance and avoidance of bermudagrasses when compared to other warm-season turfgrass species such as zoysiagrass, St. Augustinegrass and centipedegrass (Carrow, 1995, 1996; Qian and Fry, 1997). Although bermudagrass are adapted to various soil and climatic conditions and considered to be relatively a drought resistant (Beard and Sifers, 1997), to maintain acceptable growth and TQ, adequate soil moisture is required (Taliaferro, 2003). Intra and interspecific variation in drought resistance have been reported in the past for bermudagrasses tested in the field and controlled environment. The superior drought performing bermudagrass cultivars maintained higher canopy temperature, soil moisture content, and leaf relative water content in the earlier stage of the dry-down period in 40 cm deep PVC pots (Zhou et al., 2013a).

Field evaluation of drought stress response of 15 bermudagrass cultivars maintained under a fixed poly covered rain-out shelter revealed that even the least drought resistant cultivars showed no sign of wilting until 45 days of drought stress (Richardson et al., 2010). Similarly, initiation of LF in bermudagrass happened later in the dry-down period in bermudagrass compared with the other warm-season species St. Augustinegrass, and zoysiagrass, and manillagrass (*Zoysia matrella* (L.) Merr.) when grown in 90 cm long acrylic pots (Zhang et al., 2017). The NTR, gas exchange rate, and LF responses of these turfgrass species to the fraction of transpirable soil water (FTSW) were characterized through threshold and midpoint. The threshold was defined as the FTSW value at which the response variables started to decline, and midpoint was defined as the FTSW value at which the response variables declined to 50% of their original values. Bermudagrasses demonstrated the lowest threshold and higher midpoint values for LF, indicating that the initiation of LF occurred later but with a fast progression. The lower threshold for LF in bermudagrass was attributed to a lower gas exchange rate threshold and midpoint, which helped in maintaining carbon assimilation through photosynthesis during drought stress. The carbohydrates that are produced through the assimilation of carbon provide energy and carbon precursors for plant growth and metabolic processes, thus delaying LF (Huang et al., 2014).

Etemadi et al. (2005) reported that drought resistance had a significant relationship with total root length and no significant correlation with root diameter and proline content in common bermudagrass accessions collected from Iran. However, Zhou et al. (2014) suggested that drought resistance of bermudagrass accessions had no relationship with root length density measured before or after drought, or average root diameter (ARD) before drought; instead, it was associated with ARD after the drought. Also, higher rhizome production was associated with drought resistance. Rhizomes could serve as a storage organ and supply the roots with additional carbohydrates nutrients or water to keep higher root turgor and activity (Suzuki and Stuefer, 1999)

Baldwin et al. (2006) conducted a 30-day study in the greenhouse to evaluate the drought response of six bermudagrass cultivars grown in 45 cm lysimeters by implementing four irrigation treatments (5-, 10-, 15-day interval, and control). All the cultivars showed a TQ less than 7 by week 4 for the 5-day irrigation interval and by week 1 for the 10- and 15-day intervals. Celebration and ‘Aussie green’ were the top-performing cultivars for all three irrigation treatments. The root weight of Celebration was the highest when the root weights of all the irrigation treatments were pooled together. Higher root weight was reported for all cultivars for the 15-day interval due to the increase in root length in the downward direction of the soil profile in search of water. Celebration has shown the highest resistance to drought by resisting LF during dry-down and has recovered more rapidly after the drought when grown in a 76-cm PVC pipe

(Thapa, 2011). In the same study, ‘Premier’ and Latitude 36 had earlier LF and loss of live cover, indicating low drought resistance.

The physiological changes in three bermudagrass cultivars, grown in 45 cm pots, under well-watered and drought stress (by withholding irrigation) conditions was evaluated in a controlled environment (Su et al., 2013). The cultivars used in this study were the drought resistant cultivar, Celebration, a drought sensitive cultivar, Premier, and Latitude 36. Latitude 36 had the highest TQ among three cultivars in both well-watered and drought stress conditions. The EL of Latitude 36 was lower than Premier, indicating higher drought resistance than Premier. However, Celebration demonstrated superior drought resistance when compared to Latitude 36 and Premier (Thapa, 2011). This contradiction could be due to the difference in the depth of the pots used in these studies.

A recent field study conducted under an automated rainout shelter evaluated the drought performance of three bermudagrass cultivars (Tifway, Celebration, and TifTuf) and three seashore paspalum cultivars (‘SeaIsle I’, ‘SeaStar,’ and ‘UGA1743’) (Jespersen et al., 2019). TifTuf was consistently the top-performing cultivar with the highest TQ, NDVI, leaf water content and solute accumulation, lowest EL, and canopy temperature. SeaStar was the worst performing cultivar with other cultivars generally in between these extremes. However, TifTuf had a relatively high ET rate when compared to Tifway, Premier, Latitude 36, and NorthBridge (Amgain et al., 2018). However, TifTuf can avoid drought stress through its rooting characteristics (Yurisic, 2016). TifTuf maintained higher TQ and root mass than Latitude 36 and Tifway at 8 to 20 days after withholding water when grown in 45 cm pots (Yurisic, 2016). Steinke et al. (2011) conducted a study to evaluate the drought response of bermudagrasses in restricted 10 cm soil profile and unrestricted native soil. No bermudagrass cultivars survived the 60-day drought and showed 100% LF within 20 days of imposing drought stress when planted on the restricted 10-cm soil. When subjected to 60-day drought stress in the field, Celebration and Premier bermudagrasses were the top and worst performing cultivar, respectively. Tifway bermudagrass known for its drought resistance (Carrow, 1996) failed to show acceptable TQ at the end of the recovery period after a week-long drought stress when grown in the 10 cm sandy soil root zone (Jiménez et al., 2019) This inconsistency indicates the need to evaluate the drought response of bermudagrass cultivars when grown in different soil depths or soil type.

Rationale, Goals and Objectives of Projects

Chapter 2 and 3

The large transitional zone in the central parts of the United States is a region where it is challenging to maintain turfgrass because it is too cold in the winter for warm-season turfgrasses and too warm in summer for cool-season species. Many golf courses in the transition zone are converting their creeping bentgrass putting greens to bermudagrass putting greens. The bermudagrass putting greens would help golf course superintendents to focus more on improving the playability of the green rather than on its survival during summer months, which usually coincides with the peak season of play (Hartwiger, 2009). Managing bermudagrass putting greens can be less expensive than bentgrass as fewer fans, and fungicides, and hand watering are required (Hartwiger, 2009). However, one of the greatest concerns for the use of bermudagrass in the putting green in transition zone is winterkill (Goatley et al., 2007). Previous studies have reported a narrow genetic diversity among the existing putting green bermudagrass cultivars (Wang et al., 2010), which emphasizes the need for new greens type bermudagrass genotypes with improved freeze tolerance and genetic diversity. Additionally, turf loss from winterkill in fairways or athletic fields has a significant economic impact by affecting the aesthetics and playability. Eventually, requiring labor and energy-intensive re-establishment procedures to restore lost areas of turf. The breeding program at OSU has been actively engaged in bermudagrass breeding since the mid-1980s (Taliaferro et al., 2004). One of the goals of the breeding program is to develop high quality bermudagrasses with enhanced freeze tolerance (Taliaferro et al., 2004). While field evaluations can provide plant breeders with the most accurate assessment of winter survivability, environmental conditions are often unpredictable and difficult to reproduce (Anderson and Taliaferro 2002). Therefore, laboratory-based experiments can be a reliable method to evaluate freeze tolerance.

Goal

To identify bermudagrass genotypes with superior freeze tolerance.

Objectives

1. To determine the LT_{50} value of commercially available and experimental putting green bermudagrass genotypes for freeze tolerance by subjecting them to 11 target freezing temperatures (-4 to -14 ^{o}C) under controlled environment conditions.
2. To determine the LT_{50} value of commercially available and experimental bermudagrass genotypes for freeze tolerance by subjecting them to 11 target freezing temperatures (-4 to -14 ^{o}C) under controlled environment conditions.

Hypotheses

1. There will be differences in freeze tolerance among bermudagrass genotypes.
2. The OSU experimental genotypes would have an improved freeze tolerance than industry standards Champion (for chapter 2) and Tifway (for chapter 3).

Chapter 4

Lawns and turfgrass areas represent the largest irrigated crop in the United States, accounting for approximately 163,800 km^2 (±35,850 km^2) (Milesi et al., 2005). The degradation of existing water resources and variations in rainfall patterns are straining water resources needed for a growing population. This restricts the availability of irrigation water to the turfgrass industry, emphasizing the need for drought resistant cultivars. Bermudagrass is one of the most widely used warm-season turfgrasses. Bermudagrasses made up almost 93% of all warm-season turfgrasses in 2015, and with 34% of all maintained turf acreage in the US in 2015 (Gelernter et al., 2017). Therefore, developing bermudagrass genotypes with improved drought resistance is a priority for any bermudagrass breeding program. The turf breeders, extension specialists, and researchers of the Southern US are collaborating to develop new turfgrass genotypes with improved drought and salinity tolerance using grant funding from the USDA Specialty Crop Research Initiative (SCRI). Experimental bermudagrass genotypes developed by the breeding program at UGA and OSU, which after initial screening are tested at multiple locations for multiple years. These trials will provide an opportunity to expose these new experimental genotypes to diverse environmental conditions of Southern US and identify genotypes with wider geographical adaptation. The information gained from these multi-location testing will help turfgrass developers formulate a decision as to whether the experimental bermudagrass lines tested should be further pursued for possible commercial release.

However, most research in the past have focused on screening bermudagrass cultivars for drought resistance under one soil depth. Urban environments often encounter rooting restriction due to soil compaction, shallow bedrock, etc. (Steinke et al., 2011), which would require turfgrasses to be tested at multiple soil depths to evaluate the overall drought resistance of a particular genotype.

Goal

To identify top-performing bermudagrass genotype at unrestricted and restricted soil depths when subjected to simulated drought conditions.

Objective

1. To assess the drought response of four commercially available and six experimental genotypes of bermudagrasses when subjected to drought stress when grown under a rainout shelter with unrestricted soil depth in the field.
2. To determine the drought response of these ten bermudagrasses when subjected to drought stress when grown on 17-cm (6.7 inches) deep pots with restricted rooting in a controlled environment.

Hypotheses

1. There would be differences in the drought response among bermudagrass genotypes.
2. The genotypes would perform differently under the two soil depths due to the differences in drought resistance mechanism.

Chapter II of this dissertation will be submitted for publication in HortScience, affiliated with the American Society of Horticultural Science. Chapter III will be submitted to the Agrosystems, Geosciences & Environment Journal. Chapter IV will be submitted for publication in Crop Science, the official publication of the Crop Science Society of America.

CHAPTER II

QUANTIFYING FREEZE TOLERANCE OF PUTTING GREEN TYPE BERMUDAGRASS GENOTYPES

Abstract

Bermudagrasses (*Cynodon* spp) are some of the most important, widely adapted warm-season turfgrasses and are commonly used in golf course greens in the transition zone. The susceptibility of bermudagrasses to winter injury in the transition zone is a major concern. Therefore, the objective of the study was to evaluate five putting green type experimental genotypes (OKC6318, OKC0805, OKC1609, OKC0920, and OKC3920) and three industry standards ('Champion Dwarf,' 'TifEagle,' and 'Tahoma 31^{TM}') for freeze tolerance by subjecting them to 11 freezing temperatures (-4 to -14 ºC) under controlled environment conditions. The experiment was conducted in batches, with four genotypes (two standards and two experimental genotypes) per batch, and each batch was replicated in time. The mean lethal temperature to kill 50% of the population (LT_{50}) for each genotype was determined. The freeze tolerance varied among the bermudagrass genotypes. Champion Dwarf had an LT_{50} value ranging from -5.2°C to

-5.9°C across all three batches. The experimental genotypes tested in this study had LT_{50} values ranging from -7.0 to -8.1°C and were each lower than that of Champion Dwarf. Tahoma 31, the top performing genotype, had an LT_{50} value ranging from -7.8°C to -9.0°C across all three batches. OKC 3920 (-8.1°C) was the only experimental genotype to have an LT_{50} value in the same statistical group as Tahoma 31. The information gained from this research provides an accurate estimation of the freeze tolerance of these grasses. The results will help the plant breeder to make a decision on whether the experimental bermudagrass genotypes tested should be subjected to further testing.

Introduction

Low temperature stress is divided into chilling and freezing stresses. Chilling stress is defined as the temperature below the optimum temperature that affects a plant's activity (Levitt, 1980). It is difficult to establish a specific temperature below which chilling stress occurs as different plants have different temperature ranges for this stress (Levitt, 1980). However, freezing stress occurs when the temperature drops below zero. Freezing temperatures cause the formation of ice crystals inside the cell leading to its rupture. The survival of turfgrasses during freezing temperatures depends on the magnitude of the injury within the crown (Beard, 1973). Turfgrass freezing occurs at 0° C (32 °F), and colder temperatures and the injury caused are due to the formation of ice crystals, especially in the crown, which is the regenerative regions of the plant (Beard, 1973). Maintaining the integrity of the turfgrass crown is essential for freezing stress survival since leaf, root, and lateral shoot regeneration occur from the turfgrass crown (Beard, 1973).

Warm-season turfgrasses are more susceptible to winter injury than cool-season species. Bermudagrasses (Cynodon spp) are some of the most important and widely adapted warm-season turfgrasses. They tolerate the hot and humid summers of the transitional climatic region of the United States. Many golf courses in this region are converting their putting greens from creeping bentgrass to bermudagrass. This shift is mainly because managing bermudagrass putting greens can be less expensive than bentgrass as fewer fans, fungicides, and hand watering are required during summer (Hartwiger, 2009). The bermudagrass putting greens would help golf course superintendents to focus more on improving the playability of the green rather than on its survival during summer months, which usually coincides with the peak season of play (Hartwiger, 2009). However, one of the greatest concerns of using bermudagrass in the transition zone is winterkill. Winterkill is a general term used to define turf loss during the winter (Beard, 1973). Protective covers are used to reduce winterkill of bermudagrass putting greens, but there are significant labor costs associated with covering and uncovering greens (White, 2011). Also, previous studies have reported a narrow genetic diversity among the existing putting green bermudagrass cultivars, which could be a reason for their susceptibility to prevalent diseases (Wang et al., 2010). Therefore, there is a need for new greens type bermudagrass genotypes/cultivars with improved freeze tolerance and broader genetic diversity. The breeding program at Oklahoma State University (OSU) has been actively engaged in bermudagrass breeding since the mid-1980s (Taliaferro et al., 2004). One of the goals of the breeding program is to develop high quality bermudagrasses with enhanced freeze tolerance (Taliaferro et al., 2004).

The freeze tolerance of turfgrasses has been measured in the past using the percentage of winterkill post spring green-up (Anderson et al., 1988). Such field evaluation methods consume time and sometimes provide inconsistent results due to year over year temperature fluctuations. Therefore, the artificial freezing test with a controlled rate of cooling has been successfully conducted in the past in a controlled environment to evaluate the freeze tolerance of turfgrasses. Most of the artificial freezing studies determine the lethal temperatures to kill 50% of the population (LT_{50}) based on the whole-plant regrowth following exposure to freezing temperatures (Anderson et al.,1993, 2002; Patton and Reicher, 2007). Thus, the objective of this study was to determine the LT_{50} values of experimental and commercially available, putting green bermudagrass genotypes by subjecting them to 11 freezing temperatures ranging from -4 to -14°C, under controlled environment conditions.

Materials and Methods

The experiment was conducted at the Controlled Environment Research Laboratory (CERL) at Oklahoma State University, Stillwater, OK. The freezing protocol was performed in accordance with previously conducted studies with slight modifications (Anderson et al., 1993 and 2002; Patton and Reicher, 2007). The study was conducted in three batches due to space constraints in the plant growth chamber and in the freeze chamber. Each batch consisted of four genotypes (two standards and two experimental genotypes), and each batch was repeated three times. The first batch was established in June 2018 and tested in October-November 2018 on three different dates. This batch comprised of vegetatively propagated putting green bermudagrasses genotypes, OKC6318, 'Tahoma 31^{TM}', 'Champion Dwarf,' and 'TifEagle.' Champion Dwarf (hereafter stated as Champion) and TifEagle served as the freeze susceptible and freeze tolerant standards, respectively, for this experiment based on the study conducted by Anderson et al. (2002). The second batch was established in January 2019 and was tested in June 2019. This batch consisted of OKC0805, OKC1609, Tahoma 31, and Champion. The third batch was established in March 2019 and was tested in September 2019. The third batch comprised of OKC3920, OKC0920, Tahoma 31, and Champion. Bermudagrasses were propagated in Berger BM 3 germination mix potting media in cone-tainers (RayLeach Cone-tainer Nursery, Canby, OR), which were 21 cm in depth and 3.8 cm in diameter.

Plants were grown in a plant growth chamber (PGC Flex Growth Chamber, Conviron, Winnipeg, Canada) at 32/28°C day/night temperatures for 13 weeks with a photoperiod of 14 hours and a light intensity of 900 µmol m^{-2} s^{-1}. Plants were fertilized with a soluble fertilizer (Peters 20N-10P-20K) at 0.6 g L^{-1} and trimmed with scissors as needed, to maintain a height of 1.2 cm. During the establishment phase, the cone-tainers were treated with Bifenthrin (Talstar insecticide, FMC Corporation Agricultural Products Group, Philadelphia, PA) every 14 days at labeled rates as a precautionary measure. Following 13 weeks of establishment, the temperature inside the plant growth chamber was reduced to 24/20°C for one week. Plants were then subjected to cold-acclimation by lowering the temperature to 8/2°C day/night for four weeks with a photoperiod of 10 hours and a light intensity of 400 µmol m^{-2} s^{-1}. During the final week of cold acclimation, each cone-tainer was wrapped with a thin layer of paper napkin to serve as a physical barrier between the plastic cone-tainer and the plastic trays. The paper napkin facilitated the process of removing the cone-tainers from the trays as plastics tend to contract at low temperatures making it difficult to remove the cone-tainers at target temperatures. At the end of four weeks of cold acclimation, the plants were transferred to a freeze chamber (E8, Plant growth chamber, Conviron, Winnipeg, Canada) maintained at 1°C. During the final week of acclimation, each cone-tainer was hand-watered to maintain uniform moisture content. Watering was stopped two days prior to loading samples into the freeze chamber. Once the samples were loaded into the freeze chamber, ten thermocouple sensors were inserted into random cone-tainers to monitor the soil temperature. Crushed ice was placed on top of each cone-tainers to prevent supercooling of the plants and initiate ice formation. Then the temperature inside the freeze chamber was gradually reduced to -3°C. The chamber temperature was held at -3°C for 18 hours for latent heat to dissipate from the soil. The freeze chamber was then programmed to cool linearly at the rate of 1°C per hour. Four cone-tainers were removed for each genotype at target temperatures ranging from -4 ºC to -14°C which is the anticipated span from complete survival to complete mortality. The removed cone-tainers were immediately transferred to a plant growth chamber set at 4 °C overnight to induce thawing. Following thawing, the temperature in the plant growth chamber was increased to 24/20°C for a week and then to 32/28°C to encourage recovery.

The regrowth of the plants was visually evaluated after five weeks using binary values; 1= alive, 0 = dead. The LT_{50} value of each genotype was determined using a logistic regression model using the PROC PROBIT procedure (SAS Institute, Cary, NC) (Qian et al., 2001; Shahba et al., 2003). The probit procedure generated a table of predicted percentage survival at each temperature, and the temperature corresponding to 50% survival was used as the estimates of LT_{50} for each genotype. The Pearson's chi-square and the likelihood ratio chi-square (or deviance) were insignificant due to high p values for all the models generated, thus failing to reject the null hypothesis that the model fits. Temperature parameter had a significant Type III effects according the Wald χ^2 test (Appendix, Tables 32, 33, and 34). The freeze test was repeated three times for each batch, thus generating three LT_{50} values for each genotype. The LT_{50} value of each replication was treated as a response variable and was subjected to the analysis of variance (ANOVA) procedure in SAS (SAS Institute, Cary, NC). Means were separated using Fisher's protected least significant difference when F tests were significant at $p \leq 0.05$.

Results and Discussion

The ANOVA results indicated that there were significant differences in the LT_{50} values among the genotypes in all three batches (Table 1). The first batch of genotypes consisting of Champion, TifEagle, OKC6318, and Tahoma 31 ranged in freeze tolerance from -5.9 to -7.8°C (Table 2). Champion being the freeze sensitive standard, had the highest LT_{50} value. This LT_{50} value was slightly lower than the LT_{50} value obtained by Anderson et al. (2002) (-4.8°C). TifEagle, the freeze tolerant standard, had an LT_{50} value, which reflects those previously reported by Anderson et al. (2002) (-6.0°C). Although the LT_{50} value of TifEagle was numerically lower than Champion in all the three runs, it was not significantly different from Champion. TifEagle and Champion have previously shown similar responses with the least winterkill of 2.6 and 2.0%, respectively, and were in the same statistical group in a field study (Liu, 2014). The low percentage of winterkill in Liu (2014) could be due to a higher average air and soil temperature in the winter of 2013 or due to the thermal buffering capacity of the soil. However, DeBoer et al. (2019)

reported that Champion consistently had lower green turf coverage compared to TifEagle for all the four covering treatments placed on the greens at four low-temperature thresholds (-9.4°C (15°F), -7.8°C (18°F), -5.6°C (22°F), and -3.9°C (25°F)) suggesting that Champion is more susceptible to low-temperature injury than TifEagle. The experimental genotype OKC6318 had an LT_{50} value of -7.0°C and was in the same statistical group as TifEagle but was significantly different from Champion. The similarity coefficient assessed with simple sequence repeat markers by Wang et al. (2010) and Fang et al. (2017) revealed that the existing industry standards used for putting greens such as Champion, 'Mini Verde', TifEagle, and 'Tifdwarf' formed one group with a genetic similarity coefficient of 1.00. However, the 15 experimental genotypes, including OKC6318, had similarity coefficients ranging from 0.64 to 0.93, indicating that these experimental genotypes were genetically distinct from the commercial standards (Fang et al., 2017).

Tahoma 31 was the top-performing genotype in this batch with the lowest LT_{50} value, which was significantly different from the other three genotypes tested. The superior performance of Tahoma 31 was in accordance with the field observation. Tahoma 31 had the least winterkill rating of 14.5% when averaged across two locations (Indiana and Kentucky) and exhibited superior post-dormancy regrowth in the field (NTEP, 2014). Tahoma 31 has also shown quick post-dormancy regrowth after exposure to prolonged chilling stress (as 8/2 °C (day/night)) under a controlled environment, indicating its superior recovery potential when subjected to low temperatures (Fontanier et al., 2020).

Due to the lower LT_{50} value of Tahoma 31 compared to TifEagle's, the former was used as the freeze tolerant standard for subsequent batches. The LT_{50} values of the second batch consisting of Champion, Tahoma 31, OKC0805, and OKC1609 ranged from -5.7 to -9.0°C (Table 3). Similar to the first batch, Champion and Tahoma 31 had the highest and lowest LT_{50} values respectively, and these were significantly different from the other genotypes tested in the second batch. The experimental genotypes OKC0805 and OKC1609 had LT_{50} values of -7.5°C and -7.9°C, respectively, and were in the same statistical group. The LT_{50} values of the experimental genotypes tested in this study were numerically lower than the commercially available putting green bermudagrasses tested by Anderson et al. (2002).

The LT_{50} values of the third batch consisting of Champion, Tahoma 31, OKC0920, and OKC3920 were calculated (Table 4). The standards exhibited a similar trend with Champion (-5.2°C) and Tahoma 31 (-8.8°C) being the worst and top-performing genotypes, respectively. The experimental genotype OKC0920 had an LT_{50} value of -7.1°C and was significantly different from all genotypes in this batch. The genotype OKC3920 had an LT_{50} value of -8.1°C and was the only experimental genotype in the same statistical group as Tahoma 31 in all the three batches.

The existing ultradwarf bermudagrasses used in putting greens, if not all, are mutations from a single clonal plant, 'Tifgreen' bermudagrass (Harris-Shultz et al., 2010). The narrow genetic diversity of these bermudagrasses could make them susceptible to new or existing disease or insect pests leading to extensive damage (Taliaferro, 1995). The release of these experimental genotypes, which are genetically distinct, could substantially increase the genetic diversity of greens-type bermudagrass cultivars and could be less vulnerable to prevalent pests and diseases. Also, these cold hardy bermudagrass genotypes could help in expanding the geographical area where it could be grown successfully. Ultradwarf bermudagrasses are preferred choice for putting greens due to their ability to produce high quality turf with faster ball rolls at a low cutting height of 3.2 mm (1/8 inch) as compared to older dwarf cultivars (Beard and Sifers, 1996). Tahoma 31, the top-performing genotype in this study, could tolerate a mowing height of 3.8 mm and above and could produce a satisfactory putting green surface (Personal communication with Dr. Justin Quetone Moss). Utilizing these freeze tolerant bermudagrasses in the putting greens could help in reducing the current USGA recommended threshold air temperature of -4.0°C (25°F) (O'Brien and Hartwiger, 2013) for covering the greens. Lowering the threshold temperature without an increase in winter injury will result in fewer covering events, thus minimizing the labor cost involved. Also, golf courses that have cold-hardy bermudagrass greens benefit financially by maintaining a high quality putting green with lesser inputs during peak season, and by keeping the facility open for play for more days in the winter. There have been instances when rankings from field studies do not entirely match laboratory studies because of the thermal buffering capacity of the soils because crowns in the field are less exposed to cold air temperature (Anderson et al., 2002). Controlled environment studies will be a useful tool for breeders to rapidly screen genotypes for freeze tolerance. However, to increase the efficacy of selection and to understand the genotype x environment interaction, multiple-year and location evaluation is needed. Currently, these experimental genotypes are being tested for their mowing, disease, and pest tolerance in replicated field trials.

Conclusion

A total of five experimental genotypes were tested in three separate batches. Each of these experimental genotypes had LT_{50} values lower than Champion. TifEagle was not significantly different from Champion in this study. Tahoma 31 was the top-performing genotype with a mean LT_{50} value of -7.8°C and below in all three batches. This study is the first to test Tahoma 31 for freeze tolerance under controlled environment conditions. Tahoma 31 can be used as a freeze tolerant standard for future bermudagrass freeze tolerance studies due to its superior performance in the field and controlled environment. The experimental genotype OKC3920 was the only experimental genotype in the same statistical group as Tahoma 31. The experimental genotypes OKC3920, 0KC0805, and OKC0920 have been entered into the National Turfgrass Evaluation Program's 2019 - 2024 warm-season putting green trial for the multiyear and multi-location testing. These testing will provide an opportunity to expose these new experimental genotypes to diverse environmental conditions to identify genotypes with wider geographical adaptation. If found to have a promising turf quality, disease resistance, and low mowing tolerance, these OSU experimental genotypes could be pursued for possible commercial release.

Table 1. Analysis of variance for lethal temperature to kill 50% population of bermudagrass genotypes in batch 1, 2 and 3

Source	Batch 1		Batch 2		Batch 3	
	df	F value	df	F value	df	F value
Genotype (G)	3	20.1***	3	49.5***	3	39.2***

*, **, *** significant at *P* = 0.05, 0.01, and 0.001 respectively.

Table 2. Mean lethal temperature resulting in 50% survival (LT_{50}) of bermudagrass genotypes in first batch when exposed to temperatures ranging from -4 to -14°C under controlled environment conditions

Genotype[z]	LT_{50} (°C)[y]
Champion Dwarf	-5.9a[x]
TifEagle	-6.3a
OKC6318	-7.0b
Tahoma 31™	-7.8c
LSD (0.05)	0.62
CV	4.9%

[z] The first batch consisted of Champion, TifEagle, Tahoma 31, and OKC 6818 was established in June 2018 and tested in October-November 2018 on three different dates

[y] LT_{50} values were calculated using the PROC PROBIT procedure in SAS 9.4 version

[x] Fisher's protected LSD test: within columns, means followed by the same letter is not significantly different at the p=0.05 level

Table 3. Mean lethal temperature resulting in 50% survival (LT_{50}) of bermudagrass genotypes in second batch when exposed to temperatures ranging from -4 to -14°C under controlled environment conditions

Genotype[z]	LT_{50} (°C)[y]
Champion Dwarf	-5.7a[x]
OKC0805	-7.5b
OKC1609	-7.9b
Tahoma 31™	-9.0c
LSD (0.05)	0.64
CV	4.5%

[z] The second batch consisting of OKC0805, OKC1609, Tahoma 31, and Champion was established in January 2019 and was tested in June 2019.

[y] LT_{50} values were calculated using the PROC PROBIT procedure in SAS 9.4 version

[x] Fisher's protected LSD test: within columns, means followed by the same letter is not significantly different at the p=0.05 level

Table 4. Mean lethal temperature resulting in 50% survival (LT_{50}) of bermudagrass genotypes in third batch when exposed to temperatures ranging from -4 to -14°C under controlled environment conditions

Genotype [z]	LT_{50} (°C)[y]
Champion Dwarf	-5.2a[x]
OKC0905	-7.1b
OKC3920	-8.1c
Tahoma 31™	-8.8c
LSD (0.05)	0.82
CV	6.0%

[z] The third batch consisting of 'OKC3920', 'OKC0920', Tahoma 31and Champion was established in March 2019 and was tested in September 2019

[y] LT50 values were calculated using the PROC PROBIT procedure in SAS 9.4 version

[x] Fisher's protected LSD test: within columns, means followed by the same letter is not significantly different at the p=0.05 level

CHAPTER III

EVALUATING THE FREEZE TOLERANCE OF NEWLY DEVELOPED BERMUDAGRASS GENOTYPES

Abstract

Bermudagrasses (*Cynodon* spp) are commonly used in golf courses, athletic fields, and home lawns in the transitional climatic region of the United States. Cold hardiness is a major limiting factor for bermudagrasses grown in this region. Controlled environment based testing is a reliable method to evaluate freeze tolerance and can contribute to the selection of freeze tolerant genotypes. Therefore, this study aims at testing the freeze tolerance of two industry standards (‘Tifway’ and ‘Tahoma 31^{TM}’) and two experimental genotypes OKC1873 and OKC1406 by exposing these to various freeze temperatures (-4 to -14°C) in a controlled environment. The freezing test was repeated three times, and the mean lethal temperature to kill 50% of the population (LT_{50}) for each genotype was determined. Tifway (freeze sensitive standard) had an LT_{50} value of -7.0°C. The experimental genotype, OKC1873 (-7.2°C), was in the same statistical group as Tifway. Tahoma 31 was the best performing genotype with the lowest LT_{50} value

of -9.1℃. The genotype OKC1406 (-8.1℃) was in the same statistic group as Tahoma 31. The top performing experimental genotypes from such evaluations can be pursued for further field testing, and if found to have promising turf quality and disease resistance, these genotypes could potentially be released as commercial cultivars.

Introduction

Bermudagrasses (*Cynodon* spp) are some of the most important and widely adapted warm-season turfgrasses. They tolerate the hot and humid summers of the United States transition zone; however, they enter a dormancy period with the onset of winter. They are commonly used on home lawns, athletic fields, golf courses, and other utility turf areas. Bermudagrass exceed all other turf species in land coverage (196,634 ha) within the United States (Lyman et al., 2007). Bermudagrasses are regarded as having excellent tolerance to heat and drought, but low tolerance to freezing temperature (Beard, 1973). Intensively managed areas such as golf courses and athletic fields are predisposed to winter injury due to aggressive fertilization programs, low mowing heights, and vehicular and foot traffic (Richardson, 2002; Hartwiger and Moeller, 2015). Winterkill is a general term used to define turf loss during the winter (Beard, 1973). The effects of turf loss from winterkill on the aesthetics and playability of recreational turf areas by increased weed encroachment has a significant economic impact (Dipaola and Beard, 1992). Restoring the turf that was subjected to winter kill is labor intensive and expensive. Developing bermudagrasses with better winter survival is the priority of any bermudagrass germplasm improvement program. Oklahoma State University (OSU) has been actively engaged in bermudagrass breeding since the mid-1980s (Taliaferro et al., 2004). One of the major goals of the breeding program is to develop high quality bermudagrasses with enhanced freeze tolerance (Taliaferro et al., 2004).

Past research has reported significant intraspecific variation in cold hardiness in bermudagrass, indicating that genetic improvement for freeze tolerance could be achieved (Anderson et al., 1993 and 2002; Dunne et al., 2019). Many studies have been conducted to determine the freeze tolerance of bermudagrass in controlled environments by estimating the temperature to kill 50% of the population (LT_{50}). The controlled environment studies showed significant correlations to spring green-up and winterkill estimated in the field (Dunne et al., 2019; Patton and Reicher, 2007). While field evaluations can provide plant breeders with the most accurate assessment of winter survivability, environmental conditions are often unpredictable and difficult to replicate (Anderson and Taliaferro 2002). Therefore, laboratory-based experiments can be a reliable method to evaluate freeze tolerance. Thus the objective of this study was to determine the LT_{50} value of two commercially available and two experimental bermudagrass genotypes by subjecting them to 11 target freezing temperatures (-4 to -14°C) under controlled environment conditions.

Materials and Methods

The study consisted of two industry standards, ‘Tifway’ (freeze sensitive standard) and ‘Tahoma 31^{TM}’ (freeze tolerant standard), and two experimental genotypes, OKC1873 and OKC1406 developed by the bermudagrass breeding program at Oklahoma State University. All genotypes were clonally propagated in Berger BM 3 germination mix potting media in cone-tainers (RayLeach Cone-tainer Nursery, Canby, OR), 21 cm in depth and 3.8 cm in diameter. The bermudagrasses were established in June 2019 in a plant growth chamber (PGC Flex Growth Chamber, Conviron, Winnipeg, Canada) at the Controlled Environment Research Laboratory (CERL), Stillwater, Oklahoma. The chamber was maintained at 32/28°C day/night temperatures for 13 weeks with a photoperiod of 14 hours and a light intensity of 900 µmol m^{-2} s^{-1}.

The grasses in the cone-tainers were fertilized with a soluble fertilizer (Peters 20N-10P-20K) at 0.6 g L^{-1} and were trimmed as needed to maintain a height of 2.5 cm. During the establishment phase, as a precautionary measure the cone-tainers were treated every 14 days with bifenthrin (Talstar insecticide, FMC Corporation Agricultural Products Group, Philadelphia, PA) at labeled rates. Ammonium sulfate (21-0-0) (Hi-yield Ammonium Sulfate, VPG, TX) and chelated iron (Lawnstar, FL) was applied every four weeks at the labeled rate of 1.63 g L^{-1} and 5.6 ml, respectively. At the end of 13 weeks, the cone-tainers were transferred to another chamber maintained at 24/20°C day/night for a week. The cone-tainers were then subjected to cold-acclimation by lowering the temperature to 8/2 °C day/night for four weeks with a photoperiod of 10 hours and a light intensity of 400 µmol m^{-2} s^{-1}. The cone-tainers were then placed into a freeze chamber (E8, Plant growth chamber, Conviron, Winnipeg, Canada), and ten thermocouple sensors were inserted into random cone-tainers to monitor the soil temperature. Ice chips were placed on all cone-tainers to prevent supercooling and induce freezing. The temperature inside the chamber was programmed to stay at -3°C for 18 hours and then cool linearly at the rate of 1°C per hour. The target temperatures ranged from -4 to -14°C, anticipated to span from complete survival to complete mortality. At each target temperature, four cone-tainers were removed and moved to a plant growth chamber set at 4°C overnight to induce thawing. The temperature in the plant growth chamber was increased to 24/20°C for a week and then to 32/28°C to encourage recovery.

The regrowth of the plants was visually evaluated after six weeks using binary values; 1 = alive, 0 = dead. The LT_{50} values for each genotype were determined by logistic regression using the PROC PROBIT procedure (SAS Institute, Cary, NC) (Qian et al., 2001; Shahba et al., 2003). The probit procedure generated a table of predicted percentage survival at each temperature, and the temperatures corresponding to 50% survival were used as the estimates of LT_{50} for each genotype. The Pearson's chi-square and the likelihood ratio chi-square were insignificant due to high p values for all the models generated, thus failing to reject the null hypothesis that the model fits. Temperature parameter had a significant Type III effects according the Wald χ^2 test (Appendix, Table 35). The freeze test was repeated three times in October 2019, thus generating three LT_{50} values for each genotype. The LT_{50} of each replication was treated as a response variable and subjected to the analysis of variance (ANOVA) procedure of SAS (SAS Institute, Cary, NC). Means were separated using Fisher's protected least significant difference when F tests were significant at $p \leq 0.05$.

Results and Discussion

There were significant differences among genotypes in the LT_{50} values (Table 5). The LT_{50} means of the four genotypes ranged from -7.0 to – 9.1°C (Table 6). Tifway had the highest LT_{50} numerically and was within the ranges reported for this cultivar by Anderson et al. (2002, 2003, and 2007) which was -6.7°C or greater in each prior experiment. In contrast, the LT_{50} value was notably lower that that obtained by Dunne et al. (2019) (-5.4°C). This discrepancy could be attributed to the differences in the level of acclimation and the recovery period between the two studies. The samples in their study had a shorter establishment period and were acclimated at a higher temperature in contrast to our lower acclimation temperature, which may have induced a greater level of acclimation in our study. In the same study, multiyear (2011 to 2015) field testing resulted in Tifway having the highest winter survival among four commercial standards (‘Patriot,’ Tifsport,’ ‘Quickstand,’ and Tifway) in 2013, 2014, and 2015 (Dunne et al., 2019). This conflicts with other national reports which show Tifway as having a high winter kill percentage in Indiana and Kentucky (NTEP, 2014). The inconsistency in the winter survival of genotypes could be due to the differences in environmental conditions during the acclimation period.

Tahoma 31 was one of the top-performing genotype in this study with the lowest LT_{50} value which is consistent with field observations of winterkill which averaged across two locations (Indiana and Kentucky) was 14.5% (NTEP, 2014). Tahoma 31 was also reported to exhibit superior post-dormancy regrowth (NTEP, 2014). In a controlled environment study, Tahoma 31 was similarly quick to recover and reach 50% green once chilling stress was removed (Fontanier et al., 2020), indicating its higher recovery potential when subjected low temperatures. The experimental genotype OKC1873 with an LT_{50} value of -7.2°C was not significantly different from Tifway. OKC1406, with an LT_{50} value of -8.8°C, was in the same statistical group as Tahoma 31. This genotype previously ranked 6th among 53 experimental genotypes for winter survival tested in Kansas in 2018 (Xiang et al., 2019). OKC 1406 had a higher winter survival percentage

(88.3) than industry standards Tifway (0%), 'Latitude 36' (20%), 'NorthBridge' (25%), Patriot (30%), and 'TifTuf' (23%).

Tahoma 31, with low LT_{50} value, ability to tolerate a mowing height of 3.8 mm and above (unpublished data), could be successfully used in fairways in the US transition zone and further north. The higher winter survival percentage of OKC 1406 than some of the current industry standards and the LT_{50} value similar to Tahoma 31 in this study indicates its high freeze tolerance. However, multilocation and multi-year testing are required to evaluate the turf quality, mowing tolerance, and pest and disease resistance. Utilizing freeze tolerant bermudagrass genotypes will help golf courses or athletic facilities to decrease costs associated with the re-establishment of turf lost to winter injury and increase revenues by keeping the facility open for more number of days in winter.

Conclusion

The freeze tolerance of two experimental genotypes was quantified in this study by comparing it with two industry standards, Tifway and Tahoma 31. The LT_{50} values ranged from -7.1 to -9.1°C. The experimental genotype OKC1406 was in the same statistical group as Tahoma 31. This research (chapters 2 and 3) is the first to provide an LT_{50} value for Tahoma 31 under controlled environment conditions. The accurate estimation of its LT_{50} value will make it an ideal freeze resistant standard for future testing. These controlled environment evaluations would hasten the quantitative estimation of the freeze tolerance level in turfgrasses than field evaluation and would help the plant breeder to make a decision on whether the experimental bermudagrass genotypes tested should be subjected to further testing

Table 5. Analysis of variance for LT_{50} value of four bermudagrass genotypes

Source	**df**	**F value**
Cultivar (C)	3	50.9***

*, **, *** significant at *P* = 0.05, 0.01, and 0.001 respectively.

Table 6. Mean lethal temperature resulting in 50% survival (LT_{50}) of four bermudagrass genotypes when exposed to temperatures ranging from -4 to -14℃ under controlled environment conditions

Genotype	LT_{50} (℃)†
Tifway	-7.0a‡
OKC1873	-7.2a
OKC1406	-8.8b
Tahoma 31 ™	-9.1b
LSD (0.05)	0.50
CV	3.3%

† LT50 was calculated using PROC PROBIT procedure in SAS 9.4 version

‡Fisher's protected LSD test: within columns, means followed by the same letter are not significantly different at the p=0.05 level

CHAPTER IV

DROUGHT RESPONSE OF TEN BERMUDAGRASS GENOTYPES UNDER FIELD AND CONTROLLED ENVIRONMENT CONDITIONS

Abstract

Water scarcity is increasingly affecting urban landscapes and the best strategy for sustaining the turfgrass industry would be through the selection of drought-resistant genotypes. Bermudagrasses (*Cynodon* spp) have different mechanisms to survive drought stress either by growing long roots, through stomatal regulation, or by accumulating biochemical solutes in the leaf. The drought response of ten bermudagrasses were evaluated under two environments: 1) unrestricted rootzone in the field and 2) restricted 17cm rootzone in a greenhouse. Each study consisted of four commercially available ('Latitude 36,' 'Tifway,' 'TifTuf®,' and 'Celebration®'), and six experimental genotypes (OSU1337, OSU1403, OSU1439, TifB16107, TifB16113, and TifB16120). In the field study, all the genotypes survived the 60-day and 49-day dry-down period in 2017 and 2018, respectively, without completely browning off. Results showed a range of drought performance among genotypes, with TifTuf and Latitude 36 being the best and worst performing industry standards, respectively. TIfB16107, TifB16120, and TifB16113 were consistently the top performers with a leaf firing (LF) and turf quality (TQ) above 6, lower canopy temperature (CT), a higher percentage of live cover and greater normalized difference vegetation index (NDVI) values in the field. However, when grown in 17-cm pots, TifTuf, TifB16113 and TifB16120 demonstrated a TQ below 6 within 6 and 9 days after treatment (DOD) in 2019 and 2020, respectively. The discrepancy between studies suggests that these genotypes may have the ability to extract water from deeper soil profile when rootzones are unrestricted. Furthermore, these findings reinforce the importance of soil depth in maintaining turfgrasses without supplemental irrigation successfully.

Introduction

Water crisis is now a reality and not a warning as it is increasingly prevalent all around the globe. The most significant impact of water scarcity has been on the agriculture sector as it accounts for 70% of global freshwater withdrawals (FAO Water Reports, 2012a). Water stress is a common abiotic stress that plants encounter. Water stress could arise from insufficient or excessive water, but most terrestrial plants experience insufficient water condition termed as water deficit stress or drought (Levitt, 1980). Drought stress can be defined as a condition, which plants experience after a prolonged period of water deprivation that causes depletion of moisture in the root zone (Youngner, 1985). The factors that affect the drought response of plants are drought severity, drought length, soil physicochemical conditions, and plant vigor (Hossain et al., 2016). Drought affects photosynthesis through pathway regulation by stomatal closure leading to decreased flow of CO_2 into the plant (Chaves, 2002). Cell turgor loss has been the most common indicator of drought stress, having impacts on cellular structural integrity, metabolism, and whole-plant performance (Hopkins and Huner, 2008).

The ability of a plant to survive periods of water deficit stress is called drought resistance (Levitt, 1980). Turfgrasses contain 75 to 85% water by weight (Beard, 1966) and begin to wilt with a 10% decrease in water content (Beard, 1973). According to Levitt (1980) and Beard (1973), turfgrasses adopt three major drought resistance mechanisms to combat drought stress; i) through increased metabolic activity and rapid growth stress by escape or by becoming dormant, ii) by undergoing specific morphological and anatomical modifications (drought avoidance) or iii) by maintaining turgor pressure through osmotic adjustment increasing to prevent dehydration (drought tolerance). These three drought-resistance strategies are not mutually exclusive, and the same plant species may utilize more than one strategy when adapting to drought stress (Nilsen and Orcutt, 1996).

The degradation of existing freshwater resources and variations in rainfall patterns are straining water resources needed for the growing population. This restricts the availability of irrigation water to the turfgrass industry, emphasizing the need for drought resistant cultivars. Evaluation of drought resistance among turfgrasses has been conducted by withholding irrigation, excluding rainfall from field plots or in controlled environmental conditions. Leaf firing (LF), visual turf quality (TQ), evapotranspiration rate (ET), and recovery potential are general criteria for evaluating drought resistance in turfgrass research (Beard, 1989; Beard and

Sifers, 1997; Carrow, 1996). Photosynthesis and transpiration rates, canopy temperature (CT), stomatal conductance, osmotic adjustment, leaf water potential, electrolyte leakage, and altered antioxidant are some of the physiological and biochemical responses evaluated during drought stress in turfgrasses (Kopp and Jiang, 2013). Some of the alternative techniques for assessing drought responses are digital image analysis (DIA) and the normalized difference vegetative index (NDVI). In recent years, NDVI has been used to measure the response of turfgrasses to drought stress due to its strong correlation with visual ratings such as LF and TQ (Sonmez et al., 2008, Poudel, 2015).

Warm-season turfgrasses have superior drought survival and enhanced osmotic adjustment when compared to cool-season grass (Qian and Fry, 1997). Previous studies have documented the superior drought resistance of bermudagrasses (*Cynodon* spp.) when compared to other warm-season turfgrass species such as zoysiagrass (*Zoysia japonica* Steud.), St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntz), and centipedegrass ([*Eremochloao phiuroides* (Munro) Hack.) (Carrow, 1995, 1996; Qian and Fry, 1997). Although bermudagrasses are adapted to various soil and climatic conditions and is considered a drought resistant turfgrass (Beard and Sifers, 1997), to maintain proper growth and TQ, adequate soil moisture is required (Taliaferro, 2003).

Oklahoma State University (OSU) and the University of Georgia (UGA) have been actively engaged in breeding bermudagrasses with improved drought resistance. The advanced genotypes identified by these breeding programs require further characterization and testing in the field. Also, most research in the past has focused on screening bermudagrass genotypes for drought resistance at one soil depth. Urban environments often encounter rooting restriction due to soil compaction or shallow bedrock, requiring turfgrasses to be tested at multiple soil depths to evaluate the drought resistance of a particular genotype. Therefore, the objective of this study was to assess (i) the drought response of four commercially available bermudagrasses, grown under a rainout shelter in the field when subjected to drought (ii) the drought response of these ten bermudagrasses when subjected to drought stress when grown in 17-cm deep pots in a controlled environment.

Materials and Methods

Field Trial

The experimental site was located at the Oklahoma State University Botanic Garden and Turfgrass Research Station, Stillwater, OK, on Block 16 (latitude 36°07'16.0"N longitude 97°06'14.8"W). The soil type was sandy loam (55% sand, 21.5% silt, and 22% clay). The area was originally planted in July 2016 as a single trial consisting of ten bermudagrass and ten zoysiagrass entries. However, only the bermudagrass genotypes were included in this study. The study consisted of four standards, 'Latitude 36,' 'TifTuf®' 'Celebration®,' and 'Tifway,' three OSU experimental genotypes OSU1337, OSU1403, and OSU1439, and three UGA experimental genotypes, TifB16113, TifB16120, and TifB16107.

Each whole plot measuring 1.8 m by 1.8 m was maintained at a mowing height of 3.8 cm. These plots were mowed three times a week using a reel mower. The fertilizer regime was 195 kg N ha-1 yr-1 (46-0-0, N-P-K) applied in increments during the establishment phase in 2016 and from April to July in 2017 and April to August in 2018. A basic soil test was conducted at the Soil, Water, and Forage Analytical Laboratory at OSU in 2017 and 2018 to test the pH level, nitrogen, phosphorus, and potassium. The phosphorus and potassium levels were higher than the sufficiency indices of 65 and 250 respectively, no additional applications of phosphorous and potassium were made. An application of oxadiazon herbicide in liquid form (Ronstar Flo, Bayer Environmental science, NJ) was applied at 2.2 kg oxadiazon ha^{-1} in fall to provide pre-emergent control of winter annual grasses. An application of oxadiazon herbicide in granular form (Ronstar 2G, Bayer Environmental science, NJ) was applied at 2.2 kg oxadiazon ha^{-1} in spring to provide pre-emergent control of summer annual grasses. A tank-mix combination of 2,4-D, MCPP, and dicamba (Strike 3, Winfield Solutions, MN) at the rate of 3.5 kg product ha^{-1} was applied in winter to provide post-emergent control of broadleaf and perennial winter weeds. Weeds were also manually removed during the establishment phase to prevent herbicide injury. The trial was watered frequently to avoid plant wilting and to provide optimized growing conditions. Bermudagrass encroachment into the adjacent plots of another genotype was controlled by cutting stolons or by spraying a solution of glyphosate in the borders as needed. A rainout shelter was constructed in June 2017, to protect the plots from undesired precipitation during the dry-down period. Metal rain gutters were installed to divert rainwater runoff from the roof into a downpipe to prevent spillage into the experimental plots.

Drought Treatment

The drought stress was imposed when the TQ was found to be about uniform among all the genotypes. On 26 July 2017, and 17 August 2018, the experimental area was saturated with irrigation water to ensure uniform soil moisture across the plots. Irrigation was discontinued to impose drought stress. The dry-down period lasted for 60 days in 2017 (July 27- September 24) and for 49 days in 2018 (August 17- October 5). The average maximum air temperatures recorded from the Oklahoma Mesonet weather station during the dry-down period were 31°C and 29°C in 2017 and 2018, respectively (Figures 1 and 2). During the dry-down period, management practices such as mowing and fertilization were discontinued. The volumetric water content (VWC) was measured every seven to 13 days in 2017 and 2018 using FieldScout TDR 350 (Spectrum Technologies, IL) with a 7.6 cm rod spikes to monitor the moisture level in the soil during this time (Figure 3 and 4).

Data Collection

1. Turf Quality (TQ)

Visual ratings were performed by taking into consideration the color, density, texture, and uniformity of the turf. The dry-down was initiated when the TQ was deemed to be uniform and acceptable among all the genotypes. The TQ is rated on a scale from 1 to 9 where 1 = poor quality turf, 9 = outstanding/ideal turf, and 6 = acceptable turf quality (Morris, 2000). Turf quality ratings were assigned every three days in 2017 and every seven days in 2018.

2. Leaf Firing (LF)

    Leaf firing was a visual assessment of the chlorosis in leaves due to drought stress. It is rated on a scale from 1 to 9; 1 = completely brown or dead and 9 = completely green (Morris, 2000). Leaf firing ratings were taken every three days in 2017 and every seven days in 2018.

3. Digital Image Analysis (DIA)

Digital images were taken at 0, 9, 30, 45, and 58 days of drought (DOD) during the down period in 2017. The images were taken using the Canon G15 PowerShot camera mounted on a portable standard light box. The images were analyzed using the Turf Analysis macro in SigmaScan Pro 5 software (Systat Software, Chicago, IL) to measure the number of green pixels in each image (Richardson et al., 2001). Data were converted to percent live green cover prior to analysis.

4. Canopy Temperature (CT)

Canopy temperature was measured in 2017 every nine days and on the last day of drought using a handheld infrared thermometer (Fluke 561, Fluke Corporation, Everett, WA). The measurements were taken between 1300 and 1330 HR and four temperature readings per plot were taken and averaged to get a representative reading from each plot.

5. Normalized Difference Vegetative Index (NDVI)

The NDVI was measured in 2018 using the Greenseeker handheld crop sensor (Trimble, Sunnyvale, CA). This device has a sensor that emits brief bursts of red and infrared light and then measures the amount of light that is reflected from the plant. A higher reading indicates a healthier plant.

Statistical Analysis

The experiment design was a randomized complete block design with three replications of each entry. Leaf firing, TQ, NDVI, CT, and DIA were analyzed using generalized linear mixed models (GLIMMIX) methods for repeated measures (SAS version 9.4., SAS Institute Inc., Cary, NC, USA). Blocks were considered random effects, whereas genotype and rating days were fixed effects, and the day of the drought was a repeated measure. Due to the differences in the environmental conditions and duration of drought stress, the data from 2017 and 2018 were analyzed separately. Significant means were separated via Fishers protected LSD at $p \leq 0.05$. The correlation of all the parameters (LF, TQ, CT, NDVI, and DIA) was performed for both years using SAS procedure PROC CORR (SAS version 9.4; SAS Institute, Cary, NC).

Controlled Environment Study

A controlled environment study was conducted at Oklahoma State University (OSU) at the Controlled Environment Research Laboratory (CERL) greenhouse facility located in Stillwater, OK. The study was replicated in time (2019 and 2020) and consisted of the same ten bermudagrass genotypes of the SCRI advance genotypes study. The planting materials were obtained from the field located at the Mingo Valley Research Station, Bixby, Oklahoma, in 2018, using a 10.8 cm diameter (4.25 inch) cup cutter. The pots for this study was constructed by fitting a polyvinyl chloride (PVC) pipe coupling (15.2 cm diameter and cut to 17 cm in length) to a PVC Flexible Coupling having a 1-cm diameter hole at the bottom to facilitate drainage. The growing media used for the study comprised of a 1:1 mixture (v/v) of sand (Lightle Sand and Construction, Hennessey, OK) and bagged top soil (Timberline Top Soil) which were sieved separately through a 0.2-cm mesh before being mixed in a barrel type concrete mixer (Su et al., 2013). Sprigs of genotype was washed and planted into the pots in December 2018 and August 2019.

A 20-20-20 NPK water soluble fertilizer (J.R Peters Inc., Allentown, PA) was applied once a week at 1.14 g N L^{-1}. The grasses were trimmed manually with scissors at 3.8 cm height every three days. Rhodesgrass mealybug (*Antonina graminis*) were detected in some pots in the first study. Therefore, all grasses were treated on seven-day (d) intervals, with imidacloprid (Mallet® 2F T&O Nufarm, Alsip, IL). A preventive application of bifenthrin (Talstar Insecticide, FMC Agricultural Solutions, Pennsylvania, PA) was made to prevent Bermudagrass mites (*Eriophyes cynodoniensis*) on a 14-d interval.

Drought Treatment

Before the initiation of drought, all the genotypes were allowed to reach complete establishment and about uniform visual turf quality. After the six-month establishment period, all the pots were subjected to a pre-trial conditioning treatment by watering every two days. This pre-conditioning treatment was performed to acclimate grasses to mild drought stress prior to the more severe stress later on. Following the conditioning treatments, all the pots were saturated to field capacity of loam soil, i.e., 35-45% VWC, and thereafter irrigation was withheld. The drought commenced on 25 June 2019, and 5 February 2020. The dry-down period in the controlled environment lasted for 14 days and 27 days in 2019 and 2020, respectively. The longer duration of the dry-down period in 2020 could be due to lower solar radiation during February and March, resulting in reduced evapotranspiration, as compared to the study conducted in June and July of 2019.

Data Collection

All measurements were taken on the day the pots were saturated to field capacity (0 DOD). There on, the pots were closely monitored to detect any sign of wilting. The data were collected every day during the dry-down periods in 2019 and 2020. However, the days in which the ratings stayed the same for all the genotypes were omitted from the statistical analysis. Measurements collected for this study were as follows:

1. TQ
2. LF
3. Normalized Difference Vegetative Index (NDVI)

The NDVI was measured using the FieldScout TCM 500 NDVI Turf Color Meter (Spectrum Technologies, Aurora, IL). The device measures the reflected light from approximately a 7.6 cm diameter section of turfgrass in the red (660 nm) and near-infrared (850 nm) spectral bands. The NDVI meter consists of an inbuilt light to negate the effect of external light.

Moisture measurement

The soil VWC was measured during the dry-down period in 2019 by fully inserting a 12 cm probe (HydroSense^{TM} CS655-L, Campbell Scientific, Logan, UT) at the surface of each pot (Figure 7). However, as the drought progressed, it was difficult to insert the moisture probe. Therefore, for study 2 in 2020, the cumulative evapotranspiration rate (ET) were calculated using a scale to weigh the pots every third day between 1230 and 1300 HR to determine water loss.

The mean temperature and relative humidity in the greenhouse during the dry-down period of the first study were 33°C and 66%, respectively (Figure 5). For the dry-down period during the second study, the temperature and humidity were 31°C and 41%, respectively (Figure 6). Supplemental light was provided using high pressure sodium (HPS) lamps during each study. The photosynthetically active radiation (PAR) was measured at 15-minute intervals using light quantum sensors (Mini WatchDog, 2475 Plant growth station, Spectrum Technologies, Aurora, IL). The average PAR for the 14-hour photoperiod (0700 to 2100 HR) was 930 μ mol m^{-2} s^{-1} and 417 μ mol m^{-2} s^{-1} for study 1 and study 2, respectively during the dry-down period. The lower PAR for study 2 was due to decreased solar radiation during the study period (February – March).

Statistical Analysis

The analytical design for this study was a completely randomized design with five replications. All the parameters were analyzed using generalized linear mixed models (GLIMMIX) methods for repeated measures (SAS version 9.4., SAS Institute Inc., Cary, NC, USA). Replications were considered random effects, whereas genotype and rating day were fixed effects, and the day of the drought was a repeated measure. Due to the difference in the environmental conditions and duration of drought stress, the data from 2019 and 2020 were analyzed separately. Significant means were separated via Fishers protected least significant difference at $p \leq 0.05$. The correlation of all the parameters (LF, TQ, and NDVI) was performed for both years using SAS procedure PROC CORR (SAS version 9.4; SAS Institute, Cary, NC).

Results

Field Trial 2017

The analysis of fixed effects revealed that genotype, day, and their interaction had a significant effect on TQ, LF, CT, and DIA (Table 7). There were no significant differences among genotypes for TQ from 0 to 9 DOD (Table 8 and 9). However, genotypic differences were apparent as the drought progressed from 12 DOD. Turf quality among genotypes ranged from 7.1 to 8.1 and 2.1 to 7.5 at 0 and 60 DOD, respectively. TifTuf and the experimental genotypes from UGA maintained a TQ rating above the minimum acceptable during the 60-d dry-down period. The first experimental genotype to fall below 6 was OSU1403 at 15 DOD followed by OSU1439, and OSU1337 at 24 and 36 DOD, respectively. Latitude 36, Tifway, and Celebration dropped below acceptable TQ at 27, 36, and 45 DOD, respectively. TifB16113, TifB16120, and TifTuf had significantly higher TQ than OSU experimental genotypes and Latitude 36 from 33 DOD. There were no significant changes in the TQ ratings of TifB16113 from 0 to 60 DOD.

Significant differences in LF were not found among genotypes at the beginning of the drought trial but were apparent from 12 DOD (Table 10 and 11). The LF values ranged from 2.7 to 8.0 at 60 DOD. Experimental genotypes from UGA and TifTuf maintained LF values above 7.0 and were in the same statistical group throughout the dry-down period. The three OSU experimental genotypes along with Latitude 36, and Tifway showed significantly higher firing than TifTuf, and UGA experimental genotypes from 12, 21, and 27 DOD, respectively. There was no significant decrease in LF ratings of TifTuf and TifB16113 from 0 to 60 DOD.

Significant differences among genotypes in CT were observed on five rating days with TifTuf and three experimental genotypes from UGA maintaining lower CT at 27, 36, 54, and 60 DOD (Table 12). All genotypes experienced a decline in percentage live cover as the trial progressed (Table 13). Genotypic differences were not apparent at the start of the dry-down period. However, as the drought progressed, significant differences were observed at 30, 45, and 58 DOD. At 58 DOD, the percentage live cover ranged from 17.2% to 84.7%. TifTuf and experimental genotypes from UGA had a significantly higher live cover than the other genotypes at three rating days (30-57 DOD). These genotypes maintained a percentage live cover greater than 76% at 57 DOD. TifB16113 and TifTuf did not show a significant difference in the percentage of live cover over the five rating days. Leaf firing, TQ, and DIA were positively correlated with a Pearson's correlation coefficient (r) greater than 0.90 (Table 14). Canopy temperature had a negative correlation with LF, and TQ (r >0.60) (Table 14). Since CT and DIA were measured mostly on different rating days, a correlation analysis was not performed between CT and DIA.

Field Trial 2018

The analysis of fixed effects revealed that genotype, day, and their interaction had a significant effect on TQ, LF, and NDVI (Table 15). There were no significant differences among genotypes until 28 DOD (Table 16). TifB16113, TifB16120, and OSU1337 showed no significant differences in TQ from 0 to 49 DOD. The TQ ranged from 7.0 to 8.0 at 0 DOD and 3.0 to 7.0 at 49 DOD. Latitude 36, Tifway, OSU1337, and OSU1439 were the first genotypes to fall below minimum acceptable quality at 28 DOD, followed by OSU1403 at 35 DOD. Latitude

36, Tifway and the three OSU experimental genotypes had significantly lower TQ than the three UGA genotypes from 28 DOD. Similar to the 2017 results, the three experimental genotypes from UGA maintained a TQ rating of 6 and above throughout the dry-down period. TifB16113 had significantly higher TQ than TifTuf and TifB16107 from 35 and 49 DOD, respectively. There were no significant changes in the TQ ratings of TifB16120 and TifB16113 throughout the 49-d drought period. Celebration and TifTuf were in the same statistical group on all rating days.

Leaf firing ratings were not significantly different among genotypes at the start of the 2018 dry-down, but as the drought progressed, significant differences were found among genotypes from 14 DOD (Table 17). At the end of the drought stress, the LF values ranged from 2.8 to 7.2. The UGA experimental genotypes maintained an LF rating of 6.2 and above. Among the standards, TifTuf had similar LF as the UGA experimental genotypes on all rating days except the final day of drought stress on which it was significantly different from TifB16113. Celebration and TifTuf were in the same statistical group throughout this trial. The UGA experimental genotypes and TifTuf had significantly higher LF ratings than OSU experimental genotypes, Latitude 36 and Tifway from 28 DOD.

Differences in genotype performances due to drought stress varied for NDVI from 21 DOD (Table 18). The NDVI values ranged from 0.73 to 0.81 and 0.32 to 0.74 at 0 and 49 DOD, respectively. The genotypes TifB16113, TifB16120, TifB16107, TifTuf, and Celebration were in the same statistical group on all rating days. These genotypes had significantly higher NDVI values than Latitude 36, OSU1337, and OSU1439 at 49 DOD. Pearson's correlation coefficient revealed a high positive correlation ($r > 0.80$ $p \leq 0.001$) for the parameters LF, TQ, and NDVI (Table 19).

Controlled Environment Study

Study 1 in 2019

The analysis of fixed effects revealed that genotype, day, and the interaction had a significant effect on TQ, LF, and NDVI (Table 20). The TQ rating ranged from 7.6 to 8.0 at 0 DOD (Table 21). Significant differences among genotypes were observed at 5-8, 11, and 12 DOD. At 6 DOD, 80% of the genotypes fell below minimum acceptable TQ. OSU1439 was the last to fall below 6 at 8 DOD. Also, OSU1439 had higher TQ than all genotypes at 6 and 7 DOD. Celebration, Latitude 36, and TifB16107 were the only genotypes that were in the same statistical group as OSU1439 at 8 DOD.

No differences in LF among genotypes was observed from 0 to 5 DOD (Table 22). Latitude 36, TifB16107, and OSU 1439 were the only genotypes that showed no decline in LF value at 5 DOD. Significant differences among genotypes were found on 5-9 DOD and 11 DOD. Celebration, TifB16107, and OSU1439 were in the same statistical group from 6-9 DOD. The first genotypes to completely brown off were TifB16113 and OSU1337 at 12 DOD. On the last day of dry-down (14 DOD), the LF ratings ranged from 1.0 to 1.2, with 90% of the genotypes scoring 1.0.

There were no differences in NDVI among genotypes at the beginning of the dry-down period (Table 23). However, post 5 DOD, significant differences were found. Significant differences among genotypes were observed on 5, 6, 8, 11, and 13 DOD. The NDVI values ranged from 0.754 to 0.787 at 0 DOD and 0.213 to 0.331 at 13 DOD. At 6 DOD Celebration, Latitude 36, Tifway, TifB16107, and OSU1439 were significantly greater than the rest of the genotypes. The NDVI value of OSU1439 was significantly higher than all other genotypes at 8 DOD. Leaf firing, TQ, and NDVI were correlated to one another with a significantly high Pearson's correlation coefficient (r > 0.9) (Table 24).

Study 2 in 2020

The analysis of the fixed effects revealed that genotype, day, and the interaction had a significant effect on TQ, LF, and NDVI (Table 25). The TQ ratings ranged from 6.4 to 8.0 at 0 DOD. Significant differences among genotypes were observed on all rating days except on 24 DOD (Table 26 and 27). At 9 DOD, 50% of the genotypes fell below the minimum acceptable TQ. These genotypes were TifB16113, TifB16120, OSU1337, Tifway, and TifTuf. All genotypes scored below 6 at 10 DOD. TifB16113 had significantly lower TQ than OSU1439 and OSU1403 on 10 and 11 DOD, respectively. TifB16120 had significantly lower TQ than OSU1439 and OSU1403 on 13 and 15 DOD, respectively. OSU1439 and OSU1403 were in the same statistical group except on the last day of drought on which OSU1403 had significantly higher TQ than all the genotypes.

The genotypes that first showed symptoms of LF were the three UGA experimental genotypes along with OSU1337, Latitude 36, and Celebration at 8 DOD (Table 28 and 29). By 9 DOD, all the genotypes showed LF. No differences in LF among genotypes were observed until 8 DOD. OSU1439 and OSU1403 had significantly lower firing than TifB16113 and OSU1337 between10 DOD to 26 DOD. TifB16107 was in the same statistical group as OSU1403 and 1439 till 26 DOD. At 27 DOD, the LF values ranged from 1.0 to 2.0, with 50% of the genotypes with an LF score of 1.0. OSU1403 had significantly higher LF value than the rest of the genotypes at 27 DOD.

Significant differences in NDVI were found from 10 DOD (Table 30). The NDVI values ranged from 0.761 to 0.810 and 0.299 to 0.360 at 0 and 27 DOD, respectively. OSU1403 and OSU1439 had significantly higher NDVI values than TifB16113, TifB16120, Tifway, TifTuf, and Celebration at 16, and 20 DOD. Latitude 36 was in the same statistical group as OSU1403 and OSU1439 on all rating days. The analysis of variance for the cumulative ET revealed that genotype had a significant effect on ET at $P \leq 0.01$. OSU1439 had significantly lower ET than all the genotypes except Latitude 36 (Figure 8). The parameters LF, TQ, NDVI, and ET, showed a highly positive correlation ($r > 0.7$, $p \leq 0.001$) (Table 31).

Discussion

TifTuf was the top-performing commercial standard in the field study, which corresponds well with the results reported by Jespersen et al. (2019) and Katuwal et al. (2020). According to Jespersen et al. (2019), TifTuf had the highest TQ, NDVI, leaf water content, and solute accumulation, and lowest electrolyte leakage and CT. The superior performance of TifTuf could be attributed to its ability to maintain higher tissue water content by maintaining the water uptake through increased rooting. Improved drought performance has been associated with deep rooting and root plasticity in warm-season grasses in the past (Hays et al., 1991; Huang et al., 1997b). TifTuf produced 41% of its root biomass from 15 to 45 cm in contrast to Latitude 36 and Tifway, respectively produced 22 and 26% of their root biomass at the 15- to 45-cm depth suggesting that TifTuf was able to extract water from deeper within the soil profile due to its rooting characteristics (Yurisic, 2016). Also, TifTuf's large root diameter and greater root dry weight could be related to its ability to maintain water uptake and leaf hydration under drought stress (Katuwal et al., 2020). However, the decline in TQ of TifTuf in 2018, fell was primarily due to the presence of seedheads.

The superior performance of these genotypes could be associated with the similar drought avoidance characteristics adopted by TifTuf. Plants that possess avoidance mechanisms can not only survive but will also continue to grow and develop in the presence of prolonged drought stress (Levitt, 1980). The relatively low CT for the UGA genotypes is indicative of sustained transpiration under declining soil water and drought avoidance mechanisms, potentially from deeper rooting. Warmer CT as a result of lower stomatal conductance has been reported previously for Kentucky bluegrass (*Poa pratensis* L.) (Bonos and Murphy, 1999). As water becomes limited, stomata close and turfgrasses do not possess the thermal moderation provided by evaporation and transpirational cooling, thus leading to an increase in CT (Kneebone et al., 1992). The lower CT is an indication that these grasses were transpiring. As stomata close, photosynthesis is reduced because CO_2 uptake is reduced. Therefore, lower CT in these genotypes suggests that gas exchange, which maintains the carbon assimilation through photosynthesis, may not have been disrupted during drought stress. The carbohydrates produced through the carbon assimilation provide energy and precursors for plant growth and metabolic processes, thus delaying LF (Huang et al., 2014).

The results of the controlled environment study revealed that TifTuf and UGA experimental genotypes exhibited poor drought performance when grown in 17 cm pots. This finding contributes to the hypothesis that extensive rooting could be the primary drought-resistance mechanism in these genotypes. Since we did not perform any root sampling for root depth and mass measurement, one can only speculate that roots were present deep in the field. Bermudagrass, a deep rooting turfgrass species, that encounter a rooting restriction does not have the opportunity to establish a vertical, multilayered root distribution, affecting its ability to utilize soil moisture found lower in the profile (Steinke et al., 2011). Surface root viability in turfgrasses during drought stress is primarily maintained by deeper roots transporting water into drying surface soil (Huang, 1999). Steinke et al. (2011) reported that bermudagrass genotypes, including Celebration and Tifway, did not survive more than 20 days without water on a 10-cm root zone. Therefore, the results from the controlled environment studies show that genotypes with excellent rooting capacity may fail to perform under drought when planted in urban environments. Such environments often encounter rooting restriction because the building processes completely remove the topsoil, leaving a hardpan of clay, thus hindering extensive rooting. Typically, a minimum of 4 to 6 inches of topsoil is needed to establish good quality turf (Beard, 1973). However, the failure of these genotypes when grown in 17 cm pots suggest that these may require a topsoil depth above 6 inches to maintain acceptable TQ during drought stress, thereby decreasing the need for supplemental irrigation.

The time taken for Celebration to fall below the minimum acceptable TQ corresponds well with the results reported by Steinke et al. (2011). Celebration had exhibited superior drought resistance by increasing root length when irrigation was withheld for a longer interval (Baldwin et al., 2006). Also, by taking a longer time to reach a 50 % green cover, it showed higher drought resistance (Thapa, 2011). Celebration had greater transpiration and stomatal conductance similar to TifTuf when grown in 55 cm deep PVC pots (Katuwal et al., 2020). However, its lower photosynthesis rate resulted in lesser leaf water use efficiency compared to TifTuf (Katuwal et al., 2020). The lower photosynthesis in Celebration could be due to metabolic limitations related to the decline in Rubisco activity and activation state, which is a major limiting factor during severe drought stress (Hu et al., 2009), thus leading to an increase in LF when compared to TifTuf (Huang et al., 2014).

The drought performance of Tifway in this study is in accordance with the results reported by Jespersen et al. (2019) in which Tifway fell below the TQ value of 6 at the end of 28 days of drought stress. Tifway also maintained a lower live cover percentage than TifTuf after 21 days of drought conditions (Schwartz et al., 2018). In this study, similar trends were observed as Tifway had a significantly lower live cover percentage than TifTuf from 30 DOD onwards. The poor performance of Tifway in shallow pots in this study agrees with the results reported by Steinke et al. (2011) and Jiménez et al. (2019). In both these studies, Tifway failed to perform when grown in the 10 cm root zone.

Latitude 36 was the worst performing commercial standard in field trials. Latitude 36 had shorter root length and lower dry weight when compared to Celebration bermudagrass (Poudel, 2010). Latitude 36 still retained substantial amounts of soil moisture at 38.1 cm and 71.2 cm depths at the end of the dry-down period (Thapa, 2011), indicating its inability to use water from the deeper soil profile. However, in the controlled environment study, Latitude 36 had significantly higher TQ at the beginning of the drought stress than TifTuf, TifB16113, and TifB16120 in both years. The higher TQ of Latitude 36, when compared to these genotypes, could be due to its significantly lower ET in 2020. Lower ET could be due to the rapid closure of stomata in the early phase of drought stress, which may result in saving water (Hu et al., 2009). Latitude 36 had the highest visual quality when compared to Celebration and Premier bermudagrass when grown in 45 cm deep lysimeters (Su et al., 2013). Also, 16- and 23-kDa dehydrin proteins were only observed in Latitude 36, indicating that the expression of these proteins could be associated with drought tolerance (Su et al., 2013).

The experimental genotypes from OSU were the worst performing experimental genotypes in the field. Stomatal closure could be the primary defense mechanism as CT was considerably higher in these genotypes when compared to TifTuf and the three UGA genotypes in the field in 2017. Stomata remaining closed too early or too long during drought stress could affect CO_2 uptake, thereby reducing photosynthesis and carbohydrate production in leaves (Huang et al., 2014), which could have led to a rapid increase in firing in these genotypes. However, OSU1439 exhibited slightly better performance than other genotypes in the controlled environment study by maintaining a significantly higher TQ at 8 DOD and 24 DOD in 2019 and 2020, respectively. The higher TQ of OSU1439 could be due to its lower cumulative ET in 2020, indicating stomatal closure early in the drought. Although not measured in the current study, the survival of this genotype during short term drought stress could also be due to the expression of proteins similar to the ones reported in Latitude 36, indicating drought tolerance (Su et al., 2013). However, a decline in solute accumulation as drought progressed has been reported (Startseva and Ishmukhametova, 1973). This decline indicates that drought tolerance merely permits the plant to survive until the stress and, therefore, cannot grow (Levitt, 1980).

The significant correlations of CT, DIA, and NDVI with LF and TQ in the field and under controlled environment suggest their mutual usefulness as drought resistance indicators. These results indicate that changes in CT, DIA, and NDVI can be used for understanding the variability in grass water status. Rapid and accurate estimates of drought response are critical for irrigation management and also for phenotypic screening of drought resistant genotypes. Laboratory assessment of relative leaf water content or electrolyte leakage is a tedious process, especially when a large number of genotypes are involved. Human evaluation of LF and TQ requires adequate training, consistency, and time (Bell et al., 2002; Trenholm et al., 1999). Therefore, implementing these tools would help turfgrass managers or superintendents with little training, to quickly and efficiently identify areas of potential drought stress.

Conclusion

The study was conducted in an unrestricted soil depth in the field in 2017 and 2018 and restricted soil depth in 17 cm pots under controlled environment conditions in 2019 and 2020. The three experimental genotypes developed by UGA demonstrated superior drought resistance in the field by maintaining a TQ above minimum acceptable throughout the dry-down period in both the years. TifTuf was the top-performing commercial standard in the field trial. Celebration, and Tifway showed moderate drought resistance by exhibiting a delayed decrease in TQ and LF ratings. Latitude 36, and the OSU experimental genotypes were the least performing genotypes in the field trial and fell below the minimum acceptable TQ between 24-34 DOD in both the years. However, under controlled environment conditions, most genotypes exhibited a similar decline in LF and TQ, in contrast to large differences observed in the field. The best performing genotypes in the field failed to perform when grown in 17 cm pots, indicating that these genotypes require a greater soil depth to survive prolonged drought stress.

Thus, this study has identified superior performing experimental bermudagrass genotypes that can withstand the short and long duration of drought in the field, which will subsequently reduce the need for supplemental irrigation and conserve water resources. Canopy Temperature, NDVI, and DIA were correlated with LF, TQ, indicating their usefulness as relative drought resistance measurements. The information gained from this multi-year testing in the field and controlled environment will better inform OSU and UGA turfgrass breeders to make a decision on whether additional testing should be pursued on these experimental bermudagrass genotypes for possible commercial release. However, the inconsistent performance of the genotypes in the restricted and unrestricted soil highlights the importance of soil depth for turfgrasses with greater rooting ability. Thus further research is needed to better understand the specific drought resistance mechanism found in bermudagrass genotypes and how soil depth and texture influence the expression of these mechanisms. Also, understanding the genetic factors governing the drought tolerance or avoidance traits could be utilized by turf breeders for developing turfgrasses with overall drought resistance.

Table 7. Tests of fixed effects for Turfgrass Quality, Leaf Firing, Canopy Temperature (CT) and Digital Image Analysis (DIA) using PROC GLIMMIX for field trial in 2017

Source	**Turf Quality**		**Leaf Firing**		**CT**		**DIA**	
	df	F value	df	F value	df	F value	df	F value
Genotype (G)	9	360.63***	9	338.33***	9	24.38***	9	51.10***
Rating Days (D)	20	82.30***	20	121.88***	7	24.63***	4	108.22***
G X D	180	8.18***	180	6.66***	63	2.24**	36	5.57***

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 8. Mean turfgrass quality collected from 0 DOD to 30 DOD for field trial in 2017

	Turfgrass Quality †										
Cultivar	26-Jul	29-Jul	1-Aug	4-Aug	7-Aug	10-Aug	13-Aug	16-Aug	19-Aug	22-Aug	25-Aug
DOD‡	0	3	6	9	12	15	18	21	24	27	30
Latitude 36	8.1a§	7.8ab	7.8ab	7.8ab	7.5abc	6.8b-e	6.8b-e	6.8b-e	6.5b-e	5.5e-h	5.5e-h
OSU1337	7.8ab	7.8ab	7.5abc	7.5abc	7.1a-d	6.8b-e	6.8b-e	6.8b-e	6.5b-e	6.1c-f	6.1c-f
OSU1439	7.8ab	7.5abc	7.5abc	7.1a-d	6.8a-e	6.1b-f	6.1b-f	6.1b-f	5.8c-g	4.8f-j	4.8f-j
OSU1403	8.1a	8.1a	7.8ab	7.5abc	6.8b-e	5.8d-g	5.8d-g	5.8d-g	4.8f-j	4.5g-k	3.8jkl
TifB16107	7.5abc	7.5abc	7.5abc	7.5abc	7.1a-d	6.8b-e	6.8b-e	6.8b-e	6.8b-e	6.8b-e	6.8b-e
Tifway	8.1a	8.1a	8.1a	7.8ab	7.8ab	7.1a-d	7.1a-d	7.1a-d	7.1a-d	6.1c-f	6.1c-f
TifTuf	8.1a	7.8ab	7.8ab	7.8a	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab
Celebration	7.1a-d	7.5abc	7.1a-d	7.1a-d	7.1a-d	6.1c-f	6.1c-f	6.1c-f	6.1c-f	6.1c-f	6.1c-f
TifB16120	8.1a	8.1a	8.1a	8.1a	8.1a	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab
TifB16113	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab
	NS¶	NS	NS	NS	**#	***	***	***	***	***	***

†Turfgrass quality was rated on a 1 to 9 scale where 9 was considered to have exceptionally high quality and 1 was considered to have exceptionally low quality

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at $P = 0.05$, 0.01, and 0.001, respectively

Table 9. Mean turfgrass quality collected from 33 DOD to 60 DOD for the field trial in 2017

	Turfgrass Quality†									
Cultivar	28-Aug	31-Aug	3-Sep	6-Sep	9-Sep	12-Sep	15-Sept	18-Sep	21-Sep	24-Sep	
DOD‡	33	36	39	42	45	48	51	54	57	60	
Latitude 36	5.5e-h§	4.5g-k	3.8jkl	3.8jkl	3.5j-m	3.1klm	3.1klm	2.8lm	2.5lm	2.5lm	***
OSU1337	6.1c-f	4.5g-k	4.5g-k	4.5g-k	4.5g-k	4.5g-k	4.1i-k	4.1ijk	2.8lm	2.8lm	***
OSU1439	4.5f-k	4.5f-k	3.8i-l	3.8i-l	3.8i-l	3.5j-m	3.1j-m	3.1j-m	3.1j-m	3.1j-m	***
OSU1403	3.5j-m	3.1klm	2.8lm	2.8lm	2.8lm	2.8lm	2.8lm	2.5lm	2.1m	2.1m	***
TifB16107	6.8b-e	6.8b-e	6.8b-e	6.8b-e	6.8b-e	6.8b-e	6.8b-e	6.8b-e	6.1c-f	6.1c-f	***
Tifway	6.1c-f	5.8d-g	5.5e-h	5.5e-h	4.8f-j	4.1h-k	4.1i-k	4.1h-k	4.1h-k	4.1h-k	***
TifTuf	7.8ab	7.5abc	7.5abc	7.5abc	7.5abc	7.5abc	7.5abc	7.5abc	6.5b-e	6.5b-e	**
Celebration	6.1c-f	6.1c-f	6.1c-f	6.1c-f	5.8d-g	5.5e-h	5.5e-h	5.1f-i	4.5g-k	4.5g-k	***
TifB16120	7.8ab	7.8ab	7.8ab	7.8ab	7.5abc	7.5abc	7.5abc	7.5abc	6.5b-e	6.5b-e	***
TifB16113	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.8ab	7.5abc	7.5ab	NS¶
	***#	***	***	***	***	***	***	***	***	***	

† Turfgrass quality was rated on a 1 to 9 scale where 9 was considered to have exceptionally high quality and 1 was considered to have exceptionally low quality

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001, respectively

Table 10. Mean leaf firing from 0 DOD to 30 DOD from the field trial in 2017

	Leaf Firing†										
Cultivar	26-Jul	29-Jul	1-Aug	4-Aug	7-Aug	10-Aug	13-Aug	16-Aug	19-Aug	22-Aug	25-Aug
DOD‡	0	3	6	9	12	15	18	21	24	27	30
Latitude 36	9.0a§	9.0a	9.0a	8.7a	7.7b-g	7.3b-h	7.0d-i	6.7f-j	6.7f-j	5.7i-m	5.7i-m
OSU1337	9.0a	9.0a	9.0a	9.0a	7.0c-i	7.0c-i	7.0c-i	7.0c-i	6.7e-j	6.3h-k	6.3g-k
OSU1439	9.0a	9.0a	9.0a	9.0a	7.0b-i	7.0b-i	7.0b-i	6.7d-j	6.0h-k	5.7i-m	5.0j-o
OSU1403	9.0a	9.0a	9.0a	9.0a	7.0d-i	6.7f-j	6.0i-l	6.0i-k	4.7l-o	4.3m-p	4.3m-o
TifB16107	9.0a	9.0a	9.0a	9.0a	9.0a	8.3ab	8.0a-d	8.0a-d	8.0a-d	8.0a-d	8.0a-d
Tifway	9.0a	8.7a	8.7a	8.7a	8.3abc	8.0a-e	7.7a-g	7.7a-g	7.7a-g	6.7f-j	6.7f-j
TifTuf	9.0a	9.0a	9.0a	9.0a	9.0a	9.0a	8.7a	8.7a	8.7a	8.3abc	8.3abc
Celebration	9.0a	9.0a	9.0a	9.0a	9.0a	8.0a-d	7.7a-f	7.7a-f	7.3b-h	7.0c-i	7.0c-i
TifB16120	9.0a	9.0a	9.0a	9.0a	8.7a	8.7a	8.7a	8.7a	8.0a-d	8.0a-d	8.0a-d
TifB16113	9.0a	9.0a	9.0a	9.0a	9.0a	9.0a	9.0a	9.0a	8.7a	8.7a	8.7a
	NS ¶	NS	NS	NS	***#	***	***	***	***	***	***

† Leaf firing was rated on a 1 to 9 scale where 9 equaled no firing with fully green turf and 1 equaled most complete firing with straw-brown

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at $P = 0.05$, 0.01, and 0.001 respectively.

Table 11. Mean leaf firing collected from 33 DOD to 60 DOD from the field trial in 2017

	Leaf Firing†										
Cultivar	28-Aug	31-Aug	3-Sept	6-Sept	9-Sept	12-Sept	15-Sept	18-Sept	21-Sept	24-Sept	
DOD‡	33	36	39	42	45	48	51	54	57	60	
Latitude 36	5.7i-m§	4.7l-o	4.0n-q	4.0n-q	3.7opq	3.3opq	3.3opq	3.0pq	2.7q	2.7q	***
OSU1337	6.3g-k	6.0i-l	5.3i-n	5.0j-o	4.7l-o	4.3l-p	3.7opq	3.7opq	3.3opq	3.3opq	***
OSU1439	5.0j-o	4.3l-p	4.0m-q	4.0m-q	3.7n-q	3.7n-q	3.3opq	3.3opq	3.3opq	3.3opq	***
OSU1403	4.3m-p	3.0pq	3.0pq	3.0pq	3.0pq	2.7q	2.7q	2.7q	2.7q	2.7q	***
TifB16107	8.0a-d	7.7a-f	7.7a-f	7.7a-f	7.7a-f	7.3b-h	7.0c-i	7.0c-i	7.0c-i	7.0c-h	***
Tifway	6.7f-j	6.0i-l	5.3j-n	5.3j-n	5.0k-o	4.7l-o	4.7l-o	4.7l-o	3.7opq	3.7opq	***
TifTuf	8.3abc	8.0a-e	8.0a-e	8.0a-e	8.0a-e	8.0a-e	7.7a-g	7.7a-f	7.7a-f	7.7a-f	**
Celebration	7.0c-i	7.0c-g	6.3g-j	6.3g-j	6.3g-j	5.7i-m	5.7i-m	5.3j-n	4.7l-o	4.7l-o	***
TifB16120	8.0a-d	8.0a-d	8.0a-d	8.0a-d	8.0a-d	7.7a-f	7.7a-e	7.3b-h	7.3b-h	7.3b-h	***
TifB16113	8.7a	8.7a	8.7a	8.3abc	8.3abc	8.3abc	8.3abc	8.3abc	8.0a-e	8.0a-d	NS¶
	***#	***	***	***	***	***	***	***	***	***	

† Leaf firing was rated on a 1 to 9 scale where 9 equaled no firing with fully green turf and 1 equaled most complete firing with straw-brown

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at *P* = 0.05, 0.01, and 0.001 respectively.

Table 12. Mean canopy temperature collected over eight rating days from the field trial in 2017

	Canopy Temperature (°C)†								
Cultivar	Jul 26	Aug 4	Aug 13	Aug 22	Aug 31	Sep 09	Sep 18	Sep 24	
DOD‡	0	9	18	27	36	45	54	60	
Latitude 36	38.7f-i§	37.8ghi	44.3b-e	45.4b-e	42.6def	40.4e-h	49.8a	44.5b-e	***
OSU1337	39.1f-i	41.1e-h	44.1b-f	43.1c-f	39.2f-i	39.2f-i	50.1a	46.3a-d	***
OSU1439	38.5f-i	39.8e-i	45.6a-e	46.6a-d	41.6d-h	40.1e-i	48.9ab	43.6b-f	***
OSU1403	37.5ghi	40.3e-h	45.8a-d	45.5a-e	43.7b-f	40.4e-h	49.0ab	43.6b-f	***
TifB16107	38.1ghi	38.4ghi	41.7def	39.0f-i	37.4hi	37.2hi	40.6e-h	38.4ghi	***
Tifway	39.3f-i	39.0f-i	44.8b-e	43.7b-f	41.6d-g	38.8f-i	47.1abc	42.2def	***
TifTuf	38.5ghi	38.5ghi	41.1d-h	36.1i	35.8i	36.9hi	36.6hi	38.5ghi	***
Celebration	38.2ghi	36.9hi	41.4d-h	37.9ghi	38.3ghi	38.3ghi	42.9def	40.4e-h	***
TifB16120	38.2ghi	39.7f-i	39.4f-i	37.7ghi	36.1i	36.6hi	39.5f-i	38.2ghi	***
TifB16113	38.5ghi	39.6f-i	40.4e-h	39.4f-i	36.5hi	37.1hi	37.8ghi	39.4f-i	***
	NS¶	NS	**#	***	***	NS	***	**	

†Canopy temperature was measured in 2017 every three days using a handheld Fluke 561 infrared thermometer

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at *P* = 0.05, 0.01, and 0.001 respectively.

Table 13. Mean percentage live cover collected over five rating days from the field trial in 2017

	Percentage Live Cover (%)†					
Cultivars	26-Jul	4-Aug	25-Aug	9-Sep	22-Sep	
DOD‡	0	9	30	45	58	
Latitude 36	98.3a	89.1abc	58.7ef	46.6efg	22.8h	**
OSU1337	92.0abc	86.5a-d	66.9de	47.1efg	24.5gh	***
OSU1439	94.9abc	78.4bcd	48.0efg	36.0gh	30.4gh	***
OSU1403	98.3a	79.5bcd	32.8gh	27.9gh	17.2h	**
TifB16107	98.4a	93.3abc	90.7abc	81.8bcd	77.9cd	**
Tifway	97.3abc	87.1a-d	49.5efg	40.0fgh	37.5fgh	**
TifTuf	98.9a	97.1abc	92.3abc	85.7a-d	82.2a-d	***
Celebration	939abc	79.7bcd	64.8de	56.2ef	46.2efg	***
TifB16120	98.5a	96.7abc	96.6abc	88.8abc	80.0bcd	**
TifB16113	98.1a	94.3abc	90.8abc	88.8abc	84.7a-d	**
	NS¶	NS	***#	***	***	

†Percentage live cover was measured through digital image analysis using SigmaScan Pro 5

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at *P* = 0.05, 0.01, and 0.001 respectively

Table 14. Pearson correlation coefficient for Turf quality, Leaf firing, Canopy temperature (CT), Digital Image Analysis (DIA) for the field trial in 2017

Parameter	Turf Quality	Leaf Firing	CT	DIA
Turf Quality	1.00	0.96***	-0.63***	0.93***
Leaf Firing		1.00	-0.64***	0.93***
CT			1.00	NA†
DIA				1.00

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

† CT and DIA were measured mostly on different rating days, therefore a correlation analysis was not performed between CT and DIA.

Table 15. Test of fixed effects Turf Quality, Leaf Firing, and Normalized Difference Vegetation Index (NDVI) using PROC GLIMMIX for the field trial in 2018

Source	**Turf Quality**		**Leaf Firing**		**NDVI**	
	Df	F value	df	F value	df	F value
Genotype (G)	9	28.09***	9	49.06***	9	48.15***
Rating Days (D)	7	78.57***	7	112.80***	7	112.80***
G X D	63	4.27***	63	3.39***	63	3.49***

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 16. Mean turfgrass quality collected over eight rating days for the field trial in 2018

	Turfgrass Quality†								
Cultivar	17-Aug	24-Aug	31-Aug	7-Sep	14-Sep	21-Sep	28-Sep	5-Oct	
DOD‡	0	7	14	21	28	34	42	49	
Latitude 36	8.0ab§	7.7ab	7.0a-d	6.3b-f	5.0e-i	3.3ij	3.0j	3.0j	***
OSU1337	8.0ab	7.7ab	7.0a-d	6.3b-f	4.7f-j	4.3g-j	4.0hij	4.0hij	**
OSU1439	8.0a	7.7ab	7.0abc	6.3b-e	5.3e-h	4.7f-i	4.3hij	3.7ij	***
OSU1403	8.0a	7.7ab	7.0ab	6.3b-e	6.0c-f	4.7f-i	4.7f-i	4.3hij	***
TifB16107	8.0a	7.7ab	7.7ab	7.0ab	7.0ab	6.3b-e	6.3b-e	6.0c-f	**
Tifway	7.0a-d	7.0abc	7.0abc	6.3b-e	5.7d-g	4.7f-i	3.7ij	3.7ij	***
TifTuf	7.7ab	7.7ab	7.3ab	7.0abc	6.3b-e	6.0c-f	5.7e-g	5.3e-h	**
Celebration	7.0ab	7.0ab	7.0ab	7.0ab	6.0c-f	6.0c-f	5.3e-h	5.3e-h	***
TifB16120	7.7ab	7.3ab	7.3ab	7.3ab	7.0ab	7.0ab	6.7bcd	6.3b-e	NS
TifB16113	7.3ab	7.3ab	7.0ab	7.0ab	7.0ab	7.0ab	7.0a-d	7.0ab	NS
	NS¶	NS	NS	NS	***#	***	***	***	***

† Turfgrass quality was rated on a 1 to 9 scale where 9 was considered to have exceptionally high quality and 1 was considered to have exceptional quality

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 17. Mean leaf firing collected over eight rating days for the field trial in 2018

	Leaf Firing[†]								
Cultivar	17-Aug	24-Aug	31-Aug	7-Sep	14-Sep	21-Sep	28-Sep	5-Oct	
DOD‡	0	7	14	21	28	34	42	49	
Latitude 36	9.0ab§	8.5abc	7.8abc	6.2c-f	5.0e-i	3.2hi	2.8i	2.8i	***
OSU1337	9.0ab	8.5abc	8.2abc	6.2c-f	5.2d-g	4.2f-i	3.5ghi	3.5ghi	**
OSU1439	9.0a	8.5ab	7.8abc	6.2cde	5.5def	5.2d-g	4.2ghi	3.5ghi	***
OSU1403	9.0a	8.5ab	7.5bc	6.2cde	5.8def	5.2d-g	4.8e-h	4.2f-i	***
TifB16107	9.0a	8.2abc	8.2abc	7.8abc	8.0abc	7.5bc	6.8cd	6.5cde	*
Tifway	9.0a	8.5ab	7.8abc	6.2c-f	5.8def	5.2d-g	3.8ghi	3.8ghi	***
TifTuf	9.0a	9.0a	9.0a	7.8abc	7.5bc	7.5bc	6.5cd	6.0def	***
Celebration	9.0a	8.5ab	8.5ab	7.8abc	7.0cd	6.8cd	5.5def	5.2e-h	***
TifB16120	9.0a	9.0a	9.0a	8.5ab	7.8abc	7.2bc	6.5cd	6.2def	***
TifB16113	9.0a	9.0a	9.0a	7.8abc	7.8abc	7.8abc	7.2bc	7.2bc	***
	NS¶	NS	**#	***	***	***	***	***	

† Leaf firing was rated on a 1 to 9 scale where 9 equaled no firing with fully green turf and 1 equaled most complete firing with straw-brown

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively.

Table 18. Mean Normalized Difference Vegetation Index (NDVI) collected over eight rating days for the field trial in 2018

	Normalized Difference Vegetation Index (NDVI) †								
Cultivar	17-Aug	24-Aug	31-Aug	7-Sep	14-Sep	21-Sep	28-Sep	5-Oct	
DOD‡	0	7	14	21	28	35	42	49	
Latitude 36	0.74abc§	0.74abc	0.72a-d	0.66a-e	0.64a-f	0.41f-j	0.36hij	0.32j	**
OSU1337	0.73abc	0.73abc	0.73abc	0.68a-d	0.64a-f	0.42f-j	0.38g-j	0.34ij	**
OSU1439	0.74abc	0.73abc	0.74abc	0.71a-d	0.67a-e	0.56c-g	0.53d-i	0.51e-i	**
OSU1403	0.76ab	0.76ab	0.76ab	0.73abc	0.72a-d	0.65a-e	0.63c-f	0.61c-f	*
TifB16107	0.80a	0.80a	0.80a	0.78a	0.77a	0.77ab	0.76ab	0.74abc	***
Tifway	0.73abc	0.73abc	0.73abc	0.65a-e	0.64b-f	0.56c-h	0.55c-h	0.51e-i	*
TifTuf	0.78a	0.77a	0.78a	0.75ab	0.74abc	0.72a-d	0.72a-d	0.68a-d	***
Celebration	0.79a	0.79a	0.79a	0.76ab	0.76ab	0.74abc	0.73abc	0.68a-d	***
TifB16120	0.78a	0.78a	0.78a	0.74abc	0.74abc	0.74abc	0.74abc	0.73abc	*
TifB16113	0.81a	0.81a	0.81a	0.78a	0.78a	0.77a	0.76ab	0.74abc	***
	NS¶	NS	NS	**#	***	***	***	***	

†NDVI was measured using GreenSeeker™ handheld sensor

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 19. Pearson correlation coefficient for Turf Quality, Leaf firing, and Normalized Difference Vegetation Index (NDVI) for field trial in 2018

Parameter	Turf Quality	Leaf Firing	NDVI
Turf Quality	1.00	0.94***	0.84***
Leaf Firing		1.00	0.84***
NDVI			1.00

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 20. Test of fixed effects Turf Quality, Leaf Firing, and Normalized Difference Vegetation Index (NDVI), using PROC GLIMMIX for controlled environment study in 2019

Source	**Turf Quality**		**Leaf Firing**		**NDVI**	
	Df	F value	df	F value	df	F value
Genotype (G)	9	17.34***	9	21.61***	9	7.75***
Rating Days (D)	10	1264.12***	10	1534.33***	5	718.64***
G X D	90	2.43***	90	2.49***	45	2.42***

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 21. Mean turfgrass quality rating collected over 11 rating days for the controlled environment study in 2019

	Turfgrass Quality†										
Cultivar	25-Jun	29-Jun	30-Jun	1-Jul	2-Jul	3-Jul	4-Jul	6-Jul	7-Jul	8-Jul	9-Jul	
DOD‡	0	4	5	6	7	8	9	11	12	13	14	
Latitude 36	8.0a§	7.8ab	7.8ab	5.4ef	4.4gh	2.6lmn	2.4mn	2.0no	2.0no	1.6op	1.0p	***
OSU1337	8.0a	8.0a	7.2ab	4.6gh	3.4jkl	2.2no	2.0no	1.2p	1.0p	1.0p	1.0p	***
OSU1439	8.0a	7.8ab	7.8ab	7.0abc	6.0cde	3.4i-l	2.8k-n	1.8nop	1.8nop	1.8nop	1.4op	***
OSU1403	7.8ab	7.8ab	7.2ab	4.6gh	3.4jkl	2.2no	2.0no	1.6op	1.4op	1.2p	1.0p	***
TifB16107	8.0a	8.0a	8.0a	6.0de	4.8fg	3.0klm	2.8lmn	2.0no	1.6op	1.0p	1.0p	***
Tifway	8.0a	8.0a	8.0a	5.4ef	4.2ghi	2.2no	2.2no	2.0no	1.4op	1.0p	1.0p	***
TifTuf	7.8ab	7.2ab	6.4cd	4.8fg	4.0hij	2.2no	2.0no	1.4op	1.4op	1.0p	1.0p	***
Celebration	7.6ab	7.6ab	6.8bc	5.6e	4.8fg	2.8lmn	2.8lmn	1.8nop	1.6op	1.4op	1.0p	***
TifB16120	9.0ab	9.0ab	8.2bc	4.8hij	4.0lm	3.4nop	3.0op	2.0stu	1.8tuv	1.0v	1.0uv	***
TifB16113	9.0a	9.0ab	7.6c	4.2klm	3.6m-p	3.0pqr	2.6p-s	1.6tuv	1.0uv	1.0uv	1.0v	***
	NS¶	NS	***#	***	***	**	NS	*	**	NS	NS	

† Turfgrass quality was rated on a 1 to 9 scale where 9 was considered to have exceptionally high quality and 1 was considered to have exceptionally low quality

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 22. Mean leaf firing collected over 11 rating days for the controlled environment study in 2019

	Leaf Firing†											
Cultivar	25-Jun	29-Jun	30-Jun	1-Jul	2-Jul	3-Jul	4-Jul	6-Jul	7-Jul	8-Jul	9-Jul	
DOD‡	0	4	5	6	7	8	9	11	12	13	14	
Latitude 36	9.0ab§	9.0ab	9.0ab	6.2ef	4.8hij	3.4nop	3.0opq	2.4q-t	2.0stu	1.6tuv	1.0uv	***
OSU1337	9.0ab	9.0ab	7.6c	5.2ghi	4.2j-m	2.4q-t	2.4q-t	1.4tuv	1.0uv	1.0uv	1.0uv	***
OSU1439	9.0ab	9.0ab	9.0ab	7.6cd	6.4def	4.6h-l	3.6l-p	2.4p-t	2.0r-u	1.8s-v	1.4tuv	***
OSU1403	9.0ab	9.0ab	8.6ab	5.0ghi	3.8lmn	3.0opq	2.6p-s	1.6tuv	1.4tuv	1.2uv	1.0uv	***
TifB16107	9.0ab	9.0ab	9.0ab	6.8d	5.6fg	3.8lmn	3.6l-o	2.2rs	1.6tuv	1.0uv	1.0v	***
Tifway	9.0ab	9.0ab	8.8ab	5.6fg	4.6h-j	3.0opq	3.0opq	2.0stu	1.4tuv	1.0uv	1.0uv	***
TifTuf	9.0ab	8.8ab	7.6c	4.6i-l	4.2j-m	3.0o-r	2.8p-s	1.6tuv	1.4tuv	1.0uv	1.0uv	***
Celebration	9.0ab	9.0ab	8.4abc	6.6de	5.4fgh	3.6l-o	3.2nop	1.8tuv	1.6tuv	1.6tuv	1.0uv	***
TifB16120	9.0ab	9.0ab	8.2bc	4.8hij	4.0lm	3.4nop	3.0op	2.0stu	1.8tuv	1.0v	1.0uv	***
TifB16113	9.0a	9.0ab	7.6c	4.2klm	3.6m-p	3.0pqr	2.6p-s	1.6tuv	1.0uv	1.0uv	1.0v	***
	NS¶	NS	***#	***	***	**	**	*	NS	NS	NS	

† Leaf firing was rated on a 1 to 9 scale where 9 equaled no firing with fully green turf and 1 equaled most complete firing with straw-brown

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively.

Table 23. Mean Normalized Difference Vegetation Index (NDVI) collected over 6 rating days for the controlled environment study in 2019

	Normalized Difference Vegetation Index (NDVI)†						
Cultivar	25-Jun	30-Jun	1-Jul	3-Jul	6-Jul	8-Jul	
DOD‡	0	5	6	8	11	13	
Latitude 36	0.787a§	0.794a	0.716a-d	0.523e-j	0.374k-q	0.300p-t	***
OSU1337	0.754a	0.721abc	0.574efg	0.425i-o	0.273q-t	0.223rst	***
OSU1439	0.762a	0.735ab	0.632b-e	0.458h-m	0.310o-t	0.247rst	***
OSU1403	0.775a	0.773a	0.717a-d	0.544e-i	0.358l-q	0.279q-t	***
TifB16107	0.765a	0.771a	0.734ab	0.568e-h	0.354l-q	0.265q-t	***
Tifway	0.776a	0.779a	0.709a-d	0.535e-j	0.343m-r	0.273q-t	***
TifTuf	0.763a	0.707a-d	0.610c-f	0.475g-l	0.297p-t	0.245rst	***
Celebration	0.777a	0.7801a	0.754a	0.629b-e	0.417j-p	0.331o-s	***
TifB16120	0.784a	0.736ab	0.600def	0.492f-k	0.331n-s	0.269q-t	***
TifB16113	0.759a	0.725abc	0.578efg	0.443h-n	0.267q-t	0.213t	***
	NS¶	**#	***	***	***	***	

† NDVI was measured using FieldScout® TCM 500 NDVI

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 24. Pearson's correlation coefficient for Turf Quality, Leaf firing, Turf Quality and Normalized Difference Vegetation Index (NDVI) for the controlled environment study in 2019

Parameter	Turf Quality	Leaf Firing	NDVI
Turf Quality	1.00	0.98***	0.94***
Leaf Firing		1.00	0.92***
NDVI			1.00

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 25. Test of fixed effects Turf Quality, Leaf Firing, and Normalized Difference Vegetation Index (NDVI), using PROC GLIMMIX for the controlled environment study in 2020

Source	**Turf Quality**		**Leaf Firing**		**NDVI**	
	Df	F value	df	F value	df	F value
Genotype (G)	9	69.84***	9	103.95***	9	18.79***
Rating Days (D)	17	847.64***	17	1678.34***	6	1621.69***
C X D	153	1.70***	153	2.91***	54	2.68***

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 26. Mean turfgrass quality collected from 0 DOD to 16 DOD for the controlled environment study in 2020

	Turfgrass Quality†								
Cultivar	4-Feb	11-Feb	12-Feb	13-Feb	14-Feb	15-Feb	16-Feb	20-Feb	
DOD‡	0	7	8	9	10	11	12	16	
Latitude 36	8.0a§	8.0a	7.6abc	6.2g-k	5.4mno	4.6qrs	4.4q-u	4.0t-w	***
OSU1337	8.0a	7.4bcd	7.0def	5.2nop	4.6qrs	4.0t-w	4.0t-w	4.0t-w	***
OSU1439	8.0a	7.8ab	7.2cde	6.4f-i	5.8i-m	4.6qrs	4.6qrs	4.6qrs	***
OSU1403	7.6abc	7.4bcd	6.8efg	6.8efg	5.8j-m	5.0n-q	4.8pqr	4.4q-t	***
TifB16107	8.0a	7.2b-e	6.6e-h	6.4f-i	5.8i-m	5.0n-q	4.8o-r	4.4q-t	***
Tifway	7.4bcd	7.2cde	6.8efg	5.8j-m	5.4mno	4.2s-v	4.0t-w	3.8u-y	***
TifTuf	8.0a	8.0a	7.0def	5.8j-m	5.4mno	4.6qrs	4.4r-u	4.2s-v	***
Celebration	6.4f-j	6.2g-l	5.6k-n	4.4q-u	3.8t-y	3.2x-B¶	3.0z-D	2.6B-E	***
TifB16120	7.6abc	6.8efg	6.2h-l	5.4mno	4.8pqr	4.0t-w	3.6v-z	3.6v-z	***
TifB16113	7.4bcd	6.8efg	6.6fgh	5.6lmn	5.4mno	4.0t-w	4.0t-w	4.0t-w	***
	***#	**	**	***	***	***	***	***	

† Turfgrass quality was rated on a 1 to 9 scale where 9 was considered to have exceptionally high quality and 1 was considered to have exceptionally low quality

‡ Days of Drought (DOD)

§ Means accompanied by the same letter (lower and upper case) in the same column and row are not significantly different at the P = 0.05 level

¶ Upper case letter was used because the alphabetical groups exceed beyond 26 alphabets

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 27. Mean turfgrass quality collected from 17 DOD to 27 DOD for the controlled environment study in 2020

	Turfgrass Quality†									
Cultivar	21-Feb	22-Feb	23-Feb	24-Feb	25-Feb	26-Feb	28-Feb	29-Feb	1-Mar	2-Mar	
DOD‡	17	18	19	20	21	22	24	25	26	27	
Latitude 36	4.0t-w§	4.0t-w	3.8t-x	3.4x-A	3.4x-A	3.2x-B	2.6B-E	2.4DEF	2.0E-I	1.0J	***
OSU1337	4.0t-w	4.0t-w	3.6w-z	3.2y-B	3.0A-D	3.0A-D	2.0E-I	2.0E-I	1.6HIJ	1.2J	***
OSU1439	4.2r-v	4.2r-v	4.0t-w	4.0t-w	3.8t-x	3.6v-z	3.0z-C	2.4DEF	2.0E-I	1.2J	***
OSU1403	4.2s-v	4.2s-v	4.2s-v	4.0t-w	4.0t-w	4.0t-w	3.0A-D	2.4DEF	2.4DEF	2.0E-I	***
TifB16107	4.4q-t	4.4q-t	4.2r-v	3.8t-x	3.2x-B	3.2x-B	2.2EFG	2.0E-I	1.6G-J	1.2J	***
Tifway	3.8u-y	3.8u-y	3.6w-z	3.0A-D	3.0A-D	3.0A-D	2.0E-I	2.0E-I	1.8F-I	1.0J	***
TifTuf	4.2s-v	4.2s-v	4.0t-w	3.2y-B	3.2y-B	3.0A-D	2.2E-H	2.0E-I	1.8F-I	1.0J	***
Celebration	2.4C-F ¶	2.4C-F	2.4C-F	2.2E-H	2.0E-I	2.0E-I	2.0E-I	2.0E-I	1.6G-I	1.0J	***
TifB16120	3.4x-A	3.4x-A	3.4x-A	3.0z-C	3.0z-B	3.0z-C	2.0E-I	2.0E-I	1.4IJ	1.0J	***
TifB16113	3.8u-y	3.8u-y	3.6w-z	3.2y-B	3.0A-D	3.0A-D	2.2E-H	2.0E-H	1.8F-I	1.0J	***
	***	***	***	***	***	***	***	NS#	***	***	

† Turfgrass quality was rated on a 1 to 9 scale where 9 was considered to have exceptionally high quality and 1 was considered to have exceptionally low quality

‡ Days of Drought (DOD)

§ Means accompanied by the same letter (upper and lower case) in the same column and row are not significantly different at the P = 0.05 level

¶ Upper case letter was used because the alphabetical groups exceed beyond 26 alphabets

NS nonsignificant at the 0.05 level, *, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 28. Mean leaf firing collected from 0 DOD to 16 DOD for the controlled environment study in 2020

				Leaf Firing †					
Cultivar	4-Feb	11-Feb	12-Feb	13-Feb	14-Feb	15-Feb	16-Feb	20-Feb	
DOD‡	0	7	8	9	10	11	12	16	
Latitude 36	9.0ab§	9.0ab	8.8ab	6.6f	5.4ij	5.0jk	4.4k-n	4.0m-p	***
OSU1337	9.0ab	9.0ab	8.8ab	5.6hi	4.8kl	4.6kl	4.2l-o	4.2l-o	***
OSU1439	9.0ab	9.0ab	9.0ab	7.4de	6.4fg	6.0gh	5.4ij	5.0jk	***
OSU1403	9.0ab	9.0ab	9.0ab	7.0ef	6.8f	6.0gh	5.8ghi	5.0jk	***
TifB16107	9.0ab	9.0ab	8.2bc	7.0ef	6.4fg	5.8ghi	5.2ijk	4.6klm	***
Tifway	9.0ab	9.0ab	9.0ab	6.8f	6.0gh	4.8kl	4.0m-p	3.8n-r	***
TifTuf	9.0ab	9.0ab	9.0ab	6.6f	5.8ghi	5.2ijk	5.0jk	4.2l-o	***
Celebration	9.0ab	9.0ab	8.0cd	5.2ijk	4.4k-n	3.4p-r	3.4p-u	3.2r-v	***
TifB16120	9.0ab	9.0ab	8.6abc	6.0gh	5.8ghi	4.6kl	4.0m-p	3.6o-s	***
TifB16113	9.0a	9.0a	8.8ab	6.6f	5.4ij	5.0jk	4.0m-p	4.0m-p	***
	NS¶	NS	***#	***	***	***	***	***	

† Leaf firing was rated on a 1 to 9 scale where 9 equaled no firing with fully green turf and 1 equaled most complete firing with straw-brown

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ NS nonsignificant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 29. Mean leaf firing collected from 17 DOD to 27 DOD 19 for the controlled environment study in 2020

Leaf Firing†											
Cultivar	21-Feb	22-Feb	23-Feb	24-Feb	25-Feb	26-Feb	28-Feb	29-Feb	1-Mar	2-Mar	
DOD‡	17	18	19	20	21	22	24	25	26	27	
Latitude 36	4.0m-p§	4.0m-p	3.8n-r	3.4p-u	3.4p-u	3.2s-v	2.6v-y	2.4w-z	2.0z-C	1.0F	***
OSU1337	4.2l-o	4.2l-o	3.6o-s	3.0s-w	3.0s-w	3.0s-w	2.6v-y	2.2y-C	1.8A-D	1.2DEF	***
OSU1439	5.0jk	4.8kl	4.2l-o	4.0m-p	3.8n-r	3.6o-s	3.4q-u	2.6v-y	2.2x-C	1.2DEF	***
OSU1403	5.0jk	5.0jk	4.8kl	4.0m-p	4.0m-p	4.0m-p	3.4p-t	3.0t-w	2.6v-y	2.0y-C	***
TifB16107	4.4k-o	4.4k-o	4.2l-p	3.6o-s	3.4p-u	3.4p-u	3.0s-w	2.4w-B	1.8z-D	1.2DEF	***
Tifway	3.8n-q	3.8n-q	3.4p-u	3.0s-w	3.0s-w	3.0s=w	2.0y-C	2.0z-C	1.8BCD	1.0F	***
TifTuf	4.2l-o	4.2l-o	4.0m-p	3.4p-u	3.4p-u	3.2s-v	2.4w-A	2.0z-C	1.8A-D	1.0F	***
Celebration	2.8u-x	2.8u-x	2.4w-A¶	2.2x-C	2.0y-C	2.0y-C	2.0y-C	2.0y-C	1.8z-D	1.0EF	***
TifB16120	3.4p-t	3.4p-u	3.2r-u	3.0s-w	3.0s-w	3.0s-w	2.0y-C	2.0z-C	1.6CDE	1.0F	***
TifB16113	4.0m-p	4.0m-p	3.6o-s	3.2r-u	3.0s-w	3.0s-w	2.2x-B	2.0z-C	1.6CDE	1.0EF	***
	***#	***	***	***	***	***	***	***	**	***	

† Leaf firing was rated on a 1 to 9 scale where 9 equaled no firing with fully green turf and 1 equaled most complete firing with straw-brown

‡ Days of Drought (DOD)

§ Means accompanied by the same small letter in the same column and row are not significantly different at the P = 0.05 level

¶ Upper case letter was used because the alphabetical groups exceed beyond 26 alphabets

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively.

Table 30. Mean Normalized Difference Vegetation Index (NDVI) collected over 6 rating days for the controlled environment study in 2020

	Normalized Difference Vegetation Index (NDVI)†						
Cultivar	4-Feb	14-Feb	16-Feb	20-Feb	24-Feb	28-Feb	2-Mar	
DOD‡	0	10	12	16	20	24	27	
Latitude 36	0.785bc	0.722gh	0.669jkl	0.559pq	0.506tuv	0.406ABC	0.349EF	***
OSU1337	0.789ab	0.706hi	0.664j-m	0.553pqr	0.487u-x	0.394BC	0.325FGH	***
OSU1439	0.793ab	0.730fgh	0.682ijk	0.580p	0.516stu	0.404ABC	0.345EFG	***
OSU1403	0.810a	0.759de	0.683ijk	0.573p	0.518st	0.414AB	0.360DE	***
TifB16107	0.787ab	0.740d-g	0.687ij	0.574p	0.502t-w	0.404ABC	0.342EFG	***
Tifway	0.785bc	0.735d-g	0.627no	0.511stu	0.445yz	0.350EF	0.301H	***
TifTuf	0.799ab	0.733efg	0.653lmn	0.541qrs	0.478vwx	0.380CD	0.320GH	***
Celebration	0.761cd	0.615o	0.567pq	0.473wxy	0.431zA¶	0.356DE	0.307H	***
TifB16120	0.787ab	0.733fgh	0.639mno	0.501t-w	0.447yz	0.349EF	0.299H	***
TifB16113	0.799ab	0.749def	0.658klm	0.528rst	0.470xy	0.380CD	0.341EFG	***
	*#	***	***	***	***	***	***	

† NDVI was measured using FieldScout® TCM 500 NDVI

‡ Days of Drought (DOD)

§ Means accompanied by the same letter (lower and upper case) in the same column and row are not significantly different at the P = 0.05 level

¶ Upper case letter was used because the alphabetical groups exceed beyond 26 alphabets

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 31. Pearson's correlation coefficient for Turf quality, Leaf firing and Normalized Difference Vegetation Index (NDVI) for the controlled environment study in 2020

Parameter	Turf Quality	Leaf Firing	NDVI
Turf Quality	1.00	0.97***	0.90***
Leaf Firing		1.00	0.90***
NDVI			1.00

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

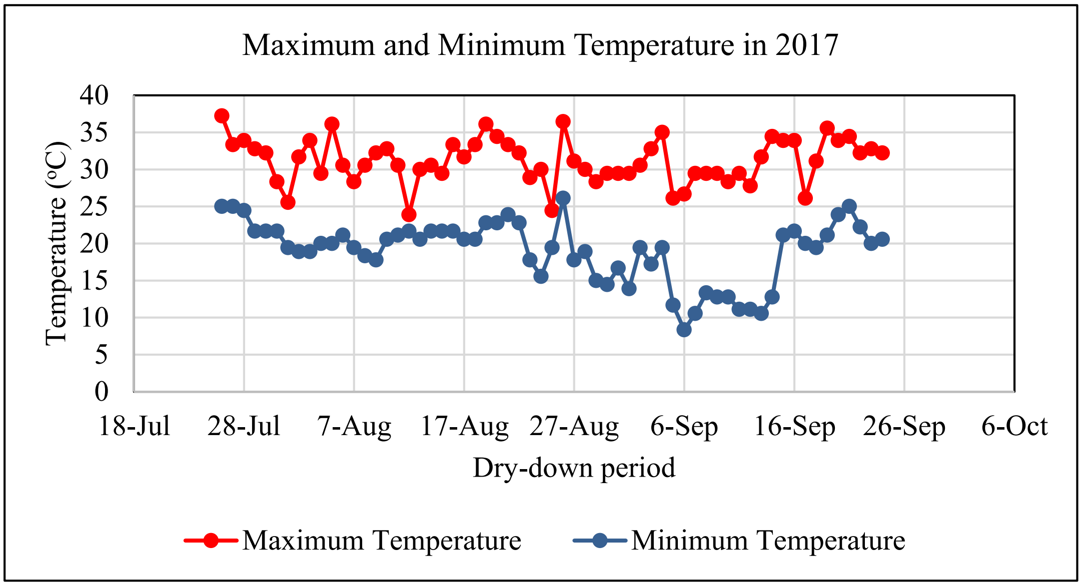

Figure 1. Average maximum and minimum air temperatures (°C) for the 60-day dry-down period (July 26 – September 24) in the field 2017

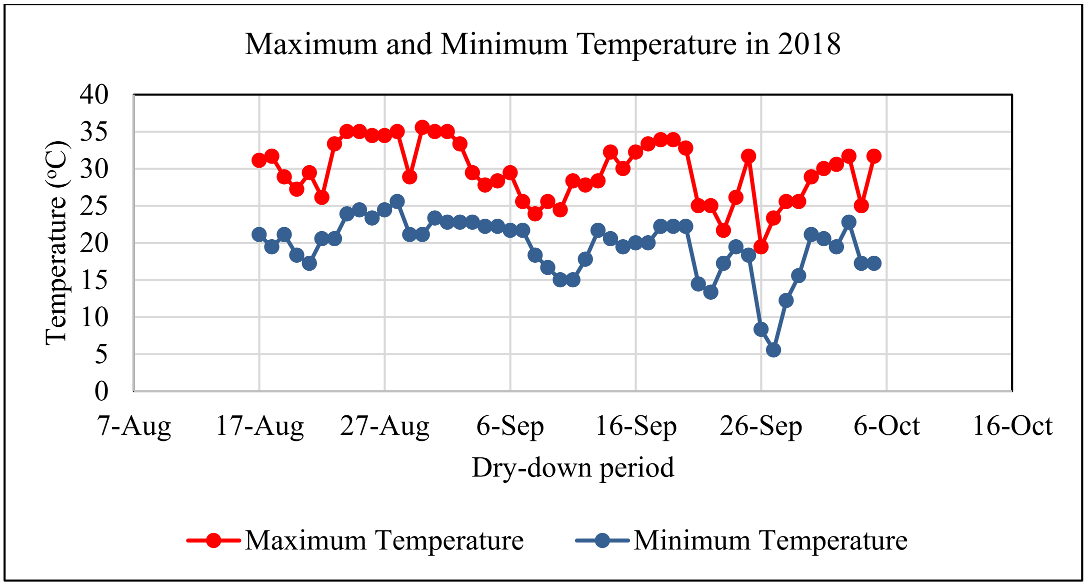

Figure 2. Average maximum and minimum air temperatures (°C) for the 49-day dry-down period (August 17 – October 5) in the field 2018

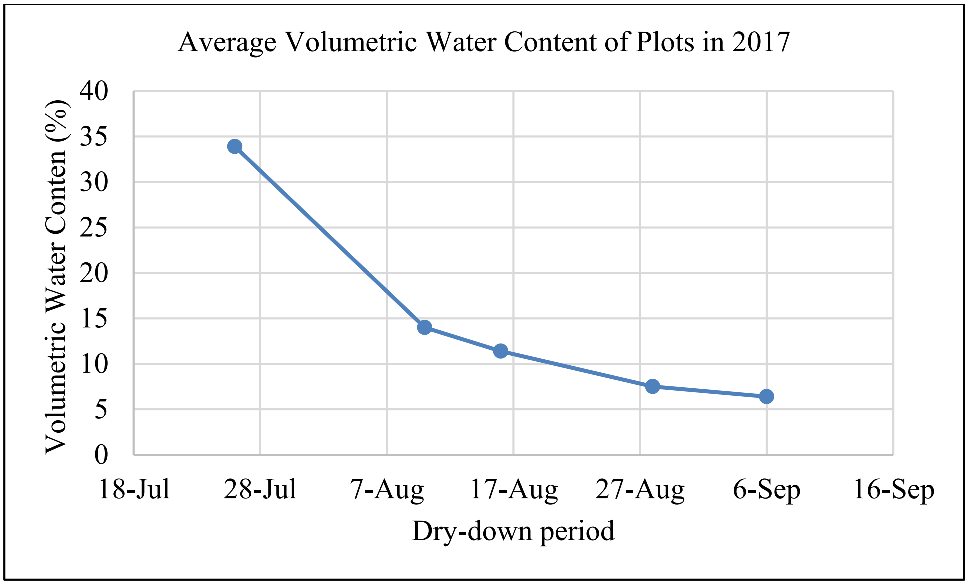

Figure 3. Average volumetric water content of bermudagrass plots measured during the 60-day dry-down period (July 26 – September-24) using FieldScout TDR 350 every seven days in the field in 2017

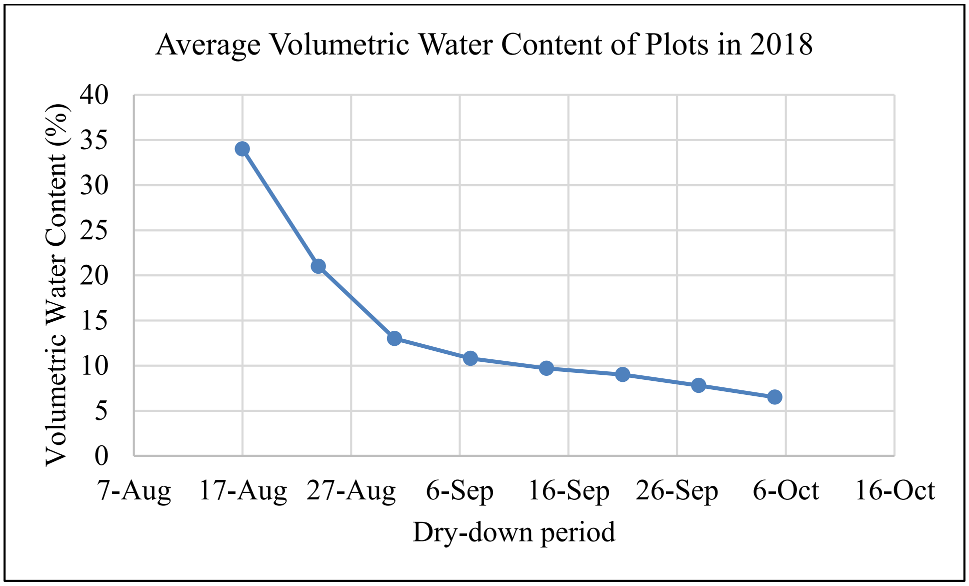

Figure 4. Average volumetric water content of bermudagrass plots measured during the 49-day dry-down period (August 17 – October 5) using FieldScout TDR 350 every seven days in the field in 2018

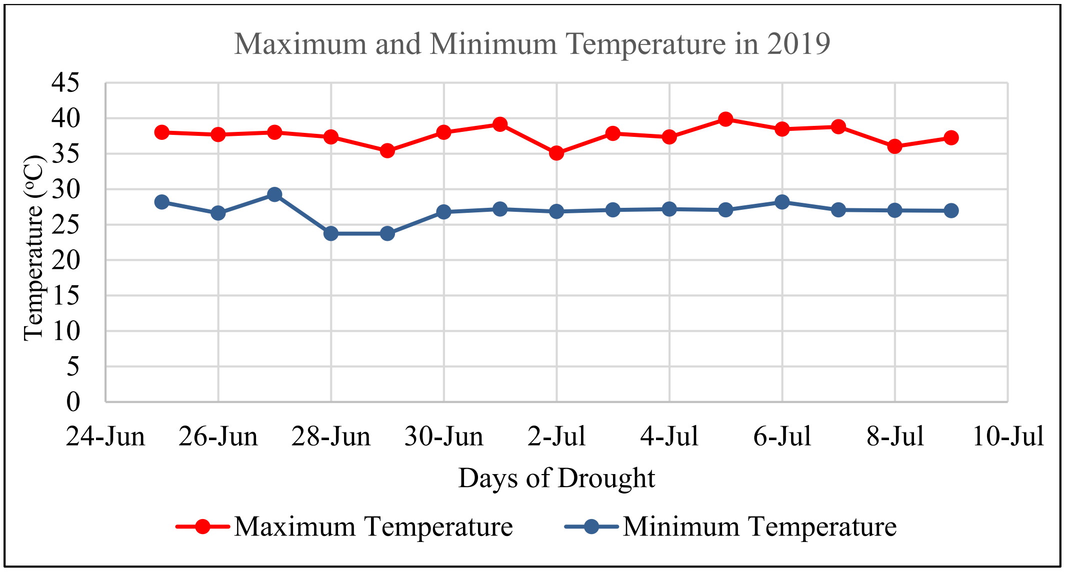

Figure 5. Average maximum and minimum air temperatures (°C) inside the controlled environment measured using WatchDog 2475 Plant Growth Station during the 14-d dry-drown period (June 25 – July 9) in 2019

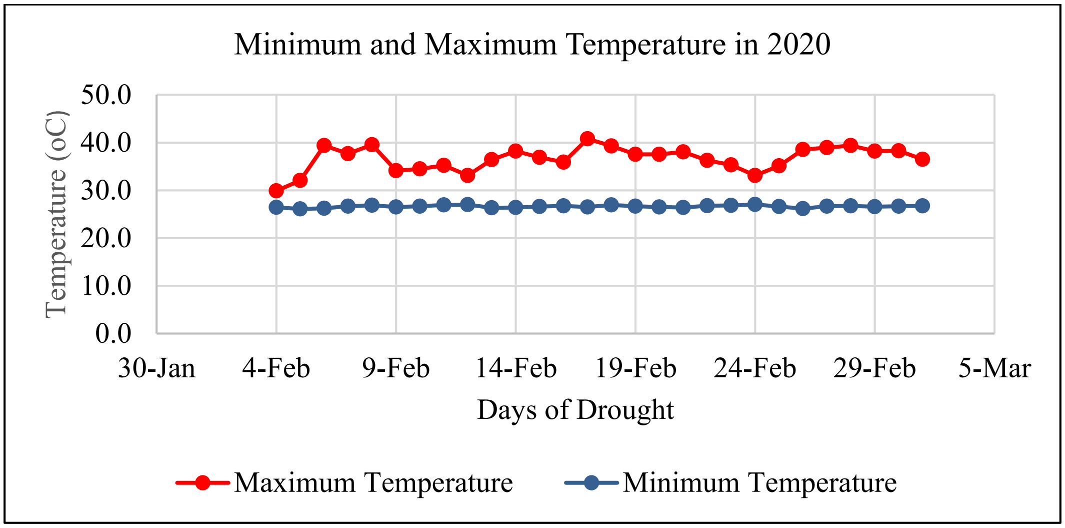

Figure 6. Figure 4. Average maximum and minimum air temperatures (°C) inside the controlled environment measured using WatchDog 2475 Plant Growth Station during the 27-d dry-down period (February 4 – March 2) in 2020

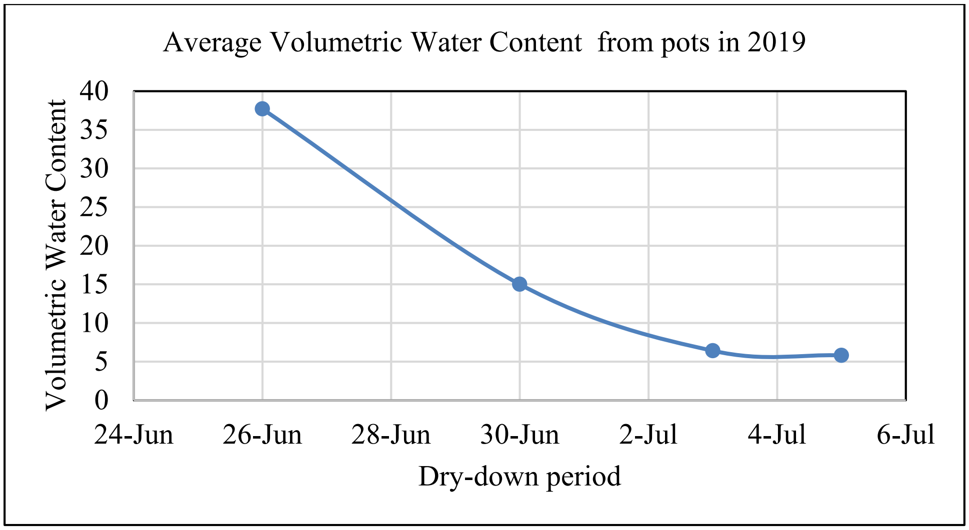

Figure 7. Average volumetric water content of pots measured during the 14-d dry-drown period (June 25 – July 9) in 2019 using HydroSenseTM moisture probe in the controlled environment in 2019

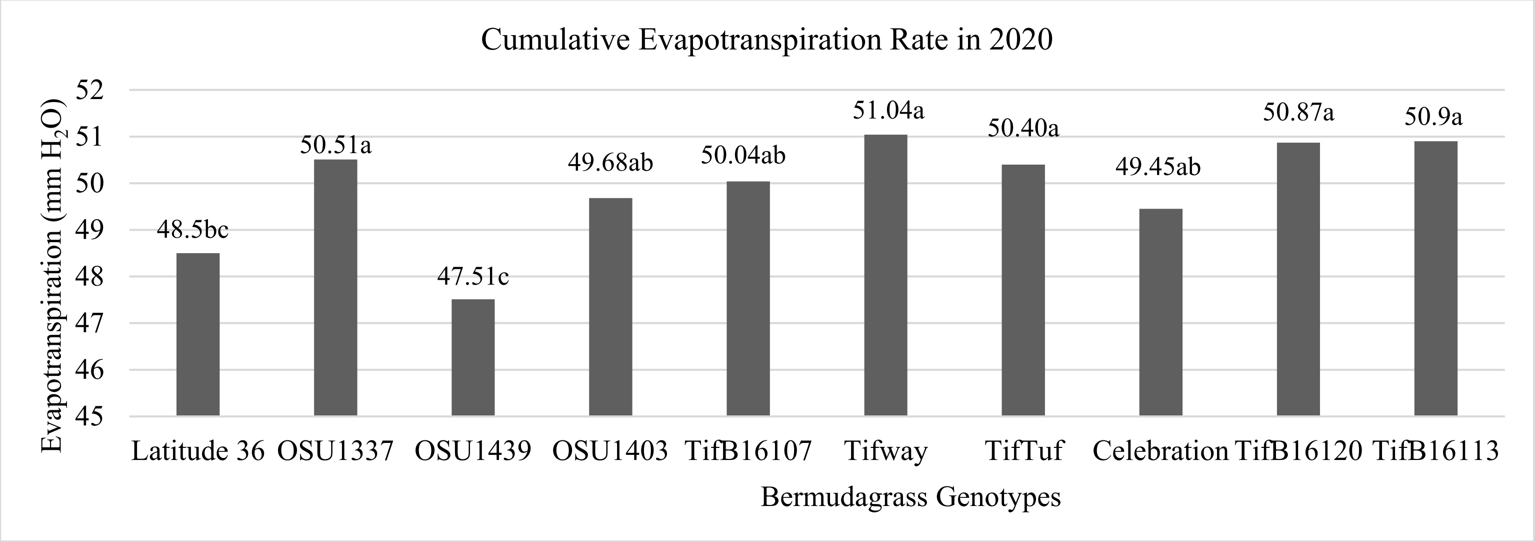

Figure 8. Cumulative evapotranspiration rate for the 27-day drought period (February 4 – March 2) in the controlled environment study in 2020. Mean data points followed by the same letter are not significantly different at Fisher's least significant difference test $p \leq 0.05$

CHAPTER V

CONCLUSION

Chapter 2 and 3

The southern United States and much of the transition zone is a geographic region not suitable for warm- and cool-season grasses as it is too cold in the winter for warm-season turfgrasses and too warm in summer for cool-season species. Bermudagrass, a warm-season turfgrass is widely grown in this region. However, turf loss from winterkill has a significant economic impact by negatively affecting the aesthetics and playability of recreational turf. Golf course superintendents spend considerable time and money on protecting the bermudagrass greens against harsh winter.

Oklahoma State University (OSU) has been actively engaged in bermudagrass breeding since the mid-1980s. One of the major goals of the breeding program is to develop bermudagrasses with improved freeze tolerance. Therefore, the goal of this study was to quantitatively estimate the freeze tolerance of newly developed bermudagrass genotypes based on their lethal temperature to kill 50% of the population (LT_{50}). The relative ranking of the genotypes in this study will help turfgrass breeders to select freeze tolerant phenotypes and improve the winter performance of future releases. The commercial release of these genotypes would help golf course facilities in the transition zone to convert their creeping bentgrass putting greens to bermudagrass putting greens without having the concerns of winterkill. The use of such genotypes would help in decreasing the threshold temperature for covering the golf course greens, which is currently -4.0°C, thereby reducing the labor cost involved. Also, the existing ultradwarf bermudagrasses used in putting greens have an extremely narrow genetic base, which could result in extensive damage from disease or insect pests. The freeze tolerant genotypes identified in this study could add to the narrow genetic diversity. Genotypes that can withstand the existing and potential pest endemics would be highly valuable to the turf industry.

Tahoma 31®, a newly released bermudagrass cultivar from Oklahoma State University, has been regarded as freeze tolerant due to its winterkill in the field. This research will be the first to provide an LT_{50} value for Tahoma 31 under controlled environment conditions. The accurate estimation of its LT_{50} values will enable Tahoma 31 to be used as a freeze resistant standard for future testing in the controlled environment conditions. The improved cold hardiness will also help in expanding the bermudagrass market in the northern states of the USA. Also, extension educators will be able to use the information on freeze tolerant bermudagrass genotypes to provide recommendations to end-users such as golf course superintendents, sportsfield managers, and homelawn owners in their cultivar selection process.

Chapter 4

Turfgrass is the major component in urban landscapes. Reducing water use of these landscapes is now a growing priority, emphasizing the need for turfgrasses with improved drought resistance. The objective of this study was to assess the drought response of four commercially available and six experimental bermudagrass genotypes in an unrestricted soil depth in the field and 17 cm pots under controlled environment condition. Our study found a range of drought performances among genotypes when subjected to 60 and 49 days of drought stress in the field in 2017 and 2018, respectively. The three experimental genotypes developed by the University of Georgia, TifB161107, TifB16113, and TifB16120, demonstrated superior drought resistance in both study years in the field by maintaining a turf quality above the minimum acceptable rating throughout the dry-down period. Recently released, 'TifTuf®' was the top performing commercial in the field. The experimental genotypes from Oklahoma State University, OSU1337, OSU1403, and OSU1439, were the relatively poor performing genotypes in the field trial and fell below the minimum acceptable TQ between 24-34 DOD in both the years. However, the results from the field study did not correspond well with controlled environment studies. The top performing genotypes in the field exhibited lower drought performance when grown in shallow 17 cm pots. This trend indicated that deep rooting would have played a critical role in minimizing drought stress in these genotypes. Genotypes capable of producing deep root when grown in unrestricted soil depth could lower the need for supplemental irrigation and conserve water resources.

One of the common issues with urban landscapes is shallow rooting depth because of the building process compacting soil. The compacted soil hinders root growth of turfgrasses, thus increasing the need for supplemental irrigation. Most research in the past have focused on screening bermudagrass genotypes for drought resistance under unrestricted soil depth, which may produce contradicting results when grown in shallow depths. Therefore, future drought resistant trials should test the genotypes at different soil depths. Also, measuring the root morphology, cuticle thickness, stomatal aperture, stomatal conductance, and solute accumulation would help in identifying the specific drought resistance mechanism adopted by the genotype.

The correlation of normalized difference vegetation index (NDVI) and canopy temperature with visual ratings in this study indicates that such technologies could be integrated for the faster screening of genotypes as it requires no prior training and is less time-consuming. On a larger scale, measuring NDVI using mobile sensor devices or unmanned aerial vehicles (UAV) would help turfgrass breeders to test a greater volume of genotypes.

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APPENDICES

Table 32. Maximum likelihood estimates of temperature parameter used to calculate the lethal temperature for 50% death (LT_{50}) for all genotypes from first batch

Replication	Cultivar	Temperature parameter estimate	Wald's chi - square	Pr > Chi-square
1	Champion D	-1.35	6.03	0.014
1	TifEagle	-0.6	9.29	0.002
1	OKC6318	-1.35	6.11	0.013
1	Tahoma 31	-1.35	6.11	0.013
2	Champion	-0.8	7.92	0.001
2	TifEagle	-1.35	6.1	0.014
2	OKC6318	-1.35	6.11	0.013
2	Tahoma 31	-0.87	9.76	0.002
3	Champion	0.95	8.04	0.005
3	TifEagle	-1.12	7.12	0.008
3	OKC6318	-0.83	9.49	0.002
3	Tahoma 31	-0.83	9.75	0.002

Table 33. Maximum likelihood estimates of temperature parameter used to calculate the lethal temperature for 50% death (LT_{50}) for all genotypes from second batch

Replication	Cultivar	Temperature parameter estimate	Wald's chi - square	Pr > Chi-square
1	Tahoma 31	-0.96	8.69	0.003
1	Champion	1.35	6.03	0.014
1	OKC0805	-1.6	5.22	0.022
1	OKC1609	-0.98	8.71	0.003
2	Tahoma 31	-0.96	8.69	0.003
2	Champion	-1.35	6.03	0.014
2	OKC0805	-0.96	8.64	0.003
2	OKC1609	-1.35	6.11	0.013
3	Tahoma 31	-0.96	8.56	0.003
3	Champion	-1.26	6.73	0.01
3	OKC1609	-1.35	6.11	0.013
3	OKC0805	-1.26	6.73	0.01

Table 34. Maximum likelihood estimates of temperature parameter used to calculate the lethal temperature for 50% death (LT_{50}) for all the genotypes from third batch

Replication	Cultivar	Temperature parameter estimate	Wald's chi - square	Pr > Chi-square
1	OKC3920	-0.87	9.76	0.002
1	OKC0920	-1.26	6.72	0.01
1	Tahoma 31	-1.12	7.17	0.007
1	Champion	-1.11	6.41	0.001
2	OKC3920	-0.96	8.69	0.003
2	OKC0920	-1.26	6.73	0.01
2	Tahoma 31	-1.26	6.73	0.01
2	Champion	-0.87	5.64	0.018
3	OKC3920	-1.26	6.73	0.01
3	OKC0920	-1.6	5.22	0.022
3	Tahoma 31	-1.27	6.73	0.01
3	Champion	-0.87	5.64	0.018

Figure 9. Conviron E8 growth chamber used for the freezing test in Chapter 2 and 3

Table 35. Maximum likelihood estimates of temperature parameter used to calculate the lethal temperature for 50% death (LT_{50}) for all genotypes tested in chapter 3

Replication	Cultivar	Temperature parameter estimate	Wald's chi - square	Pr > Chi-square
1	Tahoma 31	-1.12	7.17	0.007
1	Tifway	-0.98	8.69	0.003
1	OKC1873	-0.98	8.69	0.003
1	OKC1406	-1.26	6.67	0.01
2	Tahoma 31	-1.26	6.73	0.01
2	Tifway	-0.96	8.64	0.003
2	OKC1873	-0.96	8.64	0.003
2	OKC1406	-0.87	9.76	0.002
3	Tahoma 31	-1.26	6.73	0.01
3	Tifway	-0.96	8.64	0.003
3	OKC1873	-1.6	5.22	0.022
3	OKC1406	-1.27	6.73	0.01

Figure 10. The experimental genotypes from University of Georgia at 60 days after drought stress in 2017. From the left TifB16107, TifB16120 and TifB16113

Figure 11. The experimental genotypes from University of Georgia at 49 days after drought stress in 2018. From the left TifB16107, TifB16120 and TifB16113

Figure 12. The experimental genotypes from Oklahoma State University at 60 days after drought stress in 2017. From the left OSU1403, OSU1337 and OSU1439

Figure 13. The experimental genotypes from Oklahoma State University at 49 days after drought stress in 2018. From the left OSU1403, OSU1337 and OSU1439

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