

QUANTIFYING DROUGHT AVOIDANCE TRAITS IN ZOYSIAGRASS GENOTYPES

by

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(Under the Direction of DAVID JESPERSEN)

ABSTRACT

Zoysiagrass (Zoysia spp.) is an important warm-season turfgrass with varying drought resistance ability compared to other warm-season grasses and is becoming increasingly popular in Southeastern United States. With the increasing water shortages and imposed water restrictions in landscape irrigation, aggravated by frequent drought incidents, studying how warm-season turfgrasses respond to drought stress has become imperative. Zoysiagrass genotypes were tested for their drought avoidance ability based on morphological and physiological above-and belowground traits under drought stress conditions in both greenhouse and field environments. Genotypes differed for their drought avoidance traits like total root biomass, root to shoot ratio, relative water content, visual turf quality, evapotranspiration, and photosynthetic assimilation rates. The best performing genotypes used water more efficiently by stomatal regulation and maintained evapotranspiration rates, whereas poor performing genotypes, despite better root growth, had greater water use rates and experienced most drought symptoms on the final day of stress.

INDEX WORDS: Drought avoidance, Evapotranspiration, Root Length Density, Root to Shoot ratio

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CHAPTER 1

LITERATURE REVIEW

Zoysiagrass, (*Zoysia* spp.), is a type of hardy perennial warm-season turfgrass that forms dense and persistent ground cover and is well adapted to be used as turf for lawns, golf courses and athletic fields (Patton, Schwartz, & Kenworthy, 2017). It was introduced into United States in 1894 from east Asia and pacific islands and described as *Zoysia pungens* by Frank Lamson Scribner (Lamson-Scribner 1895). The species name *pungens* is a latin word for something that is sharp and pointed, which describes the morphology of leaf blades in these grasses. Later it got identified as *Zoysia japonica* (*Osterdamia japonica*) (Hitchcock 1920) was commonly called Japanese lawngrass and *Zoysia matrella* (*Osterdamia matrella*) (Childers and White 1947) commonly called Manila grass. The use of zoysiagrass is limited to *Zoysia japonica*, *Zoysia matrella* and *Zoysia pacifica* and interspecific hybrids of *Zoysia japonica* × *Zoysia matrella* and *Zoysia japonica* × *Zoysia pacifica* (Magni, Pompeiano et al. 2017). At present all these introductions come under the category of zoysiagrass as single common name in the US. (Patton, Schwartz, & Kenworthy, 2017). Japanese lawn grass develops thick and longer horizontal stems and is more coarse textured grass with light green leaves as compared to manila grass with denser ground cover and narrow leaves (Pompeiano, Grossi et al. 2012). Few important commercially available zoysiagrass cultivars include, ‘Zeon’, ‘Zorro’, ‘Empire’, ‘Zenith’, ‘Meyer’ and ‘El Toro’.

Zoysiagrass turf quality is considered highly valuable and has earned it the recognition of being one of the most beautiful turfs owing to its velvety dark green mat-like ground cover (Piper & Oakley, 1917; USDA Bureau of Plant Industry, 1905). It has protogynous flowers and is a highly

cross-pollinated grass like other warm-season grasses (Moser, Burson et al. 2004). It has a dense shoot growth and is mainly propagated vegetatively by stolons and rhizomes, but also as seed. It has wider adaptability to varying edaphic conditions and has superior wear, shade, drought and heat stress tolerance than cool-season grasses (Youngner 1961, Biran, Bravdo et al. 1981). Zoysiagrass doesn't grow well in poorly drained soils, and it goes dormant in prolonged dry soil conditions without irrigation (Beard 1973). One of the major limitations of zoysiagrass is its relatively slow establishment rate from sod plugs which has constrained its widespread use in the US (Magni, Pompeiano et al. 2017). One of the possible reasons behind this is relatively shallow root system in zoysiagrass when compared to other grasses. (R. Carrow, 1996; Qian, Fry, & Upham, 1997; Fuentealba et al. 2015). The slow growth rate also accounts for relatively low clipping yield or less vertical growth, when compared to bermudagrass (Trappe, Karcher et al. 2011). This however makes zoysiagrass a suitable option for commercial lawns and golf courses as it requires less frequent mowing and improved aesthetic qualities when compared to other warm-season grasses (Moser, Burson et al. 2004). In a study by (Gelernter, Stowell et al. 2017) it was estimated that as of 2015, zoysiagrass land coverage is around 10,375 ha in the U.S. Zoysiagrass acreage has increased at a high rate when compared to any other warm-season grass in the past decades. Although it is mainly planted in transitional climatic zone but in recent years with release of better cultivars it has also become increasingly popular in Southeastern U.S. (Patton, Schwartz et al. 2017).

Turfgrass area in the U.S. and landscape water use

In the continental United States, turfgrass is estimated to cover an area of 163,800 km² which makes approximately 1.9% of the entire area of continental United States and also accounts for

more than three times as much as any other irrigated crops, making turfgrass a dominating plant type in landscapes (Milesi, Running et al. 2005). Urban landscapes with turfgrass ground cover tend to reduce the impact of urban heat island effect which refers to the tendency of the urban area to be hotter than its surroundings by providing a cooling effect (Aydinsakir, Buyuktas et al. 2016; Spronken-Smith, Oke et al. 2000). It also prevents runoff, soil erosion and increases water infiltration rates compared to uncovered soil (Huang 2008). However, urban landscapes also require significant amounts of water for irrigation which impose a negative pressure on water resources.

In the United States, up to 9 billions gallons of water is used for irrigating home lawns and landscapes each day accounting for up to more than 70% of household water use (Warner, Diaz et al. 2022). Increasing frequency of summer droughts across the US has forced municipal regulation of water use in a timely manner (Hilaire, Arnold et al. 2008). For instance, the state of Georgia banned water use for outdoor landscape irrigation on September 17th, 2007, owing to a severe drought that season (Brown and Pharr 2007). This can largely be corelated to gradually increasing earth's average temperatures which could increase evapotranspirational water loss from landscapes and further aggravate the drought problem (Council 2008). From all these studies and research, it seems that one of the potential steps towards water conservation could be use of water-efficient turfgrass varieties for landscaping along with efficient irrigation practices that would avoid excessive irrigation.

Water use pattern in warm-season grasses

Turfgrass water use is a function of plant growth characteristics that involve root and shoot characteristics, root density, canopy density, vertical or lateral growth, soil edaphic factors, and environmental factors (Huang 2008). Under conditions with high ambient temperatures and vapor pressure deficits there is an increase in the water potential gradient between the soil and atmosphere which is more likely to intensify water consumption and impose water stress on turfgrasses. Water stress disrupts many cellular and whole-plant processes which could negatively affect growth and performance of turfgrasses. Water use in turfgrass is expressed as Evapotranspiration (ET), which comprises of the total amount of water required for its growth and transpiration, along with the evaporative loss of water from the underlying soil (Huang, DaCosta et al. 2014). Under non-limiting soil moisture conditions, warm-season grasses have an average ET rate ranging from 2 to 5 mm d⁻¹, which is relatively low as compared to cool-season grasses with an ET rate of 3 to 8 mm d⁻¹(Beard 1994). Among warm-season grasses, zoysiagrass (*Zoysia spp.*), bermudagrass (*Cynodon spp.*) and buffalograss (*Buchloe dactyloides*) have low evapotranspiration rates (Levitt 1980, Huang and Fry 2000).

Drought resistance mechanism

There are numerous environmental factors that might impose water stress on plants. Drought episodes can occur when there is little or no rain. The amount of time without rain during a drought event that results in significant water deficit is primarily determined by soil's water holding capacity and plant's ET rates (Kozlowski, Kramer et al. 2012; Jones 2013). During water stress, when the rate of evapotranspiration exceeds the water supply, plants will continue to lose water in absence of any supplemental irrigation. When plant water levels drop low enough, they will begin

to show visible symptoms of wilt and leaf firing (Zhang, Unruh et al. 2013). Drought resistance refers to the ability of plant species to survive periods of plant water deficit (Beard 1973). Studies on mechanism of drought resistance suggest that drought escape, drought avoidance, and drought tolerance are three possible response pathways exhibited in turfgrasses (Zhou, Lambrides et al. 2012; Turner 1986; Huang, DaCosta et al. 2014). During drought avoidance, plants maintain essential water levels by minimizing the water loss and increasing water uptake and hence avoid the situation of water deficit under water limiting conditions (Levitt 1980; Ludlow 1989). Plants exhibiting drought avoidance can develop traits like deep rooting for maximum water absorption, reduced transpiration rates to minimize water loss from leaves, leaf rolling to reduce the exposed leaf surface area, and high-water use efficiency (WUE) (Orcutt and Nilsen 2000). In the context of turfgrass, WUE is frequently defined as the ratio of clipping yield to total water use (ET) (Ebdon and Kopp 2004). In other words, it is defined as the amount of carbon fixed by photosynthesis per unit of water transpired (Lawson & Blatt, 2014). Studies on drought resistance strategies in warm-season grasses have found zoysiagrass and bermudagrass to display drought avoidance traits under drought stress conditions (Marcum, Engelke et al. 1995; Qian, Fry et al. 1997).

Zoysiagrass when exposed to severe drought, without any supplementary irrigation goes dormant (Moser et al. 2004). A relatively shallow root system is one of the reasons for early symptoms of leaf firing and wilting in zoysiagrass under drought stress as a result of early soil moisture depletion (Zhang, Poudel et al. 2019). Zoysiagrass is typically characterized as less drought resistant than bermudagrass but there is variation in rooting depth, irrigation requirement, and turf quality (Patton 2010). Also, among the zoysiagrass germplasm accessions in the U.S., the leaf texture varies drastically with cultivars ranging from coarse textured ‘Palisades’, ‘Empire’, ‘El Toro’, and ‘Zenith’ to narrow-leaved cultivars like ‘Zeon’, ‘Emerald’, ‘Diamond’, and ‘Zorro’ (National

Turfgrass Evaluation Program Trial 2002, NTEP.) There is a great potential to exploit this genetic and phenotypic variability in zoysiagrass germplasm especially among rooting traits like root length density (RLD) which is a measure of root growth and extensiveness, usually defined as total length of roots in a given soil volume, in order to screen and develop varieties that are sustainable in terms of water consumption and thus are resource efficient (Miller and McCarty 1998).

Drought tolerance involves mechanisms that help plants withstand periods of reduced tissue water potentials. Osmotic adjustment (OA) is one such technique that possibly helps plants with desiccation tolerance by the accumulation of compatible solutes in cells. As a result, solute potential decreases (becomes more negative) allowing plant cells to uptake water. The increased cellular water potential helps cells gain turgidity due to the pressure against cell walls. Therefore, OA protects membrane structures from damage caused by desiccation as it prevents drying of cells (Huang et al., 2014; Nilsen and Orcutt, 1996).

Response to Drought Stress - Zoysiagrass rooting pattern and Evapotranspiration (E)

In a study performed on 25 zoysiagrass genotypes a significant correlation was found between rooting characteristics and drought performance under both greenhouse and field conditions (Marcum, Engelke et al. 1995). Differences in rooting parameters in terms of average maximum rooting depth (AMRD), total root weight and root number at varying soil depth were found among different zoysiagrass genotypes, and future potential of improving drought resistant genotypes based on rooting characteristics through breeding programs was seen. ‘Meyer’, ‘El Torro’ zoysiagrass were among the better performing genotypes with greater AMRD, and greater root weight and root numbers. In another study by (Qian, Fry et al. 1997), comparing drought avoidance of tall fescue, bermudagrass and ‘Meyer’ zoysiagrass , it was reported that tall fescue had the

highest total root length (TRL) below 30 cm at a soil depth of 60-90 cm, and greater soil water depletion at 90 cm owing to its deeper root system, which made it a very good drought avoider. Among warm-season grasses, more than 50% of TRL was in the top 0-30 cm of soil. Meyer zoysiagrass was found to have a shallow root system and more frequent leaf wilting when compared to bermudagrass and buffalograss making it inferior drought avoider among others in the study. This result is contrasting to what was found in by (Marcum, Engelke et al. 1995). In another soil surface-level drought study, comparing shoot level responses among warm-season grasses, zoysiagrass and bermudagrass were the least drought resistant and PI 509018 seashore paspalum (*Paspalum vaginatum*) and Tifblair centipede grass (*Eremochla ophiuroides*) were equally superior in their drought performance (Huang, Duncan et al. 1997). Whereas in studies by (Carrow 1996), bermudagrass drought resistance ranking is usually stated from high to very high when compared to other warm-season grasses. Zoysiagrass ranked from low to very high, with ranking variation explained due to the use of different cultivars, assessment standards, and length of drought stress. Meyer had the lowest RLD and was associated with less genetic potential to tolerate edaphic stresses (Carrow 1996). In a 2012 study, a positive correlation was found between turfgrass visual quality and root length and mass density at greater soil depths of 25-40 cm, in bermudagrass and zoysiagrass genotypes (Rimi, Macolino et al. 2012). All these different studies have tried to explain cultivar and genotype differences in drought avoidance by studying rooting traits, but certain cultivar performance varied across these studies and further understanding of mechanisms affecting genotype response to stress is essential to get a better picture and solve discrepancies. Further research is needed to apply these results, especially for zoysiagrass genotypes that have broad range of turf quality and known differences in rooting depth (Fuentelba, Zhang et al. 2015). Studies comparing drought responses of Meyer and Emerald

zoysiagrass with other warm-season grasses, were not favorable for drought resistance in zoysiagrass, with Meyer having higher ET rates compared to bermudagrass (Kim and Beard 1988). However later studies on improved *Zoysia japonica* cultivars reported better drought performance with lower ET rates and less soil water consumption than bermudagrass and St. Augustine grass (Fuentelba, Zhang et al. 2016). However, *Zoysia japonica* and *Zoysia matrella* were found to have low root length density (RLD) at increased soil depths when compared to common bermudagrass, which could be a possible explanation for early symptoms of leaf wilting and firing in zoysiagrass under drought stress due to decreased access to soil water (Fuentelba, Zhang et al. 2015). Breeding programs aiming for selecting genotypes with low ET rates and greater RLD and depth in zoysiagrass, could provide genotypes with less soil water consumption and eventually less frequent irrigation (Patton, Schwartz et al. 2017).

Contrary to reduced shoot growth, a mild drought can stimulate root growth to access water from deeper layers under insufficient soil moisture conditions. Although more severe drought would still be detrimental to growth, this physiological response could be used to screen for plants with better drought avoidance under mild drought. The increased root depth and root to shoot ratio can be used as an important criterion to select for drought avoidance as it provides greater access to soil moisture (Kopp, 2013). Zoysiagrass has a great potential to reduce the amount of supplemental irrigation required as the ET rates have been found to be comparatively lower in modern cultivars of this species when compared to bermudagrass and St. Augustinegrass (Fuentelba et al. 2016). The studies by (Zhou et al. 2012) and (Jespersen & Schwartz 2018) provide further support to this by suggesting that among different zoysiagrass and bermudagrass genotypes screened for drought resistance, the top performers used water slowly by reducing the ET rates in the initial stages of water deficit and therefore had more available soil water in the later stage of stress. Whereas

species with higher ET rates along with deeper roots (and thereby greater access to soil water) may lead to greater soil water removal. There exists a potential to use this water usage pattern in selection for zoysiagrass varieties that are deep rooted but have lower ET rates. Present-day available zoysiagrass germplasm pool is highly variable in terms of root depth, which could be used to develop such varieties (Fumentalba et al. 2016). This combination will result in maintenance of water status and water uptake from the soil profile.

Physiological response to drought stress

Plant water deficit has been widely associated with impairment of photosynthetic responses and eventually plant growth process (Flexas, Bota et al. 2004). This photosynthetic response is used as a parameter to assess plant survival strategy in water stress conditions. One of the primary limitations to photosynthesis under drought stress is stomatal closure (Cornic 2000). Stomatal closure reduces CO₂ diffusion into the leaf carboxylation site, which has been identified as the main cause of reduced photosynthesis during drought (Cornic and Fresneau 2002). Relative water content (RWC) and leaf water potential are two important parameters employed to assess leaf hydration levels and the severity of water stress on plant systems. Stomatal conductance (gs) and photosynthetic responses to declining RWC depends on numerous factors like plant genotype, environmental conditions, and the intensity of drought stress (Tardieu and Simonneau 1998; Flexas, Escalona et al. 1999). There have been numerous studies to understand which physiological factors are directly causing reduction in photosynthetic rates, exploring metabolic impairment due to water stress, decreased mesophyll conductance owing to reduced CO₂ internal diffusion, along with stomatal closure (Flexas and Medrano 2002). However, no consensus has

been made on relative importance of each factor, but it has been tested by varying stress conditions, plant type and light intensities.

Conclusion

In the past, drought resistance studies have aimed to test for rooting capacities in various warm-season grasses and have successfully compared below ground root biomass with visual quality, ET rates, better water accessibility, and canopy responses (Zhang, Poudel et al. 2019; Marcum, Engelke et al. 1995; Carrow 1996; Jiang and Carrow 2005). There have been studies on water relations in zoysiagrass aiming for leaf level responses to stress and comparisons of morphological traits with ET rates (White, Engelke et al. 2001). However, there is a lack of a studies within zoysiagrass germplasm that compares underlying plant physiological responses in terms of shoot level responses to drought stress with below ground rooting pattern in a collection of zoysiagrasses within a single study. This would help understand basis of genotypic differences in drought response.

This thesis presents testing of recently developed zoysiagrass breeding lines against commercial standard cultivars for their drought avoidance abilities in both controlled environment studies and field experiments. The main objectives of this study were to:

1. Study morphological and physiological aboveground and belowground responses to drought stress in zoysiagrass germplasm under controlled environment conditions.
2. Evaluating drought avoidance of zoysiagrass breeding lines in field dry-down conditions and understand how drought stress affects visual quality, rooting growth and physiological responses in a single study.
3. Compare and contrast results based on the imposition of drought stress and environment plants were tested in.

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CHAPTER 2

QUANTIFYING DROUGHT AVOIDANCE AND ANALYZING ROOTING TRAITS IN NINE ZOYSIAGRASSES IN A GREENHOUSE STUDY ¹

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ABSTRACT

The objective of this study was to compare drought avoidance among zoysiagrass genotypes and to identify underlying morphological and physiological traits associated with improved drought performance. A total of nine genotypes, including 5 experimental breeding lines and 4 commercial cultivars were grown in greenhouse conditions and exposed to well-watered or drought stress treatment for 20 days in 2021 and repeated in 2022. Drought performance was evaluated by measuring change in percentage green cover, relative water content (RWC), photosynthetic assimilation rates, along with assessing total biomass production including total root dry weight, maximum rooting depth, specific root length and above ground biomass. Results found associations between visual quality and leaf hydration levels with higher drought avoidance abilities of certain genotypes and reduced water loss. 15-TZ-11777, 16-TZ-13161 were better performing genotypes among the experimental breeding lines and had higher RWC, better retention of percent green cover and had less declines in photosynthesis at the end of drought stress. Among commercial cultivars, Zeon and Meyer were better drought avoiders. Drought decreased turf quality, percentage green cover, photosynthetic performance more in *Zoysia japonica* than *Zoysia matrella* cultivars. *Z japonica* had higher resource allocation towards root biomass production and had greater above and below ground biomass, maximum root depth and more rapid declines in water content. A decline in maximum root depth within the drought treatment was observed when compared to control conditions. Studying underlying mechanisms controlling drought performance among genotypes and understanding how environmental conditions could affect drought performance within species will aid in the development of cultivars with reduced water requirements.

1. INTRODUCTION

Drought is a major abiotic stress that drastically affects turfgrass quality and overall performance particularly in semi-arid and arid regions (Pessaraki 2007). Due to increasing urbanization, water shortages and imposed water restrictions in landscape irrigation, aggravated by frequent drought incidents, studying how plant species respond to drought stress and how they use water has become a major concern in the U.S. (Patton 2010). There is a need to develop efficient water conservation strategies in turfgrass management. One of the most efficient ways to achieve this goal is by developing drought resistant turfgrass genotypes that use less irrigation and can maintain better turf quality. Various studies have tested the drought performance of warm-season turfgrasses both in field and controlled conditions, and underlying factors that may influence their performance (Huang 1999; Zhang, Unruh et al. 2013). Drought avoidance is a strategy utilized by plants under drought to sustain tissue water levels by improving plant water uptake or reducing the amount of water loss (Ervin and Koski 1998). Maintenance of leaf hydration levels may allow for plants to resist wilting and leaf firing and maintain quality during drought periods.

In zoysiagrass, previous studies have been conducted to measure drought avoidance based on rooting characteristics like maximum root depth, or total root dry weight at various soil depths (Marcum, Engelke et al. 1995). Another study evaluating vertical rooting pattern and turfgrass visual quality in both greenhouse and field conditions concluded that shallow rooting was one of the reasons for poor drought avoidance in Meyer zoysiagrass (Qian, Fry et al. 1997). The rates of root depth development have been shown to influence drought performance in bermudagrass (*Cynodon dactylon*), and *Z. matrella* and *Z. japonica* collections in a greenhouse study, in which bermudagrass had the highest rates of root development and more uniform RLD at lower depths compared to zoysiagrass which had shallower roots (Fuentelba 2015). While such comparative

studies among different warm-season turfgrass species are commonplace, zoysiagrasses have constantly been found to be on moderate side in terms of drought resistance (Carrow 1996; Patton 2010). However, extensive interspecific research on exploring relative drought avoidance among zoysiagrasses is lacking. There have been relatively fewer studies comparing different zoysiagrass species and cultivars based on aboveground physiological growth responses like photosynthetic assimilation, evapotranspiration, visual quality, and belowground rooting patterns together in a study. Newly developed breeding lines, need further exploration for assessing their potential to be released as drought resistant lines. In this study, five zoysiagrass (*Zoysia spp.*) experimental breeding lines developed by interspecific hybridization and four commercial cultivars (Empire, Meyer, Zeon, and Meyer) were used to quantify differences in their drought avoidance in greenhouse conditions. The collective goal of this study was to find a correlation between aboveground and below ground drought avoidance traits in a single study. The drought avoidance strategy exhibited by plants was tested based on visual traits like turf quality, percent green cover decline, photosynthesis rates, and the loss in leaf water content under prolonged drought, along with testing varietal differences in root and shoot biomass production. This comparison would contribute to understanding the role of various mechanisms involved in drought performance and aid in the development of cultivars with improved performance under water-limited conditions.

2. MATERIALS AND METHODS

2.1 Experimental Site description

This study was conducted in the Turfgrass Research and Education Center Greenhouses located on the University of Georgia Griffin campus, Griffin, Georgia. First trial was conducted in the Summer 2021 and repeated in Spring 2022. Nine zoysiagrass genotypes including five

experimental breeding lines from the UGA Tifton research station, 15-TZ-11777, 09-TZ-54-9, 16-TZ-13121, 16-TZ-13161, 16-TZ-13167 and four well established cultivars, Zeon, Palisades, Empire and Meyer were used in this study. Zoysia plugs were cut at 3 cm below the soil surface, washed free of all soil, and were transplanted into a 10 cm wide and 65 cm deep PVC tubes with bottoms covered with a landscape cloth. A 1:1 sand and calcinated clay (Turface, Profile Products) was used as the potting mixture. Plants were trimmed to 5 cm every week, well-watered three times a week and fertilized once a week with N-P-K (24-8-16) Miracle Gro soluble fertilizer (Scott's Miracle-Gro, Inc.). Plants were allowed to establish for a period of 45 days inside the greenhouse before the start of treatments. During the establishment and experimental periods, 1000-W high-pressure sodium lamps were set to provide supplemental light when natural lightning decreased to less than $400\mu\text{mol m}^{-2}\text{s}^{-2}$ within the 12 hr photoperiod inside the greenhouse. The measured mean greenhouse temperatures were ($36^{\circ}\text{C}/28^{\circ}\text{C}$) (maximum/minimum) and mean relative humidity was 70 % for Summer 2021 trail and ($35^{\circ}\text{C}/26^{\circ}\text{C}$) for Spring 2021 trial. Plants were either exposed to drought treatment by withholding water for a period of 20 days, or control treatments in which plants were maintained well-watered by watering three times per week.

2.2 Measurements

2.2.1 Leaf gas exchange

The leaf gas exchange parameters were measured using portable photosynthetic system (CIRAS-3, P.P. Systems). A PLC3 Universal Leaf Cuvette (25mm *18mm) was used to sample 3-4 fully expanded leaves and an LED (RGBW) light unit was placed on top to provide uniform light ($1000\mu\text{mol m}^{-2}\text{s}^{-1}$) inside the chamber. The values for photosynthetic assimilation (A),

evapotranspiration (E), stomatal conductance (g_s), water use efficiency (WUE) for the sampled leaves were recorded.

2.2.2 Relative Water Content

Leaf hydration level was determined by taking relative water content (RWC) values at 5, 10, 15, 20 days into drought by method described by (Barrs and Weatherley 1962). Fresh leaf tissue of approximately 0.25 g was taken from each pot and fresh weight was measured. The sample was then rehydrated in distilled water overnight at 4°C and weighed to get its turgid weight. The tissue was further oven dried at 72°C for 72 hours and weighed again to get its dry weight. RWC was calculated using the formula,

$$RWC = \frac{(fresh\ weight - dry\ weight)}{(turgid\ weight - dry\ weight)} * 100$$

2.2.3 Percentage Green cover

Overall turf quality was measured based on color, density, wilt and uniformity on a scale of 1 to 9, with 1 representing complete leaf firing and completely dried plants, 6 being minimum acceptable quality and 9 representing healthy, green dense ground cover (Krans and Morris 2007). Digital Image Analysis was performed to determine changes in percentage green cover under control and drought stress treatment throughout each trail. (Leinauer, VanLeeuwen et al. 2014). Digital images were taken at regular intervals using digital camera (CanonG9X, Cannon, Tokyo, Japan) and light box was used for uniform illumination. The pictures were subjected to color threshold via HSB values (hue saturation brightness) in ImageJ software (version 2.3.0/1.53f51) to measure the percentage green cover.

2.2.4 Biomass and rooting characteristics

At the end of the drought period, plant roots of control and drought treatment blocks were harvested and washed clean. All above ground biomass and rhizome sections were separated for each pot and oven dried at 72°C for 72 hours to get dry weights. For each pot, length of the longest root was measured with a ruler to analyze the differences in maximum root zone depth. Representative intact roots were then separated from the root zone of each pot, stored in 20% ethyl alcohol for further root analysis. A desktop scanner (EPSON) was used to get 2-D digital images of plant roots. Processed root images were subjected to Gia roots software (Galkovskyi, Mileyko et al. 2012) to estimate root parameters like surface area, total root length, average root width and specific root length. Specific root length (SRL) was calculated by dividing actual measured root length by root scan dry weight. The entire root section was divided into two halves of 32.5 cm each, and dry weights were taken for both top and bottom root section to analyze the variation in root density within the soil profile.

2.3 Experimental design and data analysis

The experiment was designed as a Randomized Complete Block Design with 9 genotypes, 2 treatments, and 3 replicates per treatment. The entire experiment was repeated in two separate trials. All data was subjected to analysis of variance in a mixed model, where genotype and treatment were considered as fixed effects with blocks taken as random effects, using SAS-JMP (version 16.0.0 (512340), SAS Institute Inc.). Due to significant year effects, years were analyzed separately. To account for genotypic differences at different sampling days, one-way ANOVA was performed for genotype by each sampling date and treatment. Means were compared using

Fisher's protected least significant difference ($P < 0.05$). Multivariate analysis was used to test correlation among different parameters. Significant differences were observed in drought performance of tested genotypes in both 2021 and 2022 greenhouse trials, and a significant trial effect was observed accounting for performance differences based on measured physiological and morphological traits across trials, therefore each trial was analyzed separately.

3. RESULTS

I. Physiological traits

3.1 Leaf gas exchange

3.1.1 *Photosynthetic assimilation (A):*

For both 2021 and 2022 trials, all main effects and genotype and treatment interaction effects for photosynthetic assimilation (A) were significant (Table I). In the 2021 trial, no significant difference was observed in mean A rate under control conditions with A rates ranging from 5.82 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ for 16-TZ-13161 to 4.13 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ for Meyer (Figure 1). A decline in A rate was observed for drought blocks as the dry down progressed with significant differences among breeding lines and cultivars at different sampling dates. Five days into the drought, Palisades and Empire had lowest A rates of 2.10 and 1.81 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ respectively and 16-TZ-13161 and 15-TZ-11777 were the top performers having higher A rates. On the final day of the drought stress, the greatest variation in photosynthesis was observed; Zeon, 15-TZ-11777 and 16-TZ-13161 were in top statistical group with significantly higher A rates of 1.43, 1.35 and 0.99 $\mu\text{mol m}^{-2} \text{sec}^{-1}$, respectively, whereas Empire, 09-TZ-54-9, Palisades, and Meyer had near zero or negative A rates. In 2022 trial, under control conditions, no genotype means were significantly different except for

16-TZ-13161 having highest A rate ($3.84 \mu\text{mol m}^{-2} \text{sec}^{-1}$) and Meyer having lowest A ($2.23 \mu\text{mol m}^{-2} \text{sec}^{-1}$) (Figure 2). Five days into drought stress, genotype means for drought treatment varied significantly, 16-TZ-13161, Meyer and Palisades had the highest A rates between $4.52 \mu\text{mol m}^{-2} \text{sec}^{-1}$ to $3.02 \mu\text{mol m}^{-2} \text{sec}^{-1}$, whereas Empire, Zeon, and 16-TZ-13121 had the lowest A rates ranging from 1.61 , to $0.71 \mu\text{mol m}^{-2} \text{sec}^{-1}$. At 10 DAT, means were not statistically different, however a similar trend was observed for Meyer, 16-TZ-13161 which along with 09-TZ-54-9 continued better performance with A rates ranging from 2.12 to $1.65 \mu\text{mol m}^{-2} \text{sec}^{-1}$ however, Palisades had drastic decline in A rate to $-0.09 \mu\text{mol m}^{-2} \text{sec}^{-1}$, and were bottom performers along with Zeon and 15-TZ-11777. On the final day of drought stress, all genotypes, except Meyer, had negative A rates and Palisades was the worst performer. Zeon and 15-TZ-11777 both had contrasting performance in terms of A rate decline across two trials; they were top performers in 2021 whereas had poor A rates in 2022 on final day of drought stress. 16-TZ-13161 was able to maintain higher photosynthetic assimilation rates consistently in both trials.

3.1.2 Transpiration (E) and Stomatal Conductance (gs)

For 2021 trial, no significant difference in evapotranspiration, stomatal conductance and WUE rates of different genotypes were observed, but drought pots had significantly reduced rates for the aforementioned traits when compared to control. Although not significantly different, trends in the data showed that, at 15 DAT (Figure 3), 15-TZ-11777, had the highest E rates of 1.03 compared to 16-TZ-13161 and 09-TZ-54-9 having lowest E rates of 0.46 . Meyer had the greatest WUE of 8.6 , 09-TZ-54-9, Empire and Palisades had lowest WUE of (2.22 - 0.64).

For the 2022 trial, on comparing E rates of genotypes within treatments, significant declines in E rates were observed for drought blocks in 2022. Under control conditions, 09-TZ-54-9 and Palisades had the highest mean E rate of $2.37 \text{ mmol m}^{-2} \text{ sec}^{-1}$ and $2.28 \text{ mmol m}^{-2} \text{ sec}^{-1}$ respectively; Empire, 16-TZ-13121, Zeon, and Meyer had lowest E rates in the range of $1.78\text{-}1.48 \text{ } \mu\text{mol m}^{-2} \text{ sec}^{-1}$. Under drought blocks, at the beginning of stress, no differences were observed in E rates among genotypes. However, at 5 DAT, 15-TZ-11777, 16-TZ-13161, Palisades and 16-TZ-13167 were in the higher statistical group with E rates ranging from $2.00\text{-}1.53 \text{ mmol m}^{-2} \text{ sec}^{-1}$, whereas 16-TZ-13121, Zeon, and 09-TZ-54-9 had the lowest E rates ranging from $1.02\text{-}0.37 \text{ mmol m}^{-2} \text{ sec}^{-1}$ (Figure 4). Maximum variation in E rates was observed at 15 DAT, 16-TZ-13167, Palisades and Empire had highest E rates in the range of $0.93\text{ - }0.79 \text{ mmol m}^{-2} \text{ s}^{-1}$, whereas Zeon, 15-TZ-11777, 16-TZ-13121, 09-TZ-54-9, and Meyer were in lowest statistical group with E rates ranging from $0.40\text{-}0.22 \text{ mmol m}^{-2} \text{ s}^{-1}$. On final day of drought stress, no significant differences were observed but 15-TZ-11777 and Palisades had highest recorded E rate of $2.01\text{-}1.98 \text{ mmol m}^{-2} \text{ s}^{-1}$; and Meyer and 09-TZ-54-9 had lowest E rates of $1.1\text{ - }0.72 \text{ mmol m}^{-2} \text{ s}^{-1}$. Zeon and 16-TZ-13121 were in the intermediate range for E rates. For stomatal conductance (gs) and WUE, no significant differences were observed under control conditions and at start of drought stress in the drought treatment. As drought stress progressed, a reduction in stomatal conductance and WUE was observed and gs rates were comparable to E rates at different sampling days. Maximum variation in gs was observed at 15 DAT in 2022, with 16-TZ-13167, Palisades and Empire having higher gs, ranging from $52.3\text{-}42.6 \text{ mmol m}^{-2} \text{ s}^{-1}$; whereas Zeon, 15-TZ11777, 16-TZ-13121 and Meyer had the lowest gs ($12\text{-}21 \text{ mmol m}^{-2} \text{ s}^{-1}$). For WUE, 09-TZ-54-9, Meyer, 16-TZ-13161 and 16-TZ-13121 had the greatest WUE at 10 DAT ranging from $4.9\text{-}4.5$, whereas Palisades, 15-TZ-11777 and Zeon had

the lowest WUE (-0.28-0.71). On final day of drought stress, although means were not significantly different, except Meyer (0.64) all genotypes had a negative WUE. WUE and gs rates for all genotypes during both trials are presented in Table 2 and Table 3 respectively.

II. Visual Traits and Water Content

Significant differences were observed in overall drought performance of genotypes based on drought avoidance traits during 20 days of drought treatment. The control pots were well watered throughout the drought treatment and sustained higher overall turf quality ratings of greater than 8, percentage green cover greater than 80%, and leaf RWC of more than 90%, so results will focus on differences in drought treated plants.

3.2 Relative water content (RWC)

For both trials, all main effects and two-way and three-way interactions between genotype, sampling date, and treatment were found to significantly affect RWC of genotypes (Table 4). In 2021, a range of responses were observed in RWC as the drought progressed. Five days into drought, 16-TZ-13161 and 16-TZ-13167 had highest RWC of 85.62% and 80.96% respectively, and as the drought progressed, 16-TZ-13161 shifted to being of intermediate performance (58.8%) and finally to being lowest among breeding lines at the end of drought (44.99%). At 5 DAT, Empire, Palisades, 16-TZ-13121 and Zeon had lowest RWC content ranging from 61.37 % to 69.01%. Towards middle of drought period, at 10 DAT, Zeon and 16-TZ-13121 were intermediate for RWC, however, Empire and Palisades continued to lose water and had the lowest RWC of

13.38 % and 21.04 %, respectively on final day of drought stress. On the other end, 16-TZ-13167, Zeon and 15-TZ-11777 had the highest RWC contents of 49.86 %, 48.06 %, and 44.4 %, respectively on final day of drought stress (Figure 5). In this trial, Zeon, and Meyer among cultivars and 15-TZ-11777, and 16-TZ-13167 among breeding lines were able to sustain higher leaf hydration levels under drought stress at the end of drought. Palisades and Empire lost the greatest amount of leaf RWC, with RWC < 25% at the end of drought and were the poorest performers.

In 2022, under control conditions, all genotypes maintained high leaf hydration levels with relative water contents maintained above 90% with no significant differences among genotypes. Under drought treatment as well, all genotypes had greater than 90% RWC at 0 DAT with RWC declining at variable rates as drought stress progressed. The highest statistical variation was observed at 10 DAT; Meyer and 16-TZ-13161 had highest RWC values of greater than 70%, whereas Zeon, 16-TZ-13121, Empire and Palisades, were poor performers with RWC values ranging from 40.73% to 58.21 %. At the end of drought stress (Figure 6), Meyer and 16-TZ-13161 continued to be the top performers (>40% RWC) whereas Zeon, 15-TZ-11777 and 16-TZ-13121 had the lowest RWC. Like the variation in A rate above, Zeon and 15-TZ-11777 had opposite trend in RWC across both trials as well. In 2021, Zeon and 15-TZ-11777 were two of the best performing, however in 2022 they had the lowest RWC values on final day of drought stress.

3.3 Percentage green cover:

In 2021 trial, under control conditions, no significant difference in genotype percentage green cover was observed. Genotype means for drought blocks were significantly different at start of drought stress, as ‘Empire’ and ‘Palisades’ had highest GC; 15-TZ-11777 had the lowest GC percentage. To account for these initial variations, change in percent GC was calculated to get

percent of initial GC left on final day of drought stress. Significant reductions in percentage green cover were observed as the drought treatment progressed. Zeon, 15-TZ-11777 and Meyer were top performing genotypes having highest percentage of GC left (40.5 - 27.5%) on the final day of drought stress. 16-TZ-13167, Palisades and Empire had lowest percent GC left (14 - 0.02%) (Figure 7). To observe how percentage GC varied on each sampling date by treatment, one-way analysis was done, and it showed maximum statistical differences among the genotypes at 14 DAT, 15-TZ-11777 had the highest GC (65.73%) followed by Zeon (65.06%) and 16-TZ-13161 at 61.26%, whereas Palisades and Empire had lowest GC at 34.0% and 3.84% respectively.

In 2022 trial, under control conditions, percentage GC values ranged from 92.87 % for Empire to 87.79 % for Meyer with no significant differences among genotypes. In drought blocks, maximum statistical differences were observed at 10 DAT, where 09-TZ-54-9, 15-TZ-11777 and 16-TZ-13161 had the highest GC percentages of greater than 80%. To account for initial variations, percentage of initial GC was compared and 09-TZ-54-9, 16-TZ-13161 and Meyer were top performers, having the highest percent of initial GC left on final day of drought stress (36-31%) (Figure 8). On the other end, 16-TZ-13121, Palisades, Empire and 15-TZ-11777 had lowest percent (17.1-12.6 %) of initial GC content left.

III. Morphological Traits

The ANOVA was significantly different for genotype and treatment main effect for all the analyzed root traits (

Table 5).

3.4 Biomass

In the 2021 trial, for both total root mass and the lower half of root zone biomass, Palisades had significantly higher biomass compared to all other genotypes under control treatment. However, under drought treatment, stress reduced root biomass in all genotypes to varying extent. Palisades and Empire were in top statistical group, 16-TZ-13161, 16-TZ-13167, 15-TZ-11777 were in the intermediate range while 16-TZ-13121, 09-TZ-54-9, Meyer, and Zeon were in the bottom range for total root biomass. The lower half of the root zone was measured for dry weights to see differences in root zone mass distribution among genotypes. Under drought treatment, Empire (0.81 g) and Palisades (0.73 g) had the highest root biomass in bottom half of the root zone which was approximately 31% of their total root zone biomass (Table 7). 16-TZ-13161 was in the intermediate range and all other genotypes, had relatively less biomass in lower half of the root zone ranging from 20-14% of their total root biomass. For aboveground biomass all genotypes were similar in the control treatment. In the drought treatment, however, Empire, and 16-TZ-13161 had significantly higher aboveground biomass compared to Zeon and Meyer (7.3-8.5g). Under well-watered conditions, for the rhizome and thatch layer, Palisades, 16-TZ-13121 and Empire had highest rhizome dry weights (20.2-17.7g), under drought treatment while Empire and 16-TZ-13121 were still in the top statistical group, rhizome weight reduced significantly for Palisades (10.6 g). Meyer, 15-TZ-11777, and 16-TZ-13167 (8-10 g) were in the bottom statistical group in both control and drought treatments. Significant differences were observed in maximum rooting depth of genotypes under control conditions, where 15-TZ-11777, 16-TZ-13161, 09-TZ-54-9, 16-TZ-13167, and Empire were in the top statistical group with greater maximum root depths ranging from (70-67.1 cm) whereas Zeon, 16-TZ-13121 and Meyer had shorter root depths. A trend of

reductions in rooting depth was observed under drought treatment for 15-TZ-11777, 16-TZ-13121, 16-TZ-13161, 16-TZ-13167 and Meyer when compared to control. 15-TZ-11777, 09-TZ-54-9, Empire had higher maximum root depth in range of 68.26-66.53 cm, whereas 16-TZ-13121, Meyer and Zeon had shorted root zone at depth ranging from 58-60 cm. All biomass data for 2021 trial are presented in (Table 7).

For the 2022 trial, on comparing genotype means within the control treatment for total root mass and root mass in lower half of the root zone, Palisades, 15-TZ-11777 and 16-TZ-13167 had significantly higher root mass compared to Meyer, Zeon, 16-TZ-13121, 16-TZ-13161, and 09-TZ-54-9. In the drought treatment, while Palisades, 15-TZ-11777 and 16-TZ-13167 remained in top statistical group for total root mass, there was a significant reduction in total root mass and more genotypic variation as compared to control. 16-TZ-13121 (0.89 g), Zeon (0.46 g), and Meyer (0.55 g) had the lowest total root mass (Table 8). 15-TZ-11777, Palisades had highest root biomass in lower half of root zone, accounting for approximately 30.7% to 21% of their total root zone biomass. On the other end, Zeon, Meyer and 16-TZ-13121, 16-TZ-13161 had the lowest biomass in lower half of their root zones ranging from 4.7% to 15.3%. Under control conditions, Empire (20.37 g) and 15-TZ-11777 (18.17 g) had the significantly higher mean aboveground biomass than Meyer, Zeon, 16-TZ-13161, and 16-TZ-13121 with lowest above ground biomass of 10.35 g, 11.99 g, and 12.51 g, and 13.77g respectively. Under drought conditions, an overall reduction in biomass was observed and Meyer and Zeon had the lowest above ground biomass of 8.67 g and 8.40 g, respectively.

Drought stress also reduced aboveground biomass in genotypes when compared to control treatment but higher biomass genotypes in control were also in top statistical group in the drought

treatment. In drought treatments, 15-TZ-11777 had significantly higher aboveground biomass of 16.34 g than 16-TZ-13161, Zeon and Meyer that had lowest aboveground biomass (8.4-9.7 g). In the drought treatment, 16-TZ-13167, 16-TZ-13121 and Palisades had significantly higher rhizome weights of 19.61g, 19.12g and 17.32g respectively, whereas Meyer had the lowest rhizome weight of 5.31g. Like in the 2021 trial, a significant reduction in the maximum root length was observed in genotypes under drought when compared to their control counterpart. Under drought conditions, there was no significant difference in maximum root depth among the genotypes, however the trend in genotypes shows that 09-TZ-54-9 and 15-TZ-11777 had deeper maximum root depths of 67.5 cm and 66.4 cm respectively, whereas 16-TZ-13121, Zeon and Meyer had shorter rooting depths of 55.03 cm, 50.80 cm, and 58.31 cm respectively. All biomass data for the 2022 trial are presented in (Table 8).

3.5 Root scan characteristics

For 2021 trial, high genotypic variability was observed for all measured root parameters (AGB-
Aboveground biomass

Table 6) but there was no significant treatment or interaction effect found. In 2021 trial, for average root width (root diameter), there was no difference found between control and drought treatment plots. On comparing genotype means, 15-TZ-11777 and 16-TZ-13167 had significantly highest average root width, whereas 16-TZ-13121, Meyer and Empire had lowest root width (Table 9). On comparing genotype means by treatment, no significant difference was seen under control, however under drought treatment a range of variation was observed. Zeon, 16-TZ-13121, Meyer, had the highest SRL, whereas Empire, and Palisades had lower SRL. Significant genotypic differences were observed in RLD. Empire, Palisades were significantly different from other genotypes and had highest RLD in the range of (0.17-0.12 cm/cm³), compared to Zeon, 16-TZ-13121 with RLD in the range of 0.07-0.06 cm/cm³. No genotypic variation was detected in network surface area and network volume. All root scan data for 2021 trial are presented in Table 9.

For the 2022 trial, high genotypic variability was observed for all measured root parameters but there was no significant treatment or interaction effect found, so only genotypic differences in root traits will be discussed (AGB-Aboveground biomass

Table 6). In terms of average root width, 16-TZ-13167, and 16-TZ-13121 were in the top statistical group (0.027-0.025 cm), and were significantly different from Empire, and 15-TZ-11777 (0.23-0.24 cm) that had lower average root width (Table 10). For SRL, the trend was similar for both control and drought treatments, however, like how drought effect reduced maximum root length, it also caused reduction in SRL in drought blocks compared to control. Zeon, Meyer and 16-TZ-13121 had significantly higher specific root length than 15-TZ-11777, Empire, 16-TZ-13167, and Palisades. Genotypes varied significantly based on their RLD (cm/cm^3); 15-TZ-11777, Palisades and Empire had the highest RLD in range of 0.183-0.174 cm/cm^3 , whereas Zeon, 16-TZ-13121 and Meyer had the lowest RLD values ranging from 0.06-0.05 cm/cm^3 . 16-TZ-13161 had an intermediate RLD value (0.9 cm/cm^3). On comparing genotypes for their root network surface area and network volume, significant difference was seen. 15-TZ-11777, Palisades, and 16-TZ-13167 had the highest network surface areas (3206.2-1720 cm^2) and network volumes (22.48-10.51 cm^3), whereas 16-TZ-13161, 16-TZ-13121, Meyer, and Zeon had lowest network surface areas ranging from (956.01-506.72 cm^2) and network volumes ranging from (7.17- 3.61 cm^3). The genotype variation trend was similar among treatments except that magnitude of network surface area and

network volume where drought plots had less magnitude compared to their control counterpart. All root scan data from 2022 is presented in (Table 10).

3.6 Root trait correlation analysis

In 2021, aboveground biomass, network surface area, network volume were positively correlated to total root mass, TRDW, RLD, and negatively correlated to SRL as expected (Table 11). In the 2022 trial, a significant correlation was found between different rooting traits, (Table 12) significant correlations were found between aboveground biomass and the lower half of the root zone dry weight (0.69), as well as total root mass being negatively correlated to SRL (-0.62) and positively correlated to RLD (0.68). Similar correlations were found between the lower half root zone biomass and SRL and RLD.

4. DISCUSSION

Drought avoidance traits are associated with plant's ability to maintain adequate hydration levels either by reducing water loss or increasing water uptake (Carrow 1995). Genotypes under two dry down studies exhibited a broad range of variation in their drought avoidance strategy in terms of RWC, which is an estimate of leaf's hydration levels. Certain genotypes had consistently higher RWC and overall better drought avoidance which included 16-TZ-13161 and Meyer, whereas 16-TZ-13121, Palisades and Empire were consistently poor performers with relatively lower leaf hydration levels at end of drought stress across both greenhouse studies. 15-TZ-11777 was a better performer in 2021 but showed poor shoot level responses in the 2022 trial. This difference in performance could be due to varying environmental conditions, planting variation or other factors. Low RWC in Empire and Palisades as the drought progressed could have resulted in an earlier

onset of leaf firing and greater loss of green cover. Reduced leaf water content and decline in green cover by degradation of chlorophyll has been found as prominent symptoms of water deficit in turfgrass along with leaf firing (DaCosta, Wang et al. 2004; Aydinsakir, Buyuktas et al. 2016). 16-TZ-13161 and Meyer maintained higher tissue water levels by utilizing efficient drought avoidance strategies compared to other lines. Some genotypes that were consistently able to maintain higher green cover in two trials were 16-TZ-13161, 15-TZ-11777, whereas genotypes with consistently less green cover at final day of drought stress were Empire, and Palisades. The trend of genotypic differences based on RWC and percentage green cover at final day of drought stress was comparable for most of the genotypes.

Water stress can reduce photosynthesis by causing stomatal closure. The most effective technique to prevent water loss in water limiting conditions is to close stomata; however because of restrictions on CO₂ diffusion into the leaves, this also affects net photosynthetic rates (Lawlor and Cornic 2002). In this study, we analyzed changes in leaf gas exchange parameters like g_s , WUE, A and leaf E rates when exposed to drought. Drought stress led to reduction in photosynthetic rates (A) to varying extents along with declines in g_s indicating that stomatal closure may have caused the reductions in photosynthesis (Hu, Wang et al. 2009). In the 2021 trial, higher A_{max} rates in 16-TZ-13161, 15-TZ-11777, Meyer and Zeon were comparative to their higher RWC for the initial 15 days of drought stress. Whereas genotypes like Empire, Palisades, 09-TZ-54-9, with low RWC also had almost zero to negative A rates at 15 DAT and final day of drought stress. Low RWC (<30%) on final days of drought, and consistently poor performance in visual ratings, as well as large root system indicates they may have used their plant available water. Compared to 2022, E and g_s were not significantly different among genotypes in 2021, In the 2022 trial, Meyer was

better performer in terms of their leaf gas exchange responses to drought stress and had maintained higher A rates on final day of drought stress. Significantly higher E and gs rates in Palisades than Zeon, Meyer, 15-TZ-11777, 16-TZ-13121 at 15 DAT and subsequently lowest A rates at 20 DAT, compared to Meyer, Zeon indicates that initially higher E rates in Palisades may have caused more water loss and eventual decline in A rate further into the drought. Higher WUE and lower water use has been associated with better drought resistance in bermudagrass species compared to seashore paspalums (Zhou et al., 2012). These results are also supported by similar study on zoysiagrasses, in which one of the top performing genotypes-15-TZ-11777, maintained low gs and E at the beginning of drought but also didn't have sharp declines at the end of stress, with an overall strategy to conserve more water for longer duration under drought (Jespersen and Schwartz 2018). High E rates indicate that they were actively transpiring and losing water (also evident from reduced RWC) and ultimately caused a reduction in carbon fixation rates as leaves dehydrated. Previous studies have also found that A declines due to decreasing RWC in plants under water stress (Lawlor 2002). A sharp decline in the A rate in Palisades at 10 DAT, was comparable to its decline in RWC, and its eventual loss of GC. Considering Meyer and 16-TZ-13161, were top performers with >45% RWC, >30% GC; higher A rates towards end of drought stress; along with lower water use (low E and gs rates) and were able to avoid drought stress better. This is in tune with increased WUE in wheat (*Triticum spp.*) under drought stress conditions owing to reduced ET rates (Farooq, Hussain et al. 2012). Ultimately, plants with enhanced WUE which were better able to regulate stomata appear to have performed the best in these trials.

Drought stress caused a decline in biomass production, and drought treated plants had less shoot and root biomass compared to control. In drought treatment, Palisades, 15-TZ-11777, and Empire

had highest total root biomass and higher root mass in lower areas of pots. This focus on rapid growth and production of deep tissues may have resulted in great water use rates, and a strategy that focuses on extracting more water than reducing transpiration. Root traits like root depth, root biomass, RLD are considered important factors for drought avoidance and are usually associated with a plant's ability for greater water uptake under drought conditions (Chourasia 2017; Aslam, Maqbool et al. 2015). 16-TZ-13161 and Meyer had intermediate and low root and shoot biomass, respectively; rooting depths of 63.39 and 58.31cm and low RLD. Higher leaf hydration levels by reduced ET rates and better WUE, as well as low RLD and less root biomass reflects those plants avoided extreme water deficit conditions by regulated above ground responses. Meyer has been found to have low total root biomass, low RLD compared to other *Zoysia japonicas* and other warm-season grasses (Fuentealba, Zhang et al. 2015; Carrow 1996). In previous studies RLD has been used to measure root extensiveness (Carrow 1996) and a positive correlation has been found between plants' water uptake rate and available soil water under control conditions (Huang 2000). In this study, 15-TZ-11777, Palisades and Empire had higher RLD (0.18-0.15 cm⁻²) and may have led to greater water uptake and reduced resistances in conductive tissues.

5. CONCLUSION

In summary, zoysiagrass genotypes varied in their leaf gas exchange, aboveground shoot responses along with root biomass and distribution. While some genotypes like 16-TZ-13161 and 15-TZ-11777 had favorable shoot and root responses that helped in their better avoidance of drought, cultivars like Palisades, Empire had poor drought performance despite better root growth responses. Maximum root growth and eventual access to more soil water has been previously studied as an indicator of better drought performance, but restricted growth in pots in a controlled

environment could have been the reason for their poor performance. These results may be different under field conditions. The amount of water absorbed from the soil and water lost through evapotranspiration governs the water balance in plants. So, genotypes with better drought avoidance had better regulation of water use by maintaining low to intermediate evapotranspiration rates throughout the stress periods that helped them avoid situation of extreme plant water deficit. Results may have been influenced by the limiting soil volume afforded by pots in greenhouse studies, and additional research is needed in field conditions to confirm differences among the genotypes.

6. TABLES AND FIGURES

Table 1: ANOVA results for leaf gas exchange parameters of nine Zoysiagrass genotypes during two greenhouse experiments in 2021 and 2022 at $p \leq 0.05$ level of significance

Effect	2021				2022			
	A	E	gs	WUE	A	E	gs	WUE
	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Genotype (G)	<.0001*	0.7236	0.0532	0.0713	<.0001*	<.0001*	<.0001*	0.1457
Days after treatment (DAT)	<.0001*	<.0001*	0.0054*	0.0001*	<.0001*	<.0001*	0.0036*	0.0002*
Treatment (T)	<.0001*	0.0003*	0.0017*	0.2344	0.0006*	0.0803	0.0150*	0.1772
G*DAT	0.9065	0.2538	0.9734	0.7756	0.0018*	0.9503	0.98	0.5219
G*T	<.0001*	0.0019*	0.0001*	0.2909	<.0001*	0.0954	0.1216	0.0869
DAT*T	<.0001*	<.0001*	0.0054*	0.0001*	<.0001*	<.0001*	0.0036*	0.0002*
G*T*DAT	0.9065	0.2538	0.9734	0.7756	0.0018*	0.9503	0.98	0.5219

A- photosynthetic assimilation, E-evapotranspiration, gs-stomatal conductance, WUE-water use efficiency

Table 2: Stomatal conductance (gs) rates of nine zoysiagrass genotypes at 0,5,10,15, and 20 days after drought stress during 2021 and 2022

Genotype	Stomatal Conductance (gs) (mmol m ⁻² s ⁻¹)									
	2021					2022				
	0 DAT	5 DAT	10 DAT	15 DAT	20 DAT	0 DAT	5 DAT	10 DAT	15 DAT**	20 DAT
09-TZ-54-9	185 a	211 a	48 a	30 a	20 a	172 a	124 a	128 a	23 bcd	112 a
15-TZ-11777	135 a	120 a	50 a	80 a	120 a	166 a	194 a	116 a	13 d	188 a
16-TZ-13121	121 a	137 a	24 a	57 a	46 a	72 a	34 a	65 a	17 d	144 a
16-TZ-13161	108 a	145 a	50 a	31 a	130 a	87 a	149 a	83 a	26 bcd	115 a
16-TZ-13167	218 a	119 a	73 a	41 a	29 a	241 a	142 a	122 a	52 a	184 a
Empire	162 a	75 a	16 a	52 a	96 a	113 a	120 a	105 a	42 abc	142 a
Meyer	89 a	112 a	57 a	44 a	117 a	48 a	102 a	77 a	21 cd	51 a
Palisades	176 a	126 a	7 a	50 a	25 a	211 a	148 a	113 a	45 ab	178 a
Zeon	107 a	75 a	35a	43 a	48 a	64 a	63 a	85 a	12 d	151 a

** significant at $p \leq 0.05$ level of significance

Table 3: Water use efficiency (WUE) of nine zoysiagrass genotypes at 0,5,10,15, and 20 days after drought stress during 2021 and 2022

Water Use Efficiency (WUE)										
Genotype	2021					2022				
	0 DAT	5 DAT	10 DAT	15 DAT	20 DAT	0 DAT	5 DAT	10 DAT**	15 DAT	20 DAT
09-TZ-54-9	6.62 a	6.5 a	9.75 a	2.22 a	3.94 a	11.25 a	5.27 a	4.93 a	1.39 a	-1.77 a
15-TZ-11777	9.0 a	8.98 a	16.84 a	4.72 a	1.82 a	5.83 a	3.48 a	0.69 bc	0.13 a	-2.15 a
16-TZ-13121	7.25 a	6.3 a	2.07 a	4.36 a	4.02 a	9.26 a	2.78 a	4.52 ab	2.92 a	-1.33 a
16-TZ-13161	8.43 a	9.74 a	18.20 a	3.7 a	1.78 a	8.15 a	7.59 a	4.64 ab	7.63 a	-0.34 a
16-TZ-13167	6.47 a	6.96 a	7.17 a	2.68 a	1.91 a	4.42 a	5.49 a	2.17 abc	2.50 a	-2.45 a
Empire	7.28 a	4.26 a	5.58 a	0.69 a	1.72 a	18.45 a	5.05 a	1.71 abc	-0.18	-2.14 a
Meyer	7.22 a	7.44 a	11.81 a	8.63 a	1.56 a	9.33 a	9.19 a	4.74 a	4.57 a	0.64 a
Palisades	7.28 a	4.17 a	8.89 a	0.64 a	0.47 a	6.02 a	6.86 a	-0.28 c	-0.32 a	-1.84 a
Zeon	9.23 a	6.07 a	5.38 a	4.51 a	2.62 a	1.43 a	1.96 a	0.71 a	3.09 a	-0.22 a

** significant at $p \leq 0.05$ level of significance

Table 4: ANOVA for relative water content and percentage green cover during dry down in 2021 and 2022 at $p \leq 0.05$ level of significance

Effect	2021		2022	
	RWC	GC	RWC	GC
	Prob > F	Prob > F	Prob > F	Prob > F
Genotype (G)	<.0001*	<.0001*	<.0001*	<.0001*
Days after treatment (DAT)	<.0001*	<.0001*	<.0001*	<.0001*
Treatment (T)	<.0001*	0.0011*	<.0001*	0.0001*
G*DAT	<.0010*	0.0021*	<.0001*	0.0543
G*T	<.0001*	<.0001*	<.0001*	<.0001*
DAT*T	<.0001*	<.0001*	<.0001*	<.0001*
G*T*DAT	<.0010*	0.0021*	<.0001*	0.0543

RWC-relative water content, GC-percent green cover

Table 5: ANOVA results for biomass traits of nine zoysiagrass genotypes during 2021 and 2022 at $p \leq 0.05$ level of significance

2021		AGB	Total root mass (g)	Rhizome dry wt.(g)	Max root depth (cm)	Top half root zone dry wt. (A)	Bottom half root zone dry wt. (B)
Effect	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Genotype	0.0106*	<.0001*	0.0006*	0.0008*	0.0002*	<.0001*	
Treatment	0.0010*	0.839	0.1918	0.0107*	0.991	0.5432	
G*T	0.8288	0.7505	0.2933	0.7028	0.8408	0.5453	
2022		AGB	Total root mass	Rhizome dry wt.	Max root depth	Top half root zone dry wt. (A)	Bottom half root zone dry wt. (B)
Effect	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Genotype	<.0001*	<.0001*	0.0010*	0.0724	<.0001*	<.0001*	<.0001*
Treatment	<.0001*	0.0015*	0.0171*	0.0013*	0.0019*	0.0112*	
G*T	0.5513	0.387	0.5606	0.6422	0.4908	0.3911	

AGB-Aboveground biomass

Table 6: ANOVA results for root scan traits of nine Zoysiagrass genotypes during 2021 and 2022 at $p \leq 0.05$ level of significance

2021	RLD	ARW	SRL	NSA	NV
Effect	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Genotype (G)	0.0439*	<.0001*	0.0136*	0.0974	0.1035
Treatment (T)	0.8568	0.0684	0.0174*	0.2372	0.227
G*T	0.6213	0.1836	0.6482	0.5452	0.6122

2022	RLD	ARW	SRL	NSA	NV
Effect	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Genotype (G)	<.0001*	0.0398*	<.0001*	<.0001*	<.0001*
Treatment (T)	0.8114	0.0825	0.8991	0.1636	0.1467
G*T	0.8462	0.8464	0.7979	0.9876	0.9429

RLD-root length density, ARW-average root width, SRL- specific root length, NSA-network surface area, NV-network volume

Table 7: Biomass data for nine zoysiagrass genotypes in control versus drought treatment during two greenhouse experiments in 2021.

Genotype	Biomass - 2021									
	Total root mass		Bottom-half dry wt.		AGB		Rhizome dry weight		Max root depth	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
09-TZ-54-9	0.9 b	1.03 cd	0.13 b	0.15 c	10.79 a	9.94 bc	13.73 bcd	11.12 bc	67.4 ab	68.3 a
15-TZ-11777	1.7 ab	1.45 bc	0.26 b	0.22 c	14.04 a	10.81 ab	10.23 cd	9.44 c	70.1 a	66.7 ab
16-TZ-13121	1.3 b	1.04 cd	0.22 b	0.13 c	15.09 a	11.00 ab	19.64 a	20.00 a	63.3 bc	60.01cd
16-TZ-13161	1.2 b	1.75 b	0.28 b	0.36 b	15.18 a	12.36 a	14.60 bc	10.67 bc	69.7 a	66.0 abc
16-TZ-13167	1.8 ab	1.48 bc	0.37 b	0.22 c	12.34 a	9.10 bcd	9.68 d	10.52 bc	67.1 ab	60.4 bcd
Empire	1.9 ab	2.44 a	0.55 ab	0.81 a	13.97 a	12.45 a	17.28 ab	16.03 ab	67.1 ab	66.5 ab
Meyer	1.3 b	0.95 d	0.31 b	0.14 c	10.86 a	8.54 cd	9.20 d	7.19 c	65.9 ab	59.0 d
Palisades	2.8 a	2.54 a	0.94 a	0.73 a	16.99 a	10.51 abc	20.24 a	10.60 bc	63.7 ab	62.4 abcd
Zeon	0.8 b	0.76 d	0.12 b	0.13 c	8.43 a	7.32 d	13.46 bcd	10.78 bc	59.2 c	58.0 d

AGB-Aboveground biomass. All dry weights are measured in gram (g) units and root depth is measured in cm units.

Means connected by common letter are not significantly different within the genotypes at $p \leq 0.05$ level of significance

Table 8: Biomass data for nine zoysiagrass genotypes in control versus drought treatment during greenhouse experiment in 2022

Genotype	Biomass 2022									
	Total root mass		Bottom-half dry wt.		AGB		Rhizome dry wt.		Max root depth	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
09-TZ-54-9	1.89 c	1.61 bcd	0.38 c	0.38 bc	14.5 bcd	12.1 b	12.08 bc	12.95 bc	67.52 a	67.5 a
15-TZ-11777	4.27 a	2.83 a	1.30 a	0.88 a	18.1 ab	16.3 a	19.05 ab	12.24 c	68.05 a	66.4 a
16-TZ-13121	1.23 c	0.89 de	0.17 c	0.06 d	13.7 cde	10.8 bcd	16.37 abc	19.12 ab	65.93 a	55.0 a
16-TZ-13161	1.34 c	1.11 cde	0.19 c	0.17 cd	12.5 cde	9.7 bcd	18.61 ab	14.37 abc	67.31 a	63.4 a
16-TZ-13167	3.37 ab	2.18 ab	1.05 ab	0.40 bc	15.9 bcd	11.6 bc	21.67 a	19.61 a	67.31 a	62.7 a
Empire	2.04 bc	1.76 bc	0.58 bc	0.43 bc	20.3 a	12.4 b	23.09 a	14.53 abc	69.63 a	63.0 a
Meyer	0.75 c	0.55 e	0.06 c	0.05 d	10.3 e	8.6 cd	7.42 c	5.31 d	68.36 a	58.3 a
Palisades	4.65 a	2.87 a	1.16 ab	0.61 ab	16.5 abc	12.6 b	20.44 ab	17.32 abc	66.88 a	64.5 a
Zeon	0.81 c	0.46 e	0.05 c	0.01 d	11.9 de	8.4 d	18.50 ab	10.82 cd	62.65 a	50.8 a

AGB-Aboveground biomass. All dry weights are measured in gram (g) units and root depth is measured in cm units.

Means connected by common letter are not significantly different within the genotypes at $p \leq 0.05$ level of significance

Table 9: Root length density (RLD), specific root length (SRL), network surface area (NSA), network volume (NV), and average root width (ARW) of nine zoysiagrass genotypes in greenhouse experiment during 2021.

2021					
Genotype	RLD	SRL	NSA	NV	ARW
09-TZ-54-9	0.09 b	1436 ab	1731.37 a	7.6 a	0.029 bc
15-TZ-11777	0.07 b	1192 abc	1390.30 a	10.3 a	0.032 a
16-TZ-13121	0.07 b	1578 a	1330.52 a	7.3 a	0.027 c
16-TZ-13161	0.08 b	1195 abc	1094.01 a	8.4 a	0.029 bc
16-TZ-13167	0.06 b	1186 abc	1028.01 a	8.4 a	0.031 ab
Empire	0.17 a	868 bc	945.62 a	14.7 a	0.028 c
Meyer	0.07 b	1696 a	926.28 a	11.1 a	0.026 c
Palisades	0.12 ab	701 c	907.69 a	11.8 a	0.029 abc
Zeon	0.06 b	1788 a	565.88 a	4.5 a	0.029 bc

RLD-root length density, SRL- specific root length, NSA-network surface area, NV-network volume, ARW-average root width

Means connected by common letter are not significantly different within the genotypes at $p \leq 0.05$ level of significance

Table 10: Root length density (RLD), specific root length (SRL), network surface area, network volume, and average root width (ARW) of nine zoysiagrass genotypes in greenhouse experiment during 2022.

2022					
Genotype	RLD	SRL	NSA	NV	ARW
09-TZ-54-9	0.11 b	1309 cd	1536.45 cd	11.3 cd	0.025 abc
15-TZ-11777	0.18 a	895 de	3206.25 a	22.5 a	0.024 bc
16-TZ-13121	0.06 cd	1859 bc	841.40 de	6.2 ef	0.026 ab
16-TZ-13161	0.09 bcd	1419 bcd	956.01 de	7.2 ef	0.026 ab
16-TZ-13167	0.10 bc	948 de	1720.05 bc	13.5 bc	0.027 a
Empire	0.18 a	855 de	1498.49 cd	10.5 cde	0.023 c
Meyer	0.06 cd	2101 b	506.72 e	3.6 f	0.024 bc
Palisades	0.17 a	579 e	2318.69 b	17.7 ab	0.26 ab
Zeon	0.05 d	2877 a	626.36 e	4.4 f	0.025 bc

RLD-root length density, SRL- specific root length, NSA-network surface area, NV-network volume, ARW-average root width

Means connected by common letter are not significantly different within the genotypes at $p \leq 0.05$ level of significance

Table 11: Correlation coefficients of biomass and root scan traits of nine zoysiagrass genotypes during greenhouse experiment in 2021.

	AGB	BDW	TRDW	NSA	NV	SRL	RLD	NL	ARW
AGB									
BDW	0.59**								
TRDW	0.60**	0.92**							
NSA	ns	0.37	0.72**						
NV	ns	0.38	0.31**	0.98**					
SRL	-0.32	-0.63*	-0.61*	-0.42*	-0.42*				
RLD	0.44	ns	ns	0.99**	0.95**	-0.72*			
NL	ns	ns	ns	0.99	0.96	-0.40	ns		
ARW	ns	ns	ns	ns	ns	ns	ns	0.42	
MRD	0.44	ns	ns	ns	ns	ns	ns	ns	ns

AGB- aboveground biomass (g), BDW-bottom half root dry weight(g), TRDW- total root dry weight (g), NSA- network surface area (cm²), NV-network volume (cm³), SRL-specific root length (cm g⁻¹), RLD-root length density (cm/ cm³), NL- network length (cm), ARW- average root width , MRD-maximum root depth

*Significant at $p \leq 0.05$ probability level; ns, not significant

Table 12: Correlation coefficients for root biomass and root scan traits during greenhouse experiments in 2022

	AGB	BDW	TRDW	NSA	NV	SRL	RLD	ARW
AGB								
BDW	0.69**							
TRDW	0.69**	0.93**						
NSA	0.65**	0.73**	0.72**					
NV	0.61**	0.74**	0.74**	0.98**				
SRL	-0.43	-0.59**	-0.62**	-0.76**	-0.79**			
RLD	0.66**	0.70**	0.68**	0.99**	0.95**	-0.72**		
ARW	ns	ns	ns	ns	ns	ns	ns	
MRD	0.49*	ns	ns	0.45	0.44	ns	0.44	ns

AGB- aboveground biomass (g), BDW-bottom half root dry weight(g), NSA-network surface area (cm²), NV-network volume (cm³), SRL-specific root length (cm g⁻¹), RLD-root length density (cm/ cm³), ARW- average root width , MRD-maximum root depth.

**Significant at $p \leq 0.05$ probability level; ns, not significant

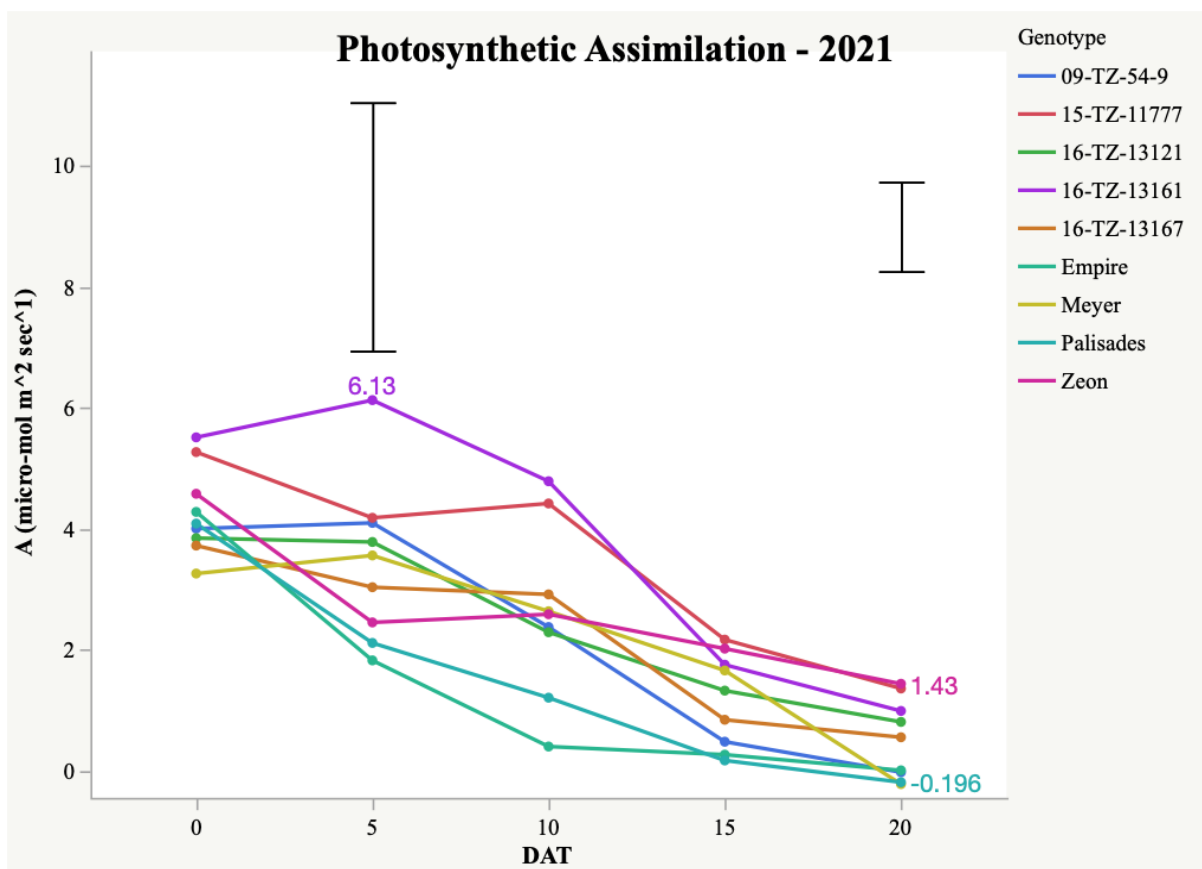


Figure 1: Change in photosynthetic assimilation rates (A) of nine zoysiagrass genotypes at 5,10,15 and 20 days after drought stress in 2021. Bars represent days when genotype means were significantly different at $p \leq 0.05$ level of significance

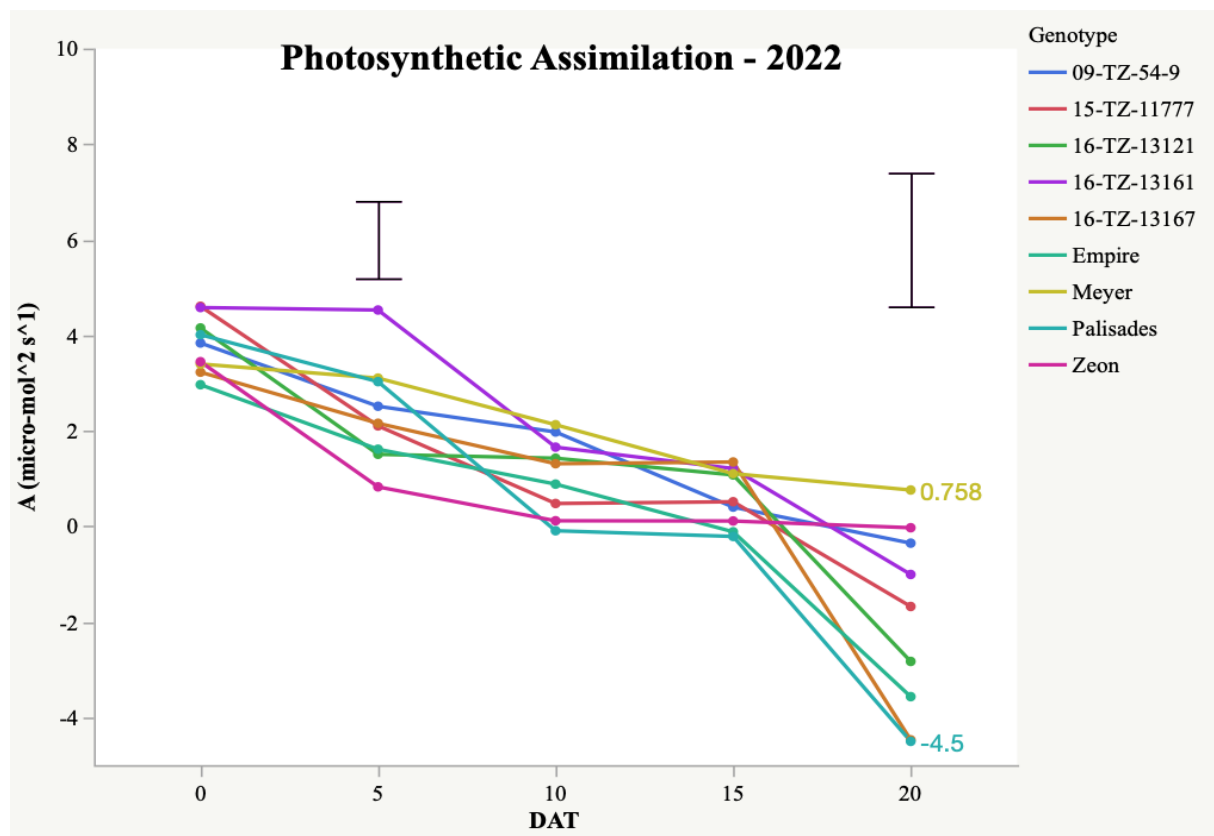


Figure 2: Change in photosynthetic assimilation rates of nine zoysiagrass genotypes at 5,10,15, and 20 days after drought stress in 2022. Bars represent days when genotypes means were significantly different at $p \leq 0.05$ level of significance

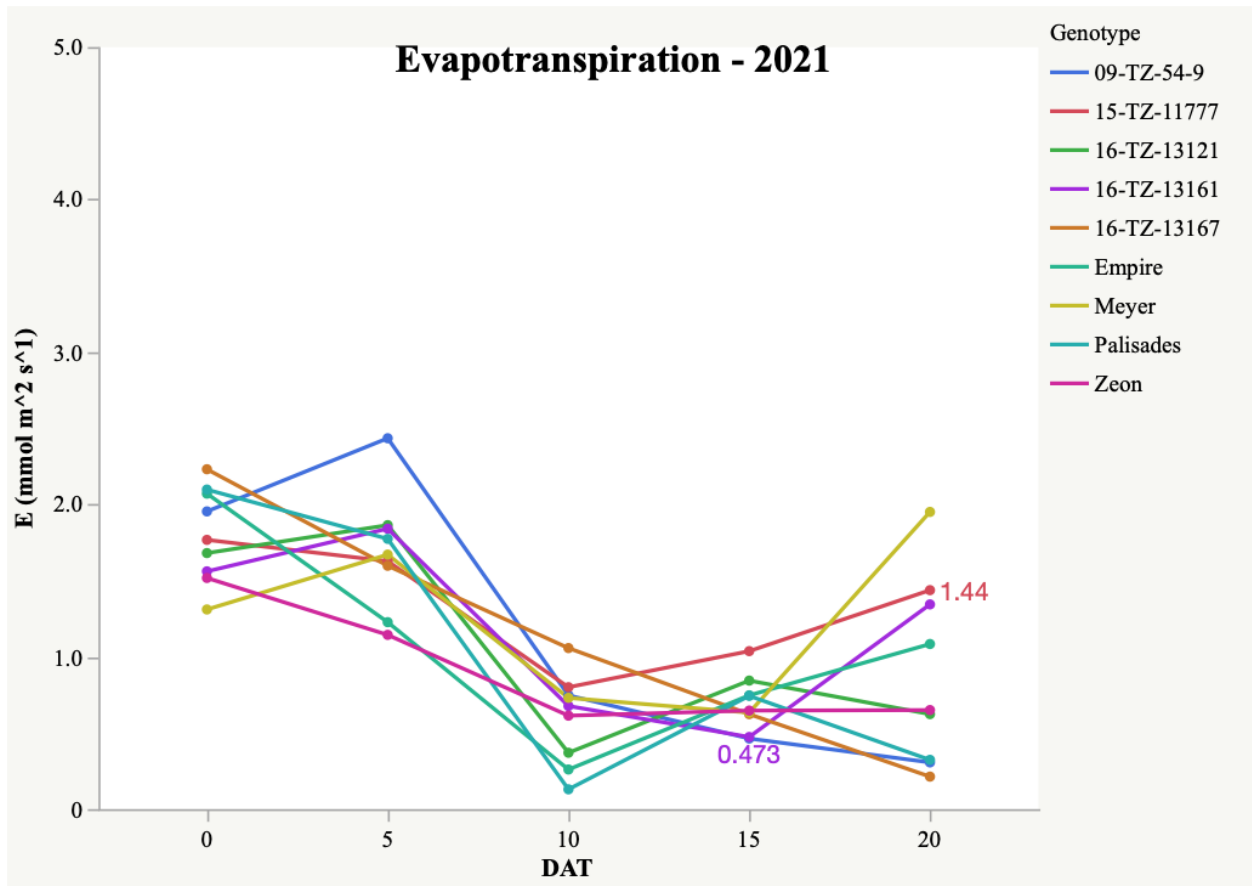


Figure 3: Change in evapotranspiration rates (E) of nine zoysiagrass genotypes at 5, 10, 15, and 20 days after drought stress in 2021.

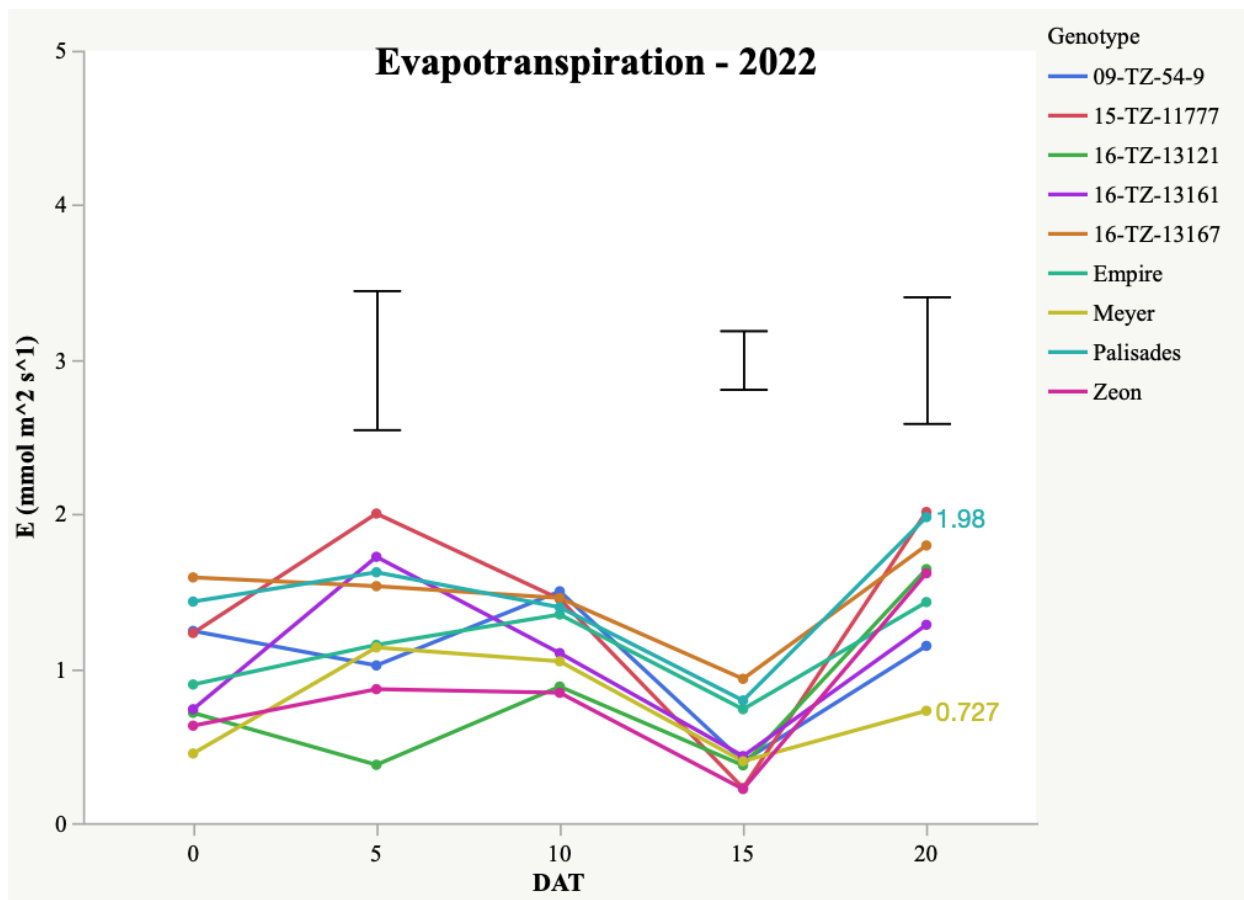


Figure 4: Change in evapotranspiration rates (E) of nine zoysiagrass genotypes at 5, 10, 15 and 20 days after drought stress in 2022. Bars represent days when genotypes means were significantly different at $p \leq 0.05$ level of significance

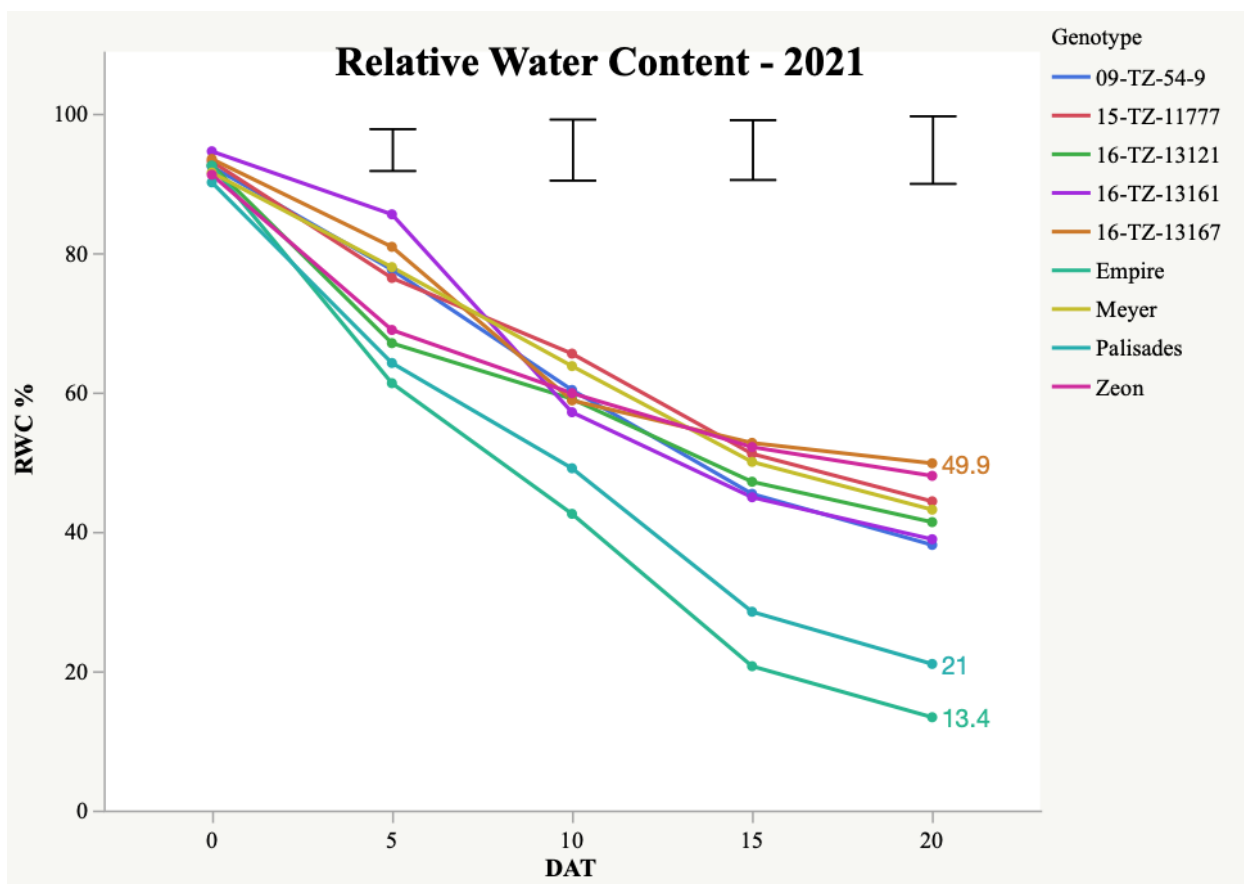


Figure 5: Change in leaf relative water content (RWC) of nine zoysiagrass genotypes at 5,10,15 and 20 days of drought stress in 2021. Bars present days when genotypes means were significantly different at $p \leq 0.05$ level of significance.

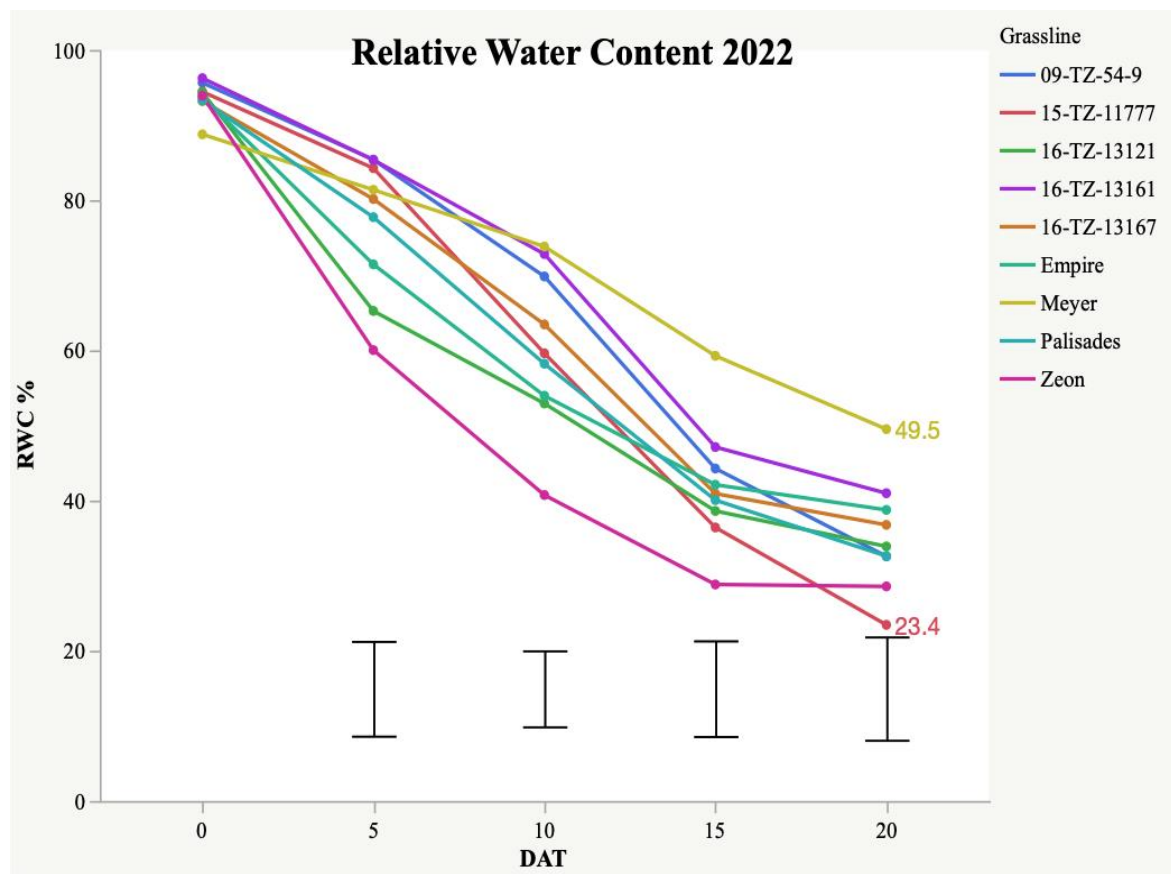


Figure 6: Change in leaf relative water content (RWC) of nine zoysiagrass genotypes at 5,10,15 and 20 days of drought stress in 2022. Bars present days when genotypes means were significantly different at $p \leq 0.05$ level of significance.

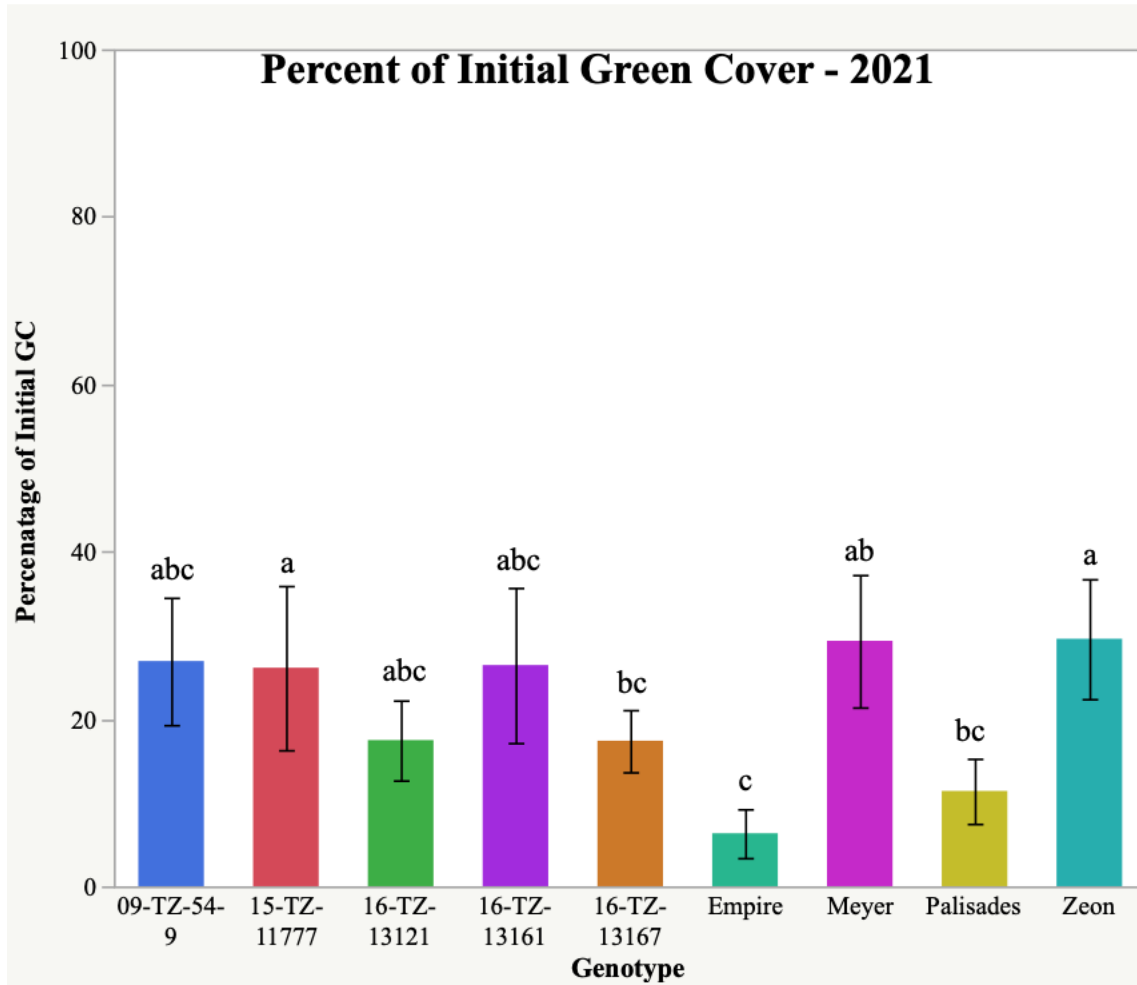
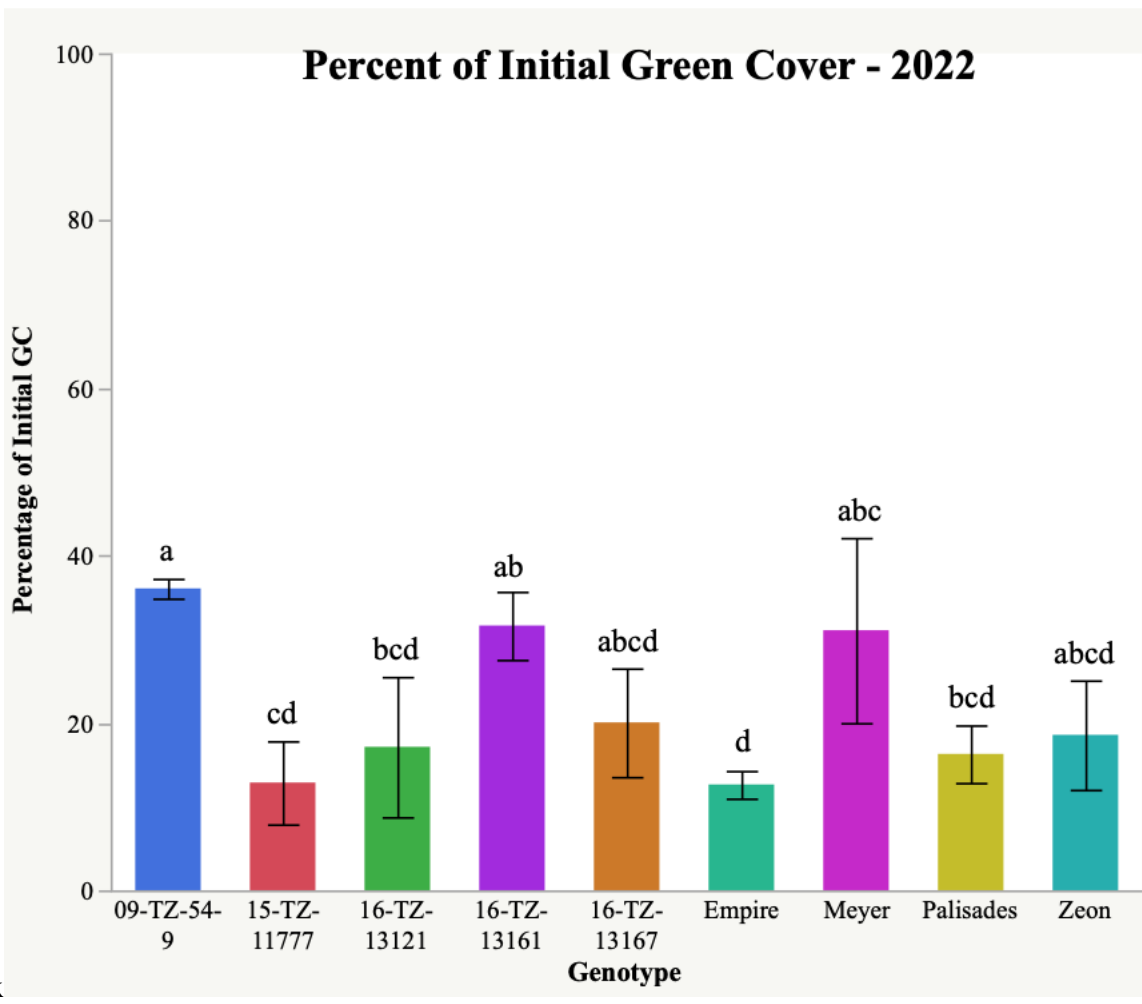


Figure 7: Percent of initial green cover at final day of drought stress for nine zoysiagrass genotypes in 2021. Errors bars represent standard error.



x

Figure 8: Percent of initial green cover at final day of drought stress for nine zoysiagrass genotypes in 2022. Error bars represent standard errors.

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CHAPTER 3

DROUGHT AVOIDANCE IN A DIVERSE COLLECTION OF ZOYSIGARSSSES UNDER FIELD CONDITIONS²

² Kaur R., Schwartz B., Waltz C., and Jespersen D. To be submitted to the Crop Science journal.

ABSTRACT

Turfgrass water consumption has come under scrutiny in recent years, and it is very likely that water use for turfgrass will be subject to stricter regulations in future. Among C4 species, zoysiagrass is becoming a very popular warm season grass with good turf quality and is used in home lawns and golf courses in US. However due to its moderate to low drought resistance, breeding efforts to develop improved genotypes with better drought avoidance are being carried out. The objective of this study was to evaluate drought performance of 8 recently developed experimental breeding lines against commercial standard cultivars in a field dry down study. The focus of this study was to find the relationship between above ground drought response traits with below ground rooting patterns by measuring turf quality, leaf gas exchange, relative water content, percentage green cover, as well as root biomass and root length density (RLD). Significant differences were seen in drought avoidance abilities of genotypes in a two-year field dry-down study. 16-TZ-13410 and 15-TZ-11777 retained >50% of their initial green cover while other genotypes like Zeon, Empire and 16-TZ-14114 lost >70% of their green cover (GC) during the drought periods. Genotypes with better water retention (higher RWC), had lower canopy temperature depression (CTD) values at the final day of drought stress. Visual quality ratings of leaf firing, turf quality (TQ), and wilt indicated 16-TZ-13410, 15-TZ-11777, 16-TZ-13685 and 16-TZ-13167 as top performers compared to standard cultivars.

1. INTRODUCTION

Water shortages are of great concern in many parts of the US as recurrent droughts last longer and water use for urban and agricultural use rises. Stricter regulations are being passed limiting irrigation use for residential landscapes to conserve water (Ozan & Alsharif, 2013). In Georgia, US, periodic droughts have impacted the state over the years, and have led to stricter regulations on landscape irrigation by The Georgia Environmental Protection Division (Bergman et al., 1986; Dublin & Grundstein, 2008; Campana et al., 2012). Along with municipal regulations on water use, breeding and screening efforts to develop genotypes with decreased susceptibility to drought stress are being carried out as a step towards sustainable turfgrass management (Cathey et al., 2011; Huang, 2016; Katuwal et al., 2022).

Turfgrasses are classified into cool-season and warm-season grasses according to their adaptability to different temperature and rainfall regimes (Beard, 1972) and are referred to as C3 and C4 plants species based on their different photosynthetic pathways (Huang et al., 2014). C4 plants due to their adaptability to arid and tropical environments are found to have greater water use efficiency when compared to C3 plants (Ward et al., 1999). Zoysiagrass is becoming an increasingly popular warm-season turfgrass species for home lawns, golf courses and sod farms in transitional and southern climatic zones (Patton, 2009). It is considered to be more drought resistant than cool-season turfgrasses (White et al., 2001). However, compared to other warm-season turfgrasses like bermudagrass (*Cynodon sp.*) zoysiagrass has been found to be less drought resistant with longer stress recovery periods. However, evaluation of drought performance in zoysiagrass germplasm is still ongoing (Hanna et al., 2013).

Drought avoidance is one of the common drought resistance strategies in turfgrasses based on enhancing water uptake through the root system or decreasing water loss from leaves with goal to

maintain adequate water status under stress conditions (Levitt, 1980). In a field study by Carrow (1996) comparing bermudagrass, centipedegrass and zoysiagrass for their drought avoidance ability, leaf wilting, leaf firing, shoot density, and rooting depth and distribution were important indicators of drought performance. Studies comparing rooting capacity with drought performance in zoysiagrass in both greenhouse and field conditions have found correlations between increased total root dry weight, deeper maximum root depth, root number, total root weight at deeper soil depths and enhanced drought performance (Marcum et al., 1995; Christensen et al., 2017). However, there have been relatively fewer studies evaluating shoot responses to water stress and their possible association with rooting traits, characterization of root morphology and physiological mechanisms involved in drought response in a single combined study. The objective of this study was to compare above and below ground drought responses in 12 zoysiagrass genotypes in prolonged field dry down conditions. The integration of above and below ground morphological and physiological traits is essential to improve our understanding of drought responses and the development of grasses with reduced water requirements.

2. MATERIALS AND METHODS

2.1 Experiment Site Description

A total of 12 zoysiagrass genotypes including 4 standard commercial cultivars and 8 experimental breeding lines (Table 13.) were evaluated for their drought performance across a 2-year field study in the Fall 2021 and Summer 2022 under two automatic rainout shelters located at the UGA Griffin campus, Griffin, Georgia (33°15'45.9"N 84°17'07.3"W). The rainout shelter plots had automatic rain out system with transparent plastic covers that utilized optical rain sensors, which sensed rain and activated an electric motor to cover the plots during any rain event. Each genotype was transplanted from plugs into a 1 m² plot in July 2020. The soil type was sandy soil (92% sand, 4%

silt, and 4% clay with 0.84% organic matter). The plots were allowed to establish for a period of 12 months. Plots were mowed once every week using rotary mower at mowing height of 5 cm and received 2.5 cm of irrigation weekly, spread across three applications. A 12-20-6 (N-P-K) Dynagreen fertilizer was applied at the rate of 31 kg per 1000 m² and was spread across three applications during the growing season in 2021. Management procedures for pest control were applied to plots in accordance with standard practices. For second year, one application of 12-20-6 (N-P-K) was applied uniformly to the plots on May 27th, 2022, at the same 10.3 kg per 1000 m². Symptoms of fungal infection were observed one week after start of dry down in 2022 and fungicide was applied to the plots to prevent disease damage. Prior to the initiation of experiments each year, 2.5 cm of water was applied to the experiment site with an irrigation boom to provide uniform soil moisture content. The automatic rainout shelter system was activated during dry-down and irrigation was completely withheld for the duration of experiment. During a rain event, the shelter would cover the plots and stay closed for one hour after the rain stopped to ensure no water reached the plots. Attempts were made to optimize observed genotypic differences in drought responses among entries and so the dry-down phase length varied by year. The length of each dry down was dictated by how rapidly turf quality and soil moisture decreased. The lengths of experiment were 45 days for Fall 2021 and 28 days for Summer 2022. During the 2021 trial, a two-week prolonged period of overcast clouds and regular rains kept the rainout shelters covered which caused the experiment to be extended. For the 45 days of 2021 trial, the total rainfall was 21.5 cm, with average maximum and minimum temperature of 21.44 and 16.05 °C. For the 2022 trial duration, the total rainfall was 8.0 cm, with average maximum and minimum temperature of 31.48 and 19.73 °C.

2.2 Measurements

2.2.1 Turf quality and digital image analysis:

Overall turf quality was measured based on color, density, and uniformity on a scale of 1 to 9, with 1 representing complete leaf firing and completely dried plants, 6 being minimum acceptable quality and 9 representing healthy, green dense ground cover (Krans & Morris, 2007). Additional 1 – 9 visual ratings of wilt and leaf firing were also collected. Digital Image analysis was performed to determine changes in percentage green cover (GC) under stress conditions during drought. Digital images were taken at regular intervals using digital camera (CanonG9X, Cannon, Tokyo, Japan) and a light box was used for uniform illumination. The pictures were subjected to color thresholding via HSB (hue saturation brightness) values in ImageJ software (2.3.0/1.53f51) to measure the percent GC (Leinauer et al., 2014). To account for initial differences in canopy coverage, percent GC was calculated as a percent of values measured on 0 days after treatment (DAT).

2.2.2 Canopy Temperature Difference (CTD):

Canopy temperature was measured per plot using infrared thermometer (Exotech) on each sampling day and the difference between ambient temperature and canopy temperature was used to get the canopy temperature difference (CTD). Values of CTD were used to account for the intensity of drought stress with a higher value indicating more stress as plants have decreased transpiration (Throssell et al., 1987).

2.2.3 Relative Water Content:

Leaf hydration levels were determined by taking relative water content (RWC) values at weekly intervals after initiation of dry down using the method described by (Barrs and Weatherley 1962). Fresh leaf tissue ranging from 0.2- 0.3 g was taken from each plot and the fresh weight was measured. The sample was then rehydrated in distilled water overnight at 4 °C and weighed to get its turgid weight. The tissue was subsequently oven dried at 72 °C for 72 hours and weighed again to get its dry weight. RWC was calculated using the formula:

$$RWC = \frac{(fresh\ weight - dry\ weight)}{(turgid\ weight - dry\ weight)} * 100$$

2.2.4 Canopy Photosynthetic Assimilation (A_{can}):

The leaf gas exchange was measured using portable photosynthetic system CIRAS-3 (Version 2.00 PP Systems). The values for canopy photosynthetic assimilation were recorded using a CPY-5 Canopy assimilation chamber as a closed system measurement of net canopy CO₂ flux during measurements (Buck, 1981; Parkinson, 1983). Delta CO₂ is the change in CO₂ concentration during a measurement sequence ($\mu\text{mol}^{-2}\text{s}^{-1}$) which can be used to calculate photosynthesis (Long & Bernacchi, 2003). Measurements were taken between 9:00 and 12:00 h on sunny days at weekly intervals throughout the dry down period. Resulting data was used to calculate net CO₂ uptake (A_{can}) at the canopy level.

2.2.5 Root and shoot biomass:

On October 16th, 2021, a 58.06 cm² square auger was used to harvest intact zoysia plugs after prior attempts to sample deeper using a longer auger failed to find intact roots at lower depths of 20-30 cm depths. For the second year, on July 5th, 2022, root cores were harvested using a 78.54 cm² cylindrical cutter to a depth of 10 cm, as a representative section of grass with intact root zone.

Each sample was washed to remove all soil and cut approximately 1 cm below the soil surface, below the majority of rhizomes, to separate the root zone. Shoot biomass was measured to include aboveground tissue and rhizomes and their dry weights were taken. The length of the longest root from root zone was measured with a ruler. Representative root samples from the root zone were stored in a 20% v/v ethanol solution for further root scan analysis and remaining root tissue was measured for dry weight. For root scan analysis, roots were floated on a transparent sheet and scanned using a desktop scanner (EPSON V550 Photo) to get digital images of plant roots. The root images were uploaded into GiA roots software (Galkovskyi et al., 2012) and computed for root parameters including network surface area, total root length, average root width, root length density (RLD), and specific root length (SRL).

2.2.6 Volumetric Soil Water Content:

The percentage of the total soil volume occupied by water is defined as VWC (SU et al., 2014). The VWC was measured using a Field Scout TDR 300 sensor probe in each plot at start of the dry down and was averaged to identify potential spatial differences between blocks (

Table 14). The moisture readings were taken again at the end of dry down to track changes in soil moisture.

2.3 Experimental Design and Data Analysis

The plots were arranged in a randomized complete block design with a total of 12 genotypes and 4 replicates per genotype spread across two rainout shelters. Each rainout shelter plot consists of 2 complete blocks. Years were analyzed separately. Data were subjected to analysis of variance (ANOVA) using mixed model in JMP Pro 16 software (SAS Institute 2021, Cary, NC) with genotype and days after treatment (DAT) considered as fixed effects and replicates (blocks) as random effects at $\alpha \leq 0.05$. A one-way ANOVA was conducted for genotypes by DAT to test for genotypic variations at a given sampling date. The genotype means were separated by Fisher's protected LSD at $\alpha \leq 0.05$ level. Multiple linear regression analysis was conducted on GC, RWC and visual traits to test for correlation between different measured parameters using to determine factors significantly associated with drought performance in genotypes (Lehman et al., 2013). Due to a significant year effect in initial models, data the two years is presented separately, except for root morphological traits as there was no significant trial effect.

3. RESULTS

3.1 Relative Water Content

For RWC significant DAT effect was observed across both trials, and genotypic separation was done by DAT. At the beginning of drought, all genotypes had leaf RWC in the range of 87-90% in 2021 and 75-80% in 2022 with no statistically significant differences among genotypes. The dry down was 45 days long due to two full weeks of rainy weather at 15 DAT and 35 DAT, with 7.8 mm of recorded rainfall from September 15th to October 8th, 2021, during which the shelters remain closed. RWC values were observed to significantly increase in samples taken after each

rainy week. Increased relative humidity conditions and possible runoff due to heavy rains might have triggered turf recovery. On comparing genotype means, greatest separation was observed at 45 DAT, ($p=0.0045^*$), where 15-TZ-11777, 16-TZ-13410 and 16-TZ-13685 had higher leaf RWC of 65.11%, 65.05%, 63.76% respectively, whereas 09-TZ-54-9 and Zeon had lower leaf RWC of 50.47% and 49.74% respectively (Figure 9). In the 2022 trial, highest statistically significant differences in genotype leaf RWC value were observed at 21 DAT ($p=0.0034^*$), where 16-TZ-13167 had higher leaf RWC of 62.83%, whereas, Zeon, 16-TZ-14114 and 09-TZ-54-9 had lower leaf RWC of 48.23%, 47.78% and 45.86%, respectively. At the end of dry down, 16-TZ-13685 had the highest leaf RWC of 59.70%, whereas Zeon continued to be poor performer with statistically lower leaf RWC of 42.5% (Figure 10). Means of RWC for different genotypes throughout the dry down for both years are shown in supplementary Table S1.

3.2 TQ, Wilt and Leaf Firing

For TQ, wilt and leaf firing both genotype and sampling date effects were significant for each parameter across both 2021 and 2022 trials with no interaction effect as shown in (

Table 15). In the 2021 trial, there was a significant block effect where blocks 1 and 2 had lower TQ and higher wilt compared to blocks 3 and 4; block was treated as a random effect. Maximum statistically significant differences in TQ rates was observed at 14 DAT ($p=0.0003^*$), where 16-TZ-13410, 15-TZ-11777 and 16-TZ-13161 were in the higher statistical group having TQ of >8.5 , whereas all the standard cultivars were on the lower end of spectrum with Empire, Meyer and Zeon having lower TQ ratings between 7.6-7.5. As the drought progressed at 28, 35 and 45 DAT, 16-TZ-13410 and 15-TZ-11777 and 16-TZ-13167 continued to be among the highest rated cultivars with TQ ranging between 8.5 to 7.2 whereas 16-TZ-14114, Meyer, Empire and Zeon continued to have lowest turf quality ratings with averages between 5.5 to 4.75. On the final day of drought stress, a similar trend was observed with 16-TZ-14114 having lowest turf quality ($TQ < 5.5$) among breeding lines and Empire was worst rated cultivar ($TQ < 5$) (Figure 11). In terms of wilt and leaf firing ratings taken throughout the drought stress, 16-TZ-13140 and 15-TZ-11777 had significantly lowest wilt (Figure 13) and leaf firing at all sampling dates compared to Zeon, Meyer, 16-TZ-14114, and Empire continuously showed greater wilt and leaf firing symptoms.

In the 2022 Trial, at 0 DAT, all plots had average TQ ratings of greater than 8.7. Maximum statistically significant differences in TQ was observed at 21 DAT ($p=0.0027^*$) where 16-TZ-13410, 16-TZ-13685 had the highest average TQ of 7.25 and 6.75 respectively. 16-TZ-13167, 15-

TZ-11777, 09-TZ-54-9 and 16-TZ-13121 were in the intermediate statistical range, whereas Empire and 16-TZ-14114 had the lowest average TQ ratings of 3.0 and 2.3, respectively. At the end of dry down, 16-TZ-13410, 16-TZ-13685 and 15-TZ-11777 continued to perform better in terms of turf quality (ranging from 6.25-4.75), however, Empire and 16-TZ-14114 had the significantly lowest visual TQ ratings of less than 2 (Figure 12). Significant differences in visible wilt (Figure 14) and leaf firing were also observed at 14, 21 and 28 DAT where Zeon, Empire and 16-TZ-14114 continued to show maximum wilt and leaf firing towards the end of the dry down and 16-TZ-13685, 16-TZ-13167, 16-TZ-13410 and 15-TZ-11777 were the best performing genotypes.

3.3 CTD

In both the 2021 and 2022 trials, no differences were observed in CTD at the beginning of dry down. In the 2021 trial, genotypes did not vary significantly based on their CTD values with DAT (Figure 15). In the 2022 trial, significant variation was observed; 16-TZ-13410 and 16-TZ-13685 had the lowest CTD at 13-14°C, whereas, Empire, and 16-TZ-14114 had the highest CTD at 19-18 °C at 14 DAT. On the final day of drought stress, 16-TZ-13685, 16-TZ-13167, Palisades and 16-TZ-13121 had significantly lower CTD values ranging from 19.5 to 21.1°C than 16-TZ-14114 and 16-TZ-13161 that had highest CTD of 27.8 and 26.3 °C respectively. 15-TZ-11777, 16-TZ-13410 and Meyer were intermediate in their response with CTD values ranging from 21.7 to 21.6 °C but not statistically different than other genotypes (Figure 16).

3.4 GC

In both year's trails, genotypes varied significantly with DAT as the dry down progressed. Also, for both years, at beginning of dry down genotypes varied significantly in their percentage green

cover so percent of initial green cover left on final day of drought stress is presented. For the 2021 trial (Figure 17), 16-TZ-13410, 15-TZ-11777 had the highest percentage of their initial green cover left on final day of drought stress (59.835 and 47.90% respectively) whereas, Empire, 16-TZ-13161 and Zeon had only 15-20% of their initial percentage green cover. For the 2022 trial (Figure 18), 16-TZ-13410, 15-TZ-11777 and 16-TZ-13685 were better performing in terms of remaining green cover that ranged from 49.03 to 41.46% of their initial canopy coverage; whereas 16-TZ-13161, Zeon, Empire and 16-TZ-14114 had poorer retention of their initial green cover, having only 19 to 14% of their initial green cover left. The percent of initial GC left at the end of dry downs during 2021 and 2022 for the 12 zoysiagrass genotypes is detailed in supplementary table S2

3.5 Root Biomass and Root scan analysis

Significant differences were observed among genotypes based on different root biomass parameters across both year's trials (Table 16). In the 2021 trial, most of the breeding lines (09-TZ-54-9, 16-TZ-13121, 16-TZ-13410 and 16-TZ-13685) had significantly greater shoot biomass than Zeon and Empire. 16-TZ-13685, and 09-TZ-54-9 had the lowest total root masses (0.20-0.24g), whereas Zeon and Meyer had higher total root masses (0.42-0.48g). 16-TZ-13161, Meyer, Zeon, and 16-TZ-13121 were deeper rooted and their maximum root depth ranged from 9.94 cm to 8.57 cm. 15-TZ-11777 had shortest root zone of 6.15cm long (Table 17). Significant genotypic differences were found in root to shoot ratios (R/S), Zeon, Meyer had higher root to shoot ratios in range of 0.08-0.09, whereas 16-TZ-13685, 09-TZ-54-9, and 15-TZ-11777 had lowest R/S ratios ranging from 0.05-0.02.

In the 2022 trial, shoot biomass from the sampled root cores ranged from 19.01 g to 8.9 g and 09-TZ-54-9, and 16-TZ-13161 ranked in top statistical group whereas Zeon, 16-TZ-13167, Palisades

and Empire had lowest shoot biomass (Table 18) which was consistent to 2021 comparisons. On comparing total root mass, 16-TZ-13121 and Palisades had statistically greater total root masses of 1.89 g and 1.55 g, respectively, compared to 16-TZ-13161, 16-TZ-13167, 16-TZ-13410, 09-TZ-54-9, with biomasses that ranged from 0.72 g to 0.54 g. 16-TZ-13685 and Meyer had the greatest root depths at 12.14 and 12.06 cm, respectively. These were significantly greater than 16-TZ-13410, 15-TZ-11777 and 09-TZ-54-9 which had the shortest root zones (8.65 cm - 8.17 cm). Root to shoot ratios varied significantly among genotypes in 2022 as well; Palisades had highest R/S ratio of 0.16 compared to 16-TZ-13161, 16-TZ-13410 and 09-TZ-54-9 that were in the lowest statistical group with R/S ranging from (0.07-0.03).

No significant trial effect was seen in terms of root morphological parameters so root morphological data from both trials were included in a single analysis. No significant differences were found for any morphological root scan traits. There were trends in root length density, average root width and SRL (Table 19) where some genotypes with better shoot physiological responses had lower root length density and SRL and variation in root thickness.

3.6 Canopy Photosynthetic Assimilation (A_{can})

In both trials significant genotype and sampling date effects for canopy photosynthesis (A_{can}) were found with no genotype and sampling date interaction observed (

Table 15). In the 2021 trial, differences among genotypes for A_{can} were only observed at 35 DAT, 16-TZ-13167, 16-TZ-13410 and 15-TZ-11777 had the highest A_{can} rates ($14.47-8.32 \mu \text{ mol}^{-2} \text{ s}^{-1}$) (Figure 19). Genotypes in the lower statistical group with the lowest photosynthesis rates were 16-TZ-14114, Empire, Zeon, Meyer, 16-TZ-13121 and 16-TZ-13685. At the beginning of the 2022 dry down, there was a significant difference in A_{can} among genotypes. 16-TZ-13685, and 15-TZ-11777, had higher photosynthetic assimilation (A_{can}) rates in the range of $20.98-14.97 \mu \text{ mol}^{-2} \text{ s}^{-1}$, whereas Zeon, had lower A_{can} rates of $4.75 \mu \text{ mol}^{-2} \text{ s}^{-1}$) (Figure 20). The greatest differences were seen at final day of dry down. 16-TZ-13685, 16-TZ-13167 and Palisades had significantly higher A_{can} rates in the range of $7.51-5.37 \mu \text{ mol}^{-2} \text{ s}^{-1}$. Zeon, 16-TZ-13121, 16-TZ-14114, 09-TZ-54-9, and 16-TZ-13161 had significantly lower A_{can} rates ranging from -0.52 to $1.32 \mu \text{ mol}^{-2} \text{ s}^{-1}$ on final day of drought stress.

3.7 Correlation among shoot response parameters:

Due to trial effects, data from each trail for all measured aboveground shoot responses were analyzed separately. In the 2021 trial, based on multivariate analysis, high positive correlation was

found between TQ, wilt and leaf firing and negative correlation with CTD values as shown in (Table 20). In the 2022 trial, again positive correlations were found between the visually scored TQ, wilt and leaf firing. Strong negative correlations (coefficients of < -0.83) with CTD were found for TQ, wilt and GC, which may be due to greater differences in CTD in the 2022 trial (Table 21).

4. DISCUSSION

Turfgrass shoot responses are often evaluated to measure turfgrass drought avoidance in various cool and warm-season grasses (Huang et al., 1997; Carrow, 1996). In this study, GC, TQ, leaf wilt, leaf firing, CTD, RWC and A_{can} were used to assess the shoot level responses in twelve zoysiagrass genotypes. During both year's trials, leaf RWC was generally higher in 16-TZ-13167, 16-TZ-13410, 16-TZ-13685, 15-TZ-11777 at the end of dry down indicating better ability to maintain leaf hydration levels. These genotypes were also among top performers in terms of visual quality ratings, having TQ >7 , and lowest leaf firing and wilt ratings >6.6 in the 2021 trial; and TQ ranging from 6.2- 4.5 in the 2022 trial, with wilt ratings >4.7 at end of the dry down. In previous studies, the ability to retain higher moisture in leaves has been correlated to less leaf firing in drought resistant genotypes of creeping bentgrass (*Agrostis stolonifera*) (Lehman et al., 1993). These higher RWC levels could be due to ability of plants to draw water from deeper in the soil profile or reduced water consumption and less evapotranspirative water loss as the dry down progressed (Jespersen et al., 2019). On evaluating percentage change in GC at the end of the dry down, the aforementioned genotypes were able to maintain 40-60% of their initial GC across both trials. Percent GC has been used as a drought resistance indicator and been highly correlated with RWC, photosynthetic rate and CTD (Zhou et al., 2014). In this study, a positive correlation was found between TQ and % GC, RWC and negative correlation with CTD. On the other hand, 16-TZ-

14114, Zeon and 09-TZ-54-9 had the lowest RWC at the end of dry down consistently in both trials. 16-TZ-14114 also had the poorest TQ ratings, below acceptable quality ratings (4.5-2); Zeon, and Empire also rated low in TQ and had greater wilt ratings and leaf firing ratings in 2022 and 2021 at the end of the dry down. These poorly performing genotypes rated consistently poor for all shoot level responses. The poor shoot responses could be due to their inability to maintain water status and could possibly indicate low drought avoidance ability.

In the 2021 trial, on comparing rooting traits with shoot responses, it was observed that genotypes with better shoot responses (15-TZ-11777, 16-TZ-13410, 16-TZ-13167) along with 09-TZ-54-9 had less total root biomass (0.3- 0.2 g) and lowest maximum rooting depth (7.7-6.1 cm) and genotypes with poor shoot responses and low shoot biomass (Meyer, Zeon, 16-TZ-14114, 16-TZ-13121) had higher total root mass and were deeper rooted at >8.5 cm. Greater drought stress symptoms as evident from their reduced A_{can} , RWC, GC and TQ, could be a results of greater soil water depletion, as more resource allocation went towards root development. This is in tune with results from a drought study comparing biomass distribution and gas exchange in switchgrass and millet seedlings under water stress, where switchgrass performed poorly in response to drought stress and water deficit caused increase in its root dry weight and reduced the shoot dry weight, increasing the root/shoot (R/S) ratio (Xu et al., 2006).

In terms of total root mass genotype results were similar across both trials. In the 2022 trial, higher total root mass in Palisades, Meyer, Zeon, and 16-TZ-13121 supports the assumption that genotypes which were poor performing had greater root biomass. For maximum rooting depth, results were different across trials. There seemed to be a general trend that the best performing genotypes (16-TZ-13410) had shallower root depths whereas poor performing genotypes were spread across intermediate to greater root depths. SRL is a measurement of how much root length

is produced per weight of biomass and higher SRL may allow plants to produce longer roots in search of water (Eissenstat, 1992). Higher SRL as thinner roots may allow plants to produce thin and longer roots in search for water in deeper soil depths. Also, a higher RLD can be associated with longer and deeper root development as demonstrated by (Christensen et al., 2017). In this study, the trend in SRL and RLD supports these assumptions but no significant differences were found for root morphological traits. These results varied from what has been found in previous studies on rooting characteristics, where rooting depth and higher biomass at lower depths has been associated with better drought resistance mechanisms in zoysiagrass (Marcum et al., 1995; Huang, 2003). The reason for differences in performance could be that under different soil edaphic stresses, the plants experiencing the greatest drought symptoms, ran out of plant available water sooner due to greater water usage rates. Also, these roots were harvested at the end of dry down, a stage when almost all plots reached at VWC contents of 1-2%, to better understand how drought stress induced root growth, harvesting roots mid-way through the dry down could have provide greatest insight to changing root dynamics.

Genotypes experiencing less drought symptoms didn't find the need to extract water from deeper soil layers as they never ran out of water completely, as indicated by their high RWC values and low CTD rates (cooler canopies indicating active evapotranspiration). Canopy temperature difference (CTD) has been used as a reliable indicator of drought stress in previous studies on Kentucky bluegrass by (Throssell et al., 1987). 16-TZ-14114 had the highest CTD of 27.80 °C, which when correlated with its TQ, RWC, wilt and leaf firing ratings, indicated its poor drought avoidance, whereas 16-TZ-13685 had the lowest average CTD of 19.5°C. Lower CTD values in 16-TZ-13410, 16-TZ-13685 and 16-TZ-13167 indicate an overall cooler canopy and maintained transpiration rates in these lines as the dry down progressed whereas genotypes with higher CTD

indicates their possible inability to maintain active transpiration rates as they may have used up the plant available water, resulting in warmer canopies. CTD has been correlated with drought performance in bermudagrass and seashore paspalum where drought tolerant lines like ‘TifTuf’ had the lowest CTD and susceptible lines like ‘Seastar’ had higher CTD (Jespersen et al., 2019). In another bermudagrass study by Zhou et al. (2013) found that drought resistant genotypes were extracting more water from soil than drought susceptible ones and it was stated as a possible explanation for their lower canopy temperatures, higher photosynthetic rates and higher leaf RWC. This explanation fits well for 16-TZ-13685, 16-TZ-13167, 16-TZ-13410 and 15-TZ-11777 as they also had higher canopy photosynthetic assimilation rates ($7.51-3.80 \mu \text{mol}^{-2} \text{s}^{-1}$) along with better RWC at end of dry down in both trials. These genotypes may have been able to sustain higher A_{can} rates due to better transpiration and sufficient water levels in the system, similar to what was found in a tall-fescue drought resistance study (Sun et al., 2013; Craine et al., 2013). These high A_{can} rates coupled with higher RWC rates and low CTD values, indicates their ability to maintain active transpiration rates and avoiding excessive transpiration earlier on in the dry down when compared to poorer performers that could have transpired excessively at the onset of drought stress. Overall, it appears the better performing genotypes used water more efficiently. This demonstrates that responses are genotype specific, and that integration of above and below ground processes will be essential for fully understanding water loss dynamics during drought.

5. CONCLUSION

This study provides a comparison of drought avoidance of twelve zoysiagrass genotypes under imposed drought stress in field conditions. In summary, findings from the two-year field dry-down studies indicate improved drought performance of some experimental breeding lines developed in

recent years over standard commercial cultivars in terms of their ability to better retain hydration levels, turf quality, green cover, and photosynthesis rates in response to water stress. Differences in rooting pattern could be due to underlying genotypic differences of these lines and slow growth rates from plugs during establishment resulted in shallow rooting in 2021. A wide range of drought avoidance based on measured above and below ground traits was observed with certain genotypes consistently performing better than others. 16-TZ-131410, 15-TZ-11777, were consistently top performers under drought; 16-TZ-14114 was a consistently poor performing. No clear separation of best and worst performance was seen in the four standard cultivars. Variation across years could be accounted for by different temperatures, evapotranspiration rates, rain events, and difference in plant maturity. 2021 had lower maximum temperature and evapotranspiration whereas during 2022 dry-down, the heat index and evapotranspiration rates for the experimental site were higher. This environmental variation affected the rate and extent of decline in each parameter across the two trails. Future research should focus on better understanding of underlying physiological mechanisms behind visible variation in shoot responses. Research on the regulation of stomata and water use rates may help better understand the unexpected trend that plants with relatively small root systems maintained better visual quality. Furthermore, studying chlorophyll degradation, could provide more information on the extent of intercellular photosystem degradation resulting in declining photosynthesis rates. Lastly, a better understanding of seasonal growth effects on root development should be considered when comparing drought avoidance in field conditions.

6. TABLES AND FIGURES

Table 13: Description of zoysiagrass cultivars and experimental selections tested in field trails

Genotype	Species	Source
09-TZ-54-9	Interspecific hybrid	Experimental breeding line
15-TZ-11777	<i>Z. matrella</i>	Experimental breeding line
16-TZ-13121	Interspecific hybrid	Experimental breeding line
16-TZ-13161	Interspecific hybrid	Experimental breeding line
16-TZ-13167	Interspecific hybrid	Experimental breeding line
16-TZ-13410	Interspecific hybrid	Experimental breeding line

16-TZ-13685	Interspecific hybrid	Experimental breeding line
16-TZ-14114	Interspecific hybrid	Experimental breeding line
Empire	<i>Z. japonica</i>	Sod Solutions, Mt. Pleasant, SC, 1990
Meyer	<i>Z. japonica</i>	USDA, 1951
Palisades	<i>Z. japonica</i>	Texas A&M, Dallas, 1996
Zeon	<i>Z. matrella</i>	Bladerunner Farms Inc., TX 1996

Table 14: VWC (%) at the end of trial during 2021 and 2022 dry down.

Block	2021		2022	
	45 days after dry down		28 days after dry down	
	12 cm soil depth	7.6 cm soil depth	12 cm soil depth	7.6 cm soil depth
1	3.8	1.2	1.5	0.8
2	1.5	0.8	3.4	1.6
3	3.4	1.6	1.8	1.3
4	1.8	1.3		

Table 15: ANOVA results for aboveground drought response parameters during 2021 and 2022 trial

2021	RWC	GC	TQ	Wilt	LF	CTD	Acan
Effect	Prob>F						
Genotype (G)	0.0100*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0180*	<0.0001*
Days (D)	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
G*D	0.8588	0.6953	0.8335	0.3021	0.9766	0.99	0.68
2022	RWC	GC	TQ	Wilt	LF	CTD	Acan
Effect	Prob>F						
Genotype (G)	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0003*	<0.0001*
Days (D)	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
G*D	0.1713	0.8662	0.8109	0.7569	0.9966	0.99	0.4384

RWC, relative water content; TQ, turf quality; LF, leaf firing; CTD, canopy temperature difference; Acan, canopy photosynthetic assimilation; GC, percent green cover.

*Genotype means and interactions significant at $p \leq 0.05$

Table 16: ANOVA results for belowground root biomass traits after dry down in 2021 and 2022

	Total root mass (g)	Shoot biomass (g)	Max root depth	S/R	R/S
2021	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Genotype	0.1114	0.0152*	0.1965	0.0018*	0.0036*
2022	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
Genotype	0.05*	<.0001*	0.4201	<.0001*	0.0410*

S/R- shoot to root ratio, R/S- root to shoot ratio

*Genotype means significant at $p \leq 0.05$

Table 17: Total root mass, aboveground shoot biomass, maximum root depth, root to shoot ratios and shoot to root ratios of twelve genotypes after dry down during 2021

Genotype	Total root mass (g)	Shoot biomass (g)	Max root depth	R/S ratio
09-TZ-54-9	0.24 cd	7.84 a	8.09 abc	0.03 de
15-TZ-11777	0.28 bcd	5.42 bcd	6.15 c	0.05 cde
16-TZ-13121	0.396 abc	6.90 ab	8.57 ab	0.06 bcd
16-TZ-13161	0.30 bcd	6.51 abc	9.94 a	0.04 cde
16-TZ-13167	0.30 bcd	6.39 abc	7.77 abc	0.04 cde
16-TZ-13410	0.29 bcd	6.96 ab	7.58 bc	0.04 cde
16-TZ-13685	0.20 d	6.73 ab	8.69 ab	0.02 e
16-TZ-14114	0.38 abc	6.26 abc	8.25 abc	0.06 abc
Empire	0.30 bcd	4.45 d	7.93 abc	0.06 abc
Meyer	0.48 a	5.20 bcd	9.20 ab	0.08 ab
Palisades	0.34 abcd	5.26 bcd	7.46 bc	0.06 abc
Zeon	0.42 ab	4.83 cd	9.20 ab	0.09 a

Note: within each dependent measure, means with different letter differ significantly at ($p < 0.05$)

Table 18: Total root mass, aboveground shoot biomass, maximum root depth, root to shoot ratios and shoot to root ratios of twelve genotypes after dry down during 2022

Genotype	Total root mass (g)	Shoot biomass (g)	Max root depth	R/S
09-TZ-54-9	0.62 c	19.01 a	8.17 d	0.03 c
15-TZ-11777	0.78 bc	11.54 bcd	8.19 cd	0.07 bc
16-TZ-13121	1.89 a	16.10 abc	9.28 bcd	0.11 ab
16-TZ-13161	0.72 c	17.62 ab	9.52 abcd	0.04 c
16-TZ-13167	0.72 c	9.80 cd	9.81 abcd	0.07 bc
16-TZ-13410	0.54 c	14.30 abcd	8.65 cd	0.03 c
16-TZ-13685	0.82 bc	11.74 bcd	12.14 a	0.07 bc
16-TZ-14114	0.93 bc	12.63 abcd	10.63 abcd	0.07 bc
Empire	0.89 bc	8.93 d	10.16 abcd	0.09 abc
Meyer	1.18 abc	11.58 bcd	12.06 ab	0.10 abc
Palisades	1.55 ab	9.11 d	11.19 abc	0.16 a
Zeon	1.10 abc	10.93 cd	10.83 abcd	0.10 abc

Note: within each dependent measure, means with different letter differ significantly at ($p < 0.05$)

Table 19: Average of Network surface area, Network volume, Specific root length (SRL), Average root width and Root length density (RLD) of twelve genotypes during 2021 and 2022

Genotype	Network Surface Area (cm ²)	Network Volume (cm ³)	SRL (cm/g)	Avg. root width (cm) (ns)	RLD (cm/cm ³) (ns)
09-TZ-54-9	339	3.31	345	0.033	0.010
15-TZ-11777	407	3.64	310	0.031	0.013
16-TZ-13121	618	5.94	282	0.034	0.011
16-TZ-13161	275	2.93	398	0.036	0.011
16-TZ-13167	269	2.79	276	0.035	0.012
16-TZ-13410	243	2.46	281	0.034	0.010
16-TZ-13685	280	3.32	233	0.038	0.015
16-TZ-14114	331	3.45	198	0.035	0.013
Empire	360	3.69	235	0.033	0.012
Meyer	581	5.73	312.2	0.033	0.015
Palisades	562	5.92	235.7	0.034	0.014
Zeon	430	3.95	233.3	0.030	0.013

SRL- specific root length (cm/g); RLD- root length density (cm/cm³)

Note: within each dependent measure, means not connected by any letter don't differ significantly at (p<0.05)

Table 20: Correlation between aboveground drought response physiological traits in twelve zoysiagrasses during field dry down in 2021 at 7, 14, 21, 28, 35 and 45d

	RWC	Wilt	TQ	LF	CTD	A_{can}
RWC						
Wilt	0.5454**					
TQ	0.5380**	0.9526**				
LF	0.5671**	0.9402**	0.9310**			
CTD	-0.1014	-0.0188	-0.8198**	-0.7908**		
A _{can}	0.3178	0.3719	0.6230**	0.6011**	-0.6538*	
GC	0.6442**	0.9060**	0.9028**	0.9059**	-0.0859**	0.3718

RWC, relative water content; TQ, turf quality; LF, leaf firing; CTD, canopy temperature difference; A_{can}, canopy photosynthetic assimilation; GC, percent green cover.

** Pearson correlation coefficient (*r*) values, Significant at $p \leq 0.05$

Table 21: Correlation between aboveground physiological drought response traits in twelve zoysiagrasses during field dry down in 2022 at 7, 14, 21 and 28d

	RWC	Wilt	TQ	LF	CTD	Acan
RWC						
Wilt	0.8862**					
TQ	0.8456**	0.9509**				
LF	0.7971**	0.8978**	0.9310**			
CTD	-0.7859**	-0.8444**	-0.8198**	-0.7908**		
Acan	0.6420**	0.6565**	0.6230**	0.6011**	-0.6538*	
GC	0.8263**	0.9003**	0.8619**	-0.8618**	-0.8231**	0.6689**

RWC, relative water content; TQ, turf quality; LF, leaf firing; CTD, canopy temperature difference; Acan, canopy photosynthetic assimilation; GC, percent green cover.

** Pearson correlation coefficient (r) values, Significant at $p \leq 0.05$

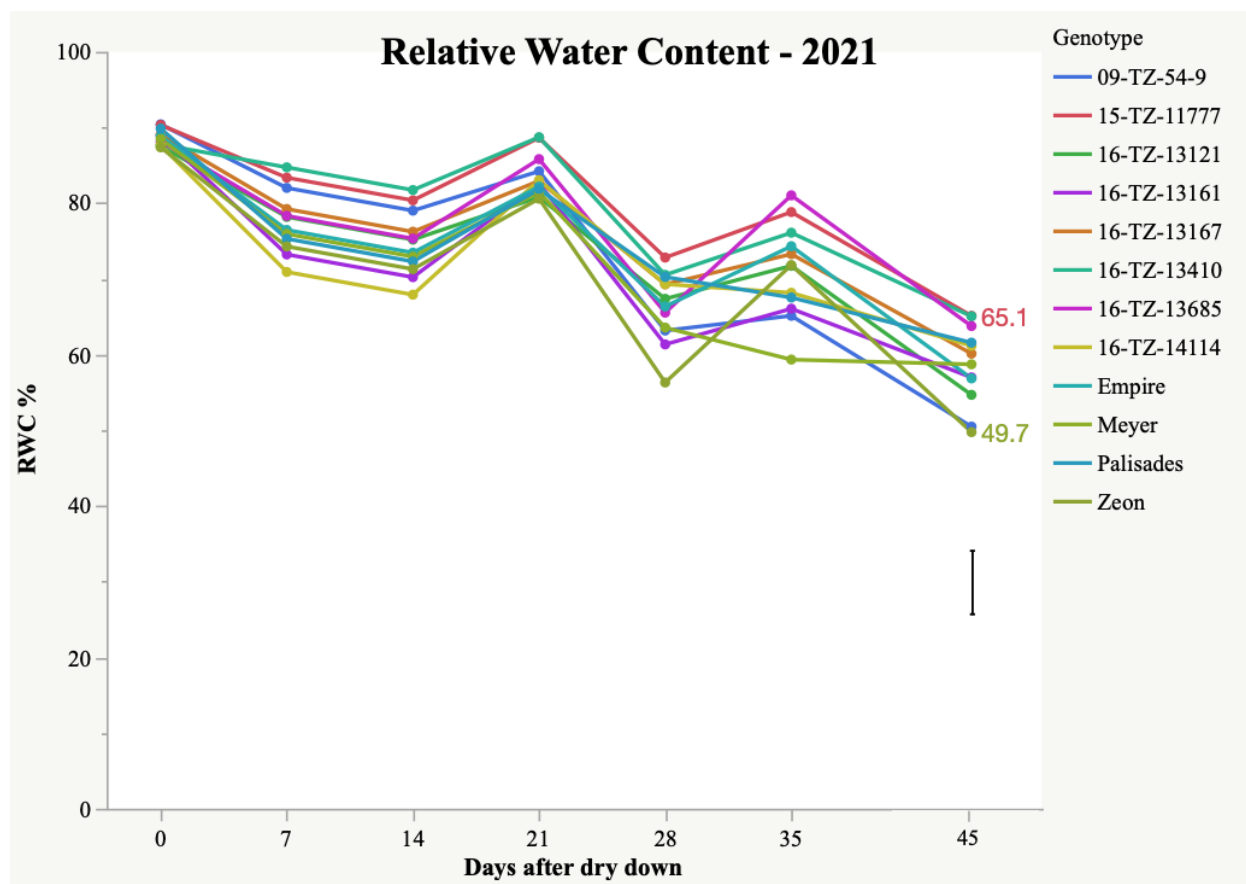


Figure 9: Changes in leaf hydration levels based on relative water content (RWC) values measured over a period of 45 days during 2021 field dry down. Bars show LSD values ($p < 0.05$) on days when significant differences were observed.

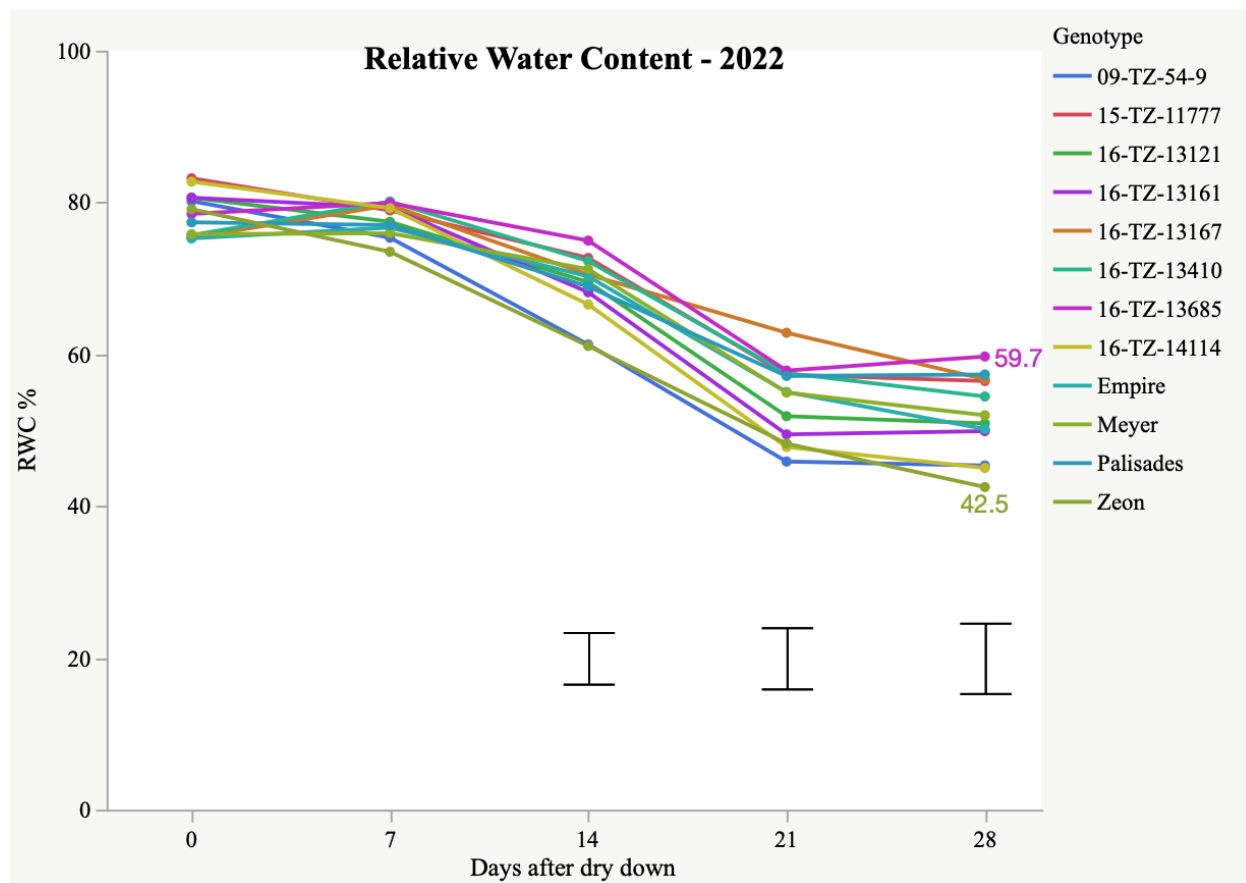


Figure 10: Changes in leaf hydration levels based on RWC values measured over a period of 28 days of field dry down in 2022. Bars show LSD values ($p < 0.05$) on days when significant differences were observed.

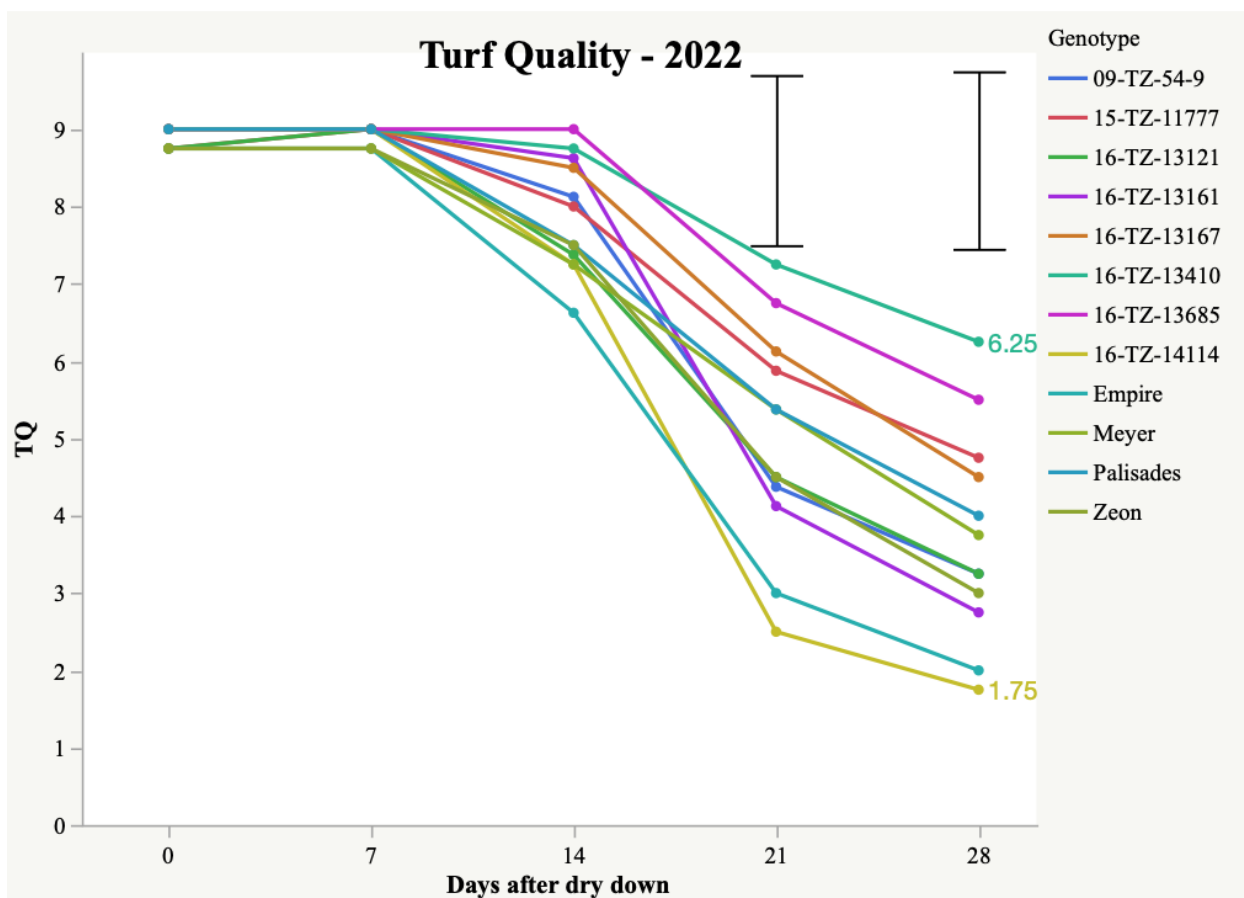


Figure 12: Visual quality based on TQ for twelve zoysiagrass genotypes during 2022 field dry down. Bars show LSD values ($p < 0.05$) on days when significant differences were observed.

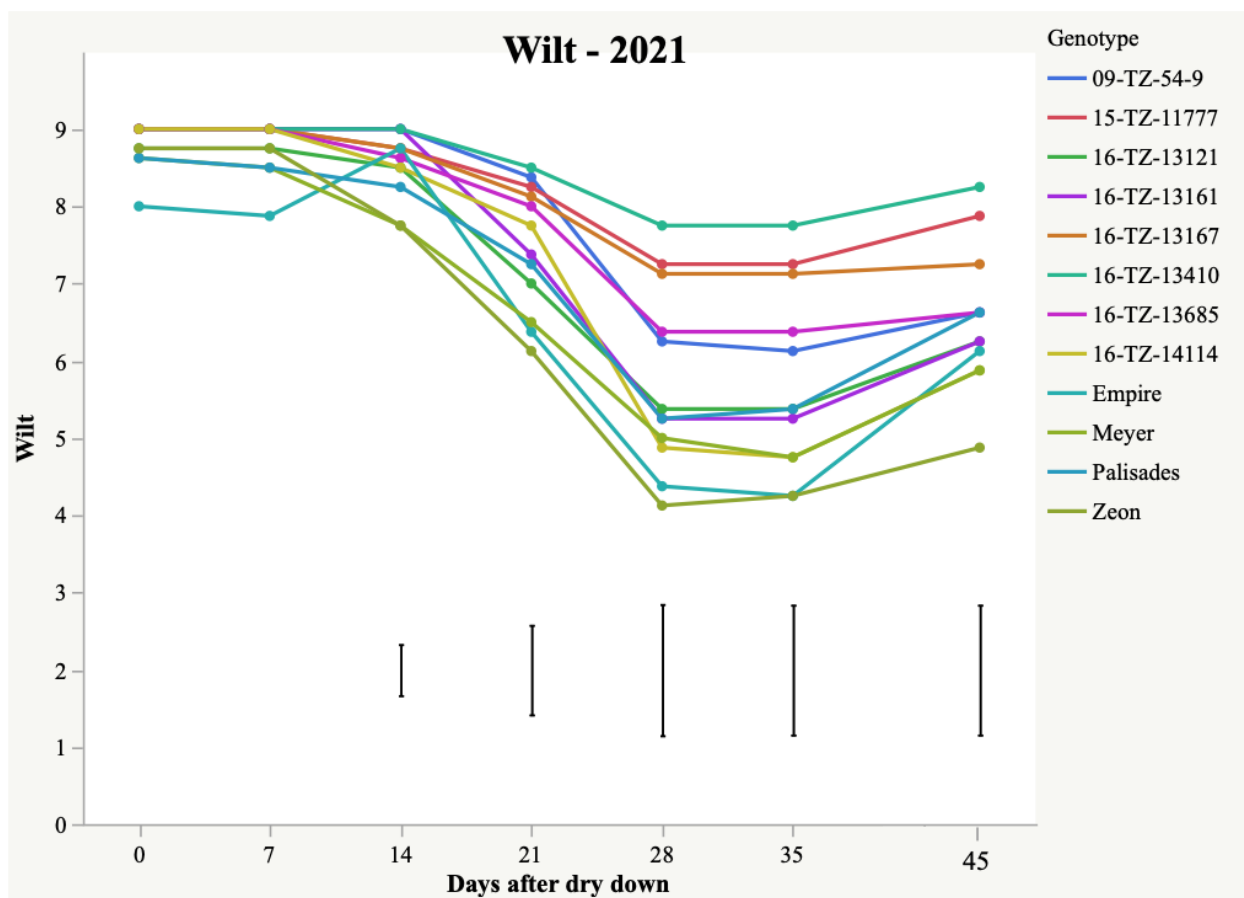


Figure 13: Wilt ratings for twelve zoysiagrass genotypes during 2021 field dry down. Bars show LSD values ($p < 0.05$) on days when significant differences were observed among genotypes

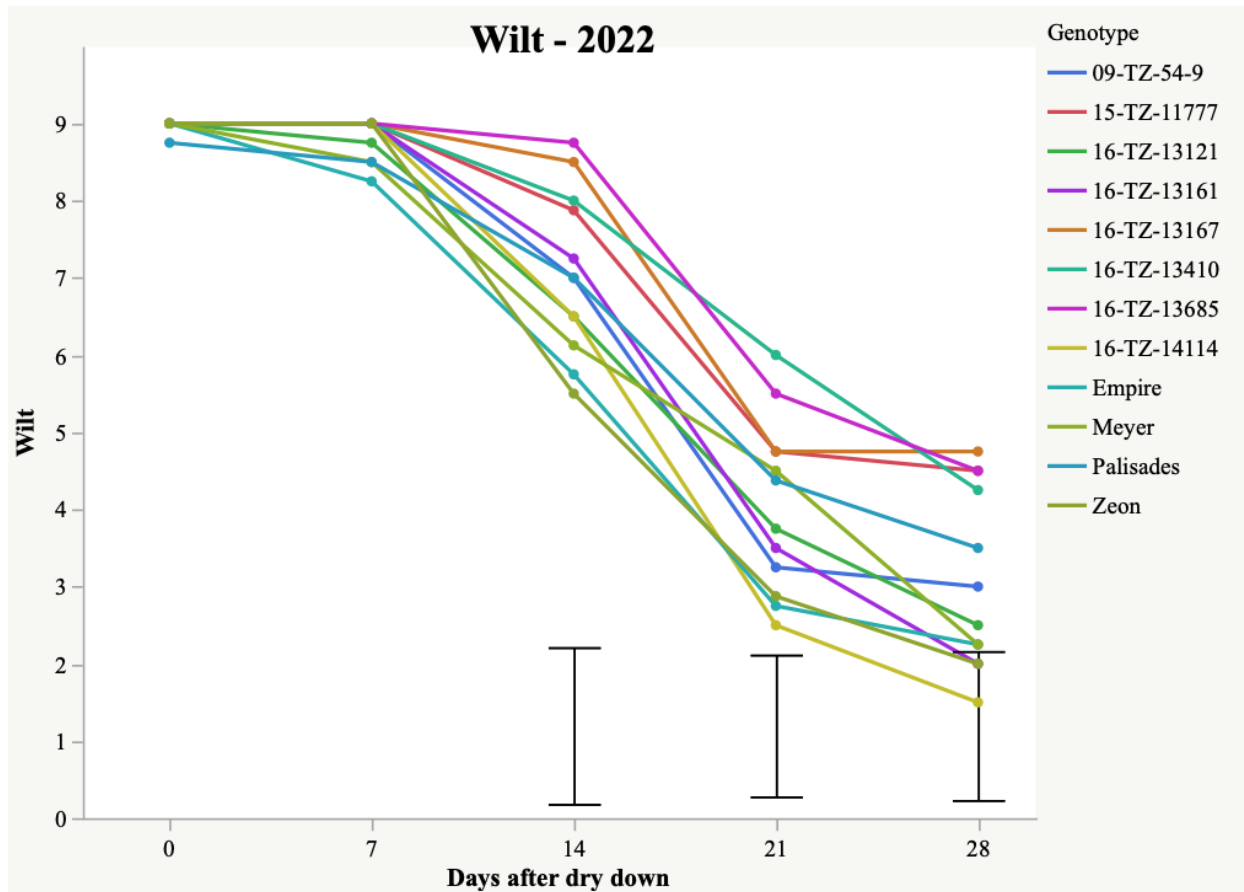


Figure 14: Visual quality based on wilt ratings for twelve zoysiagrass genotypes during 2022 field dry down. Bars show LSD values ($p < 0.05$) on days when significant differences were observed among genotypes.

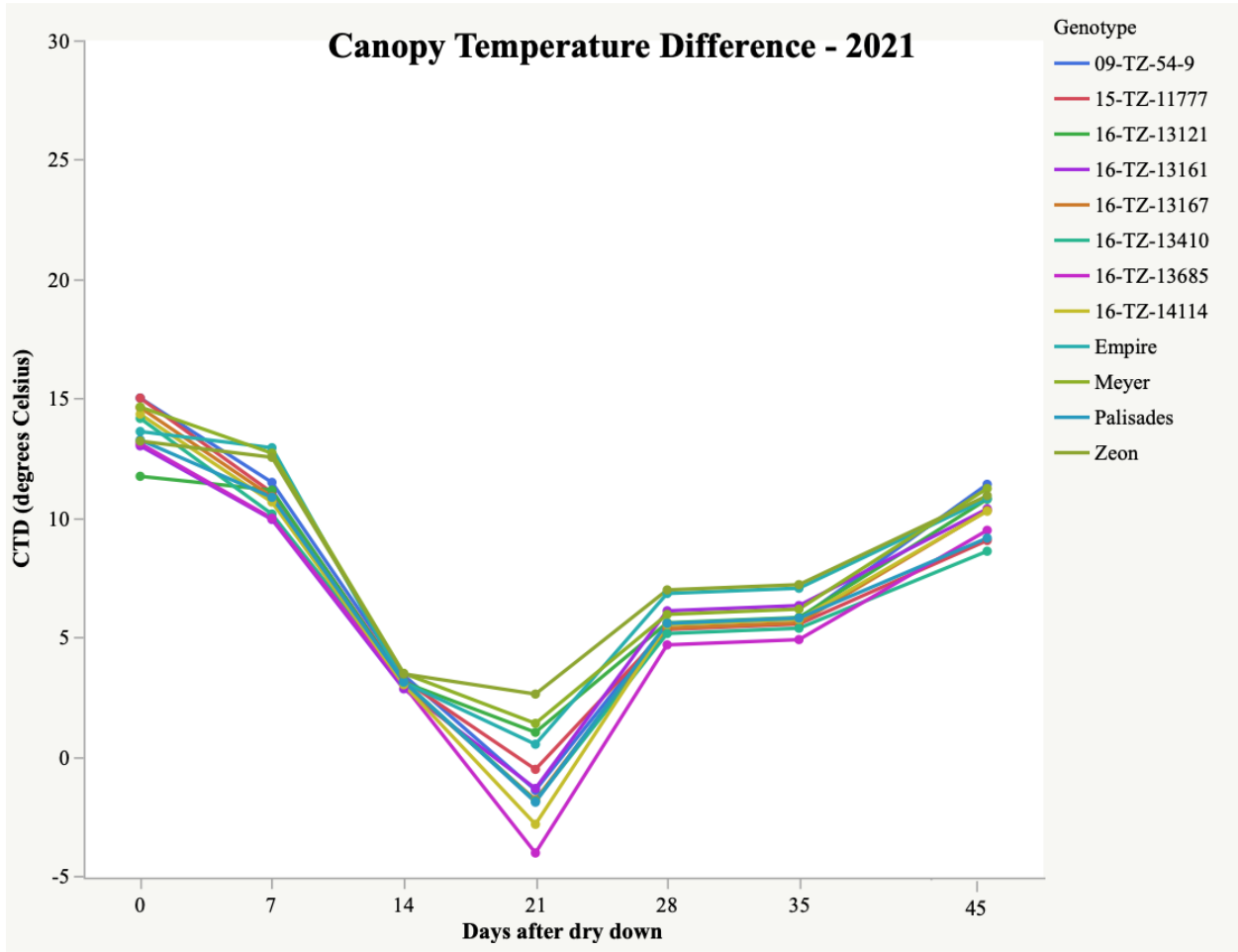


Figure 15: Change in CTD over a period of 45 days of dry down in 2021

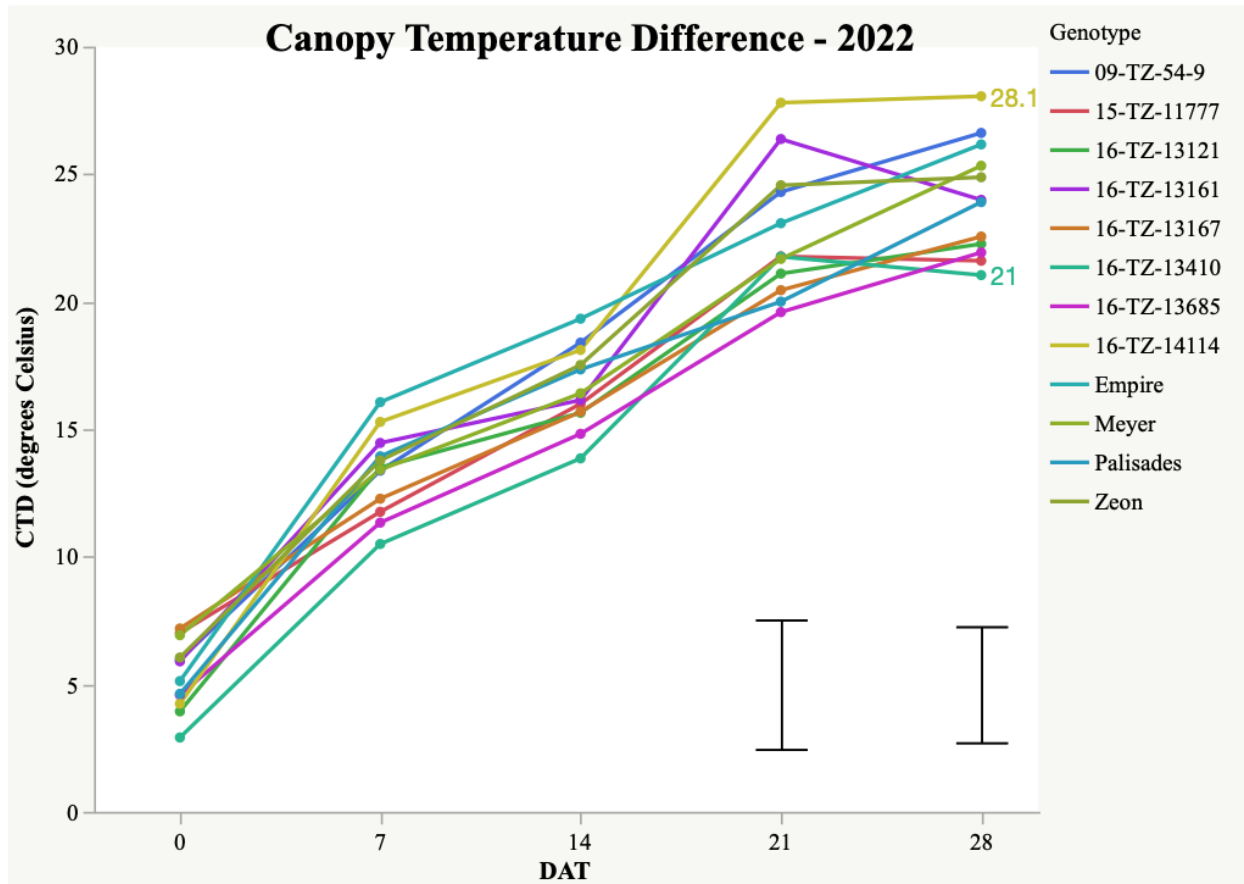


Figure 16: Changes in CTD over a period of 28 days of dry down in 2022. Bars show LSD values ($p < 0.05$) on days when significant differences were observed among genotypes.

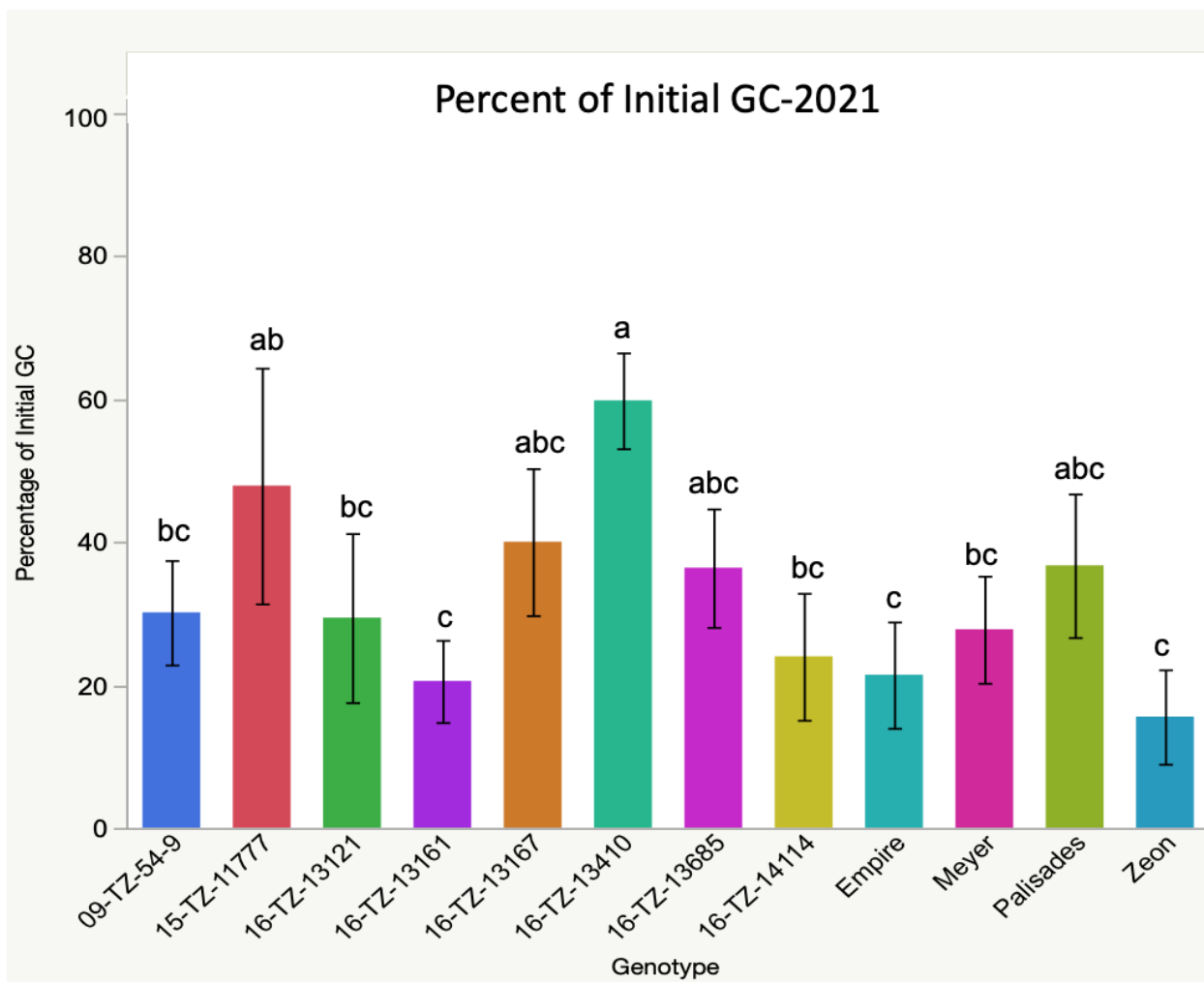


Figure 17: Percentage of initial GC at the final day of dry down in 2021. Genotypes that are connected by common letter are not significantly different at $p < 0.05$. Error bars represent standard error.

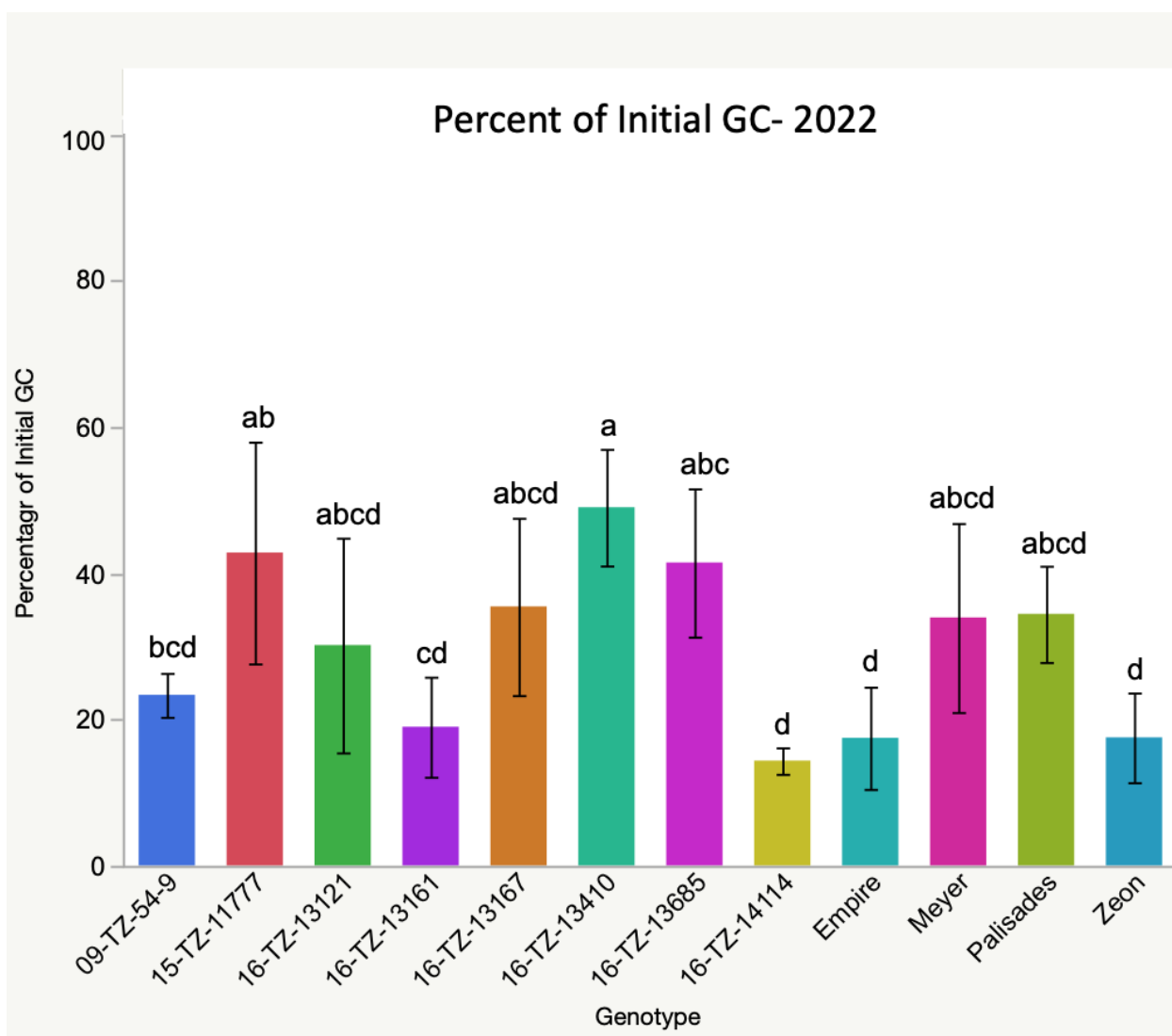


Figure 18: Percentage of initial GC at the final day of dry down in 2022. Genotypes that are connected by common letter are not significantly different at $p < 0.05$. Error bars represent standard errors.

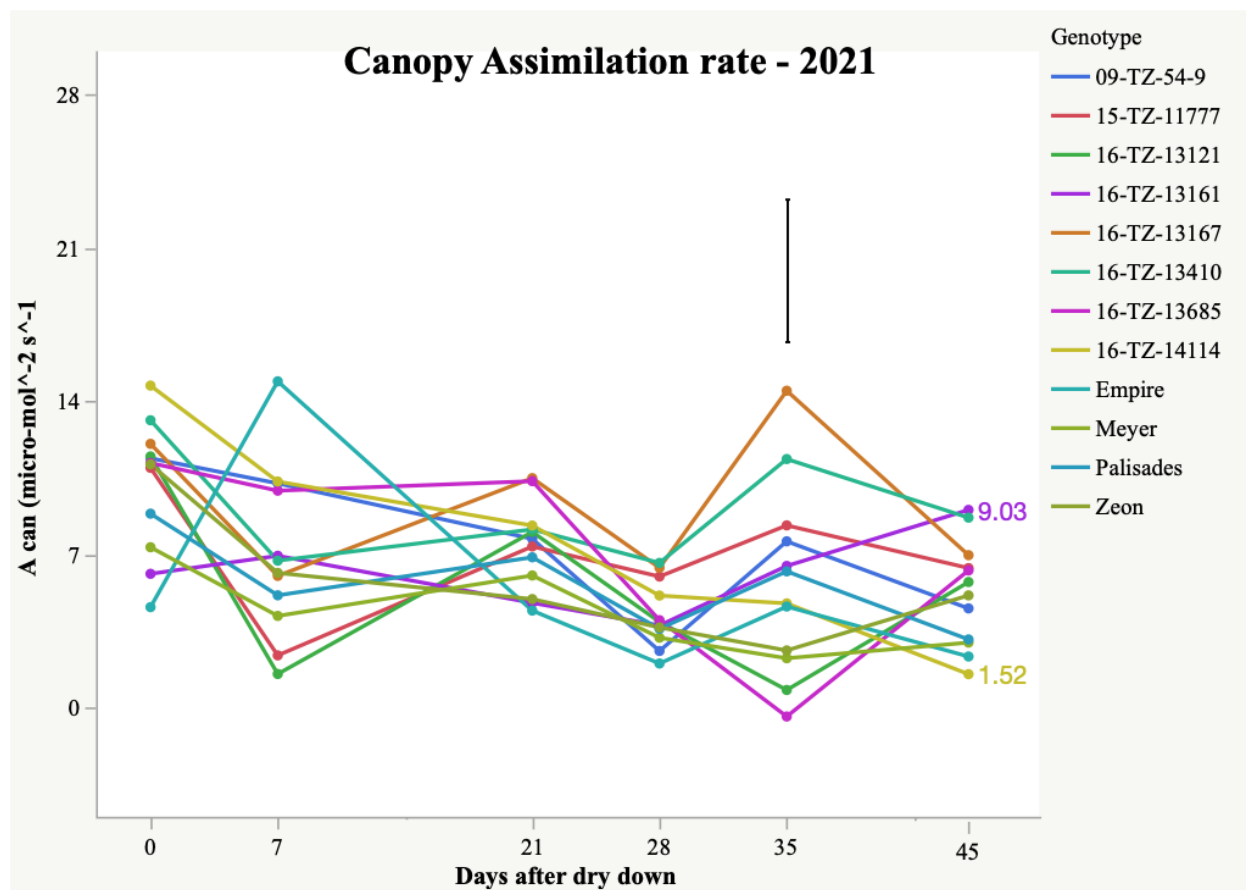


Figure 19: Change in Acan rates at 7, 21, 28, 35 and 45 days after dry down in 2021. Bars show LSD values ($p < 0.05$) on days when significant differences were observed among genotypes.

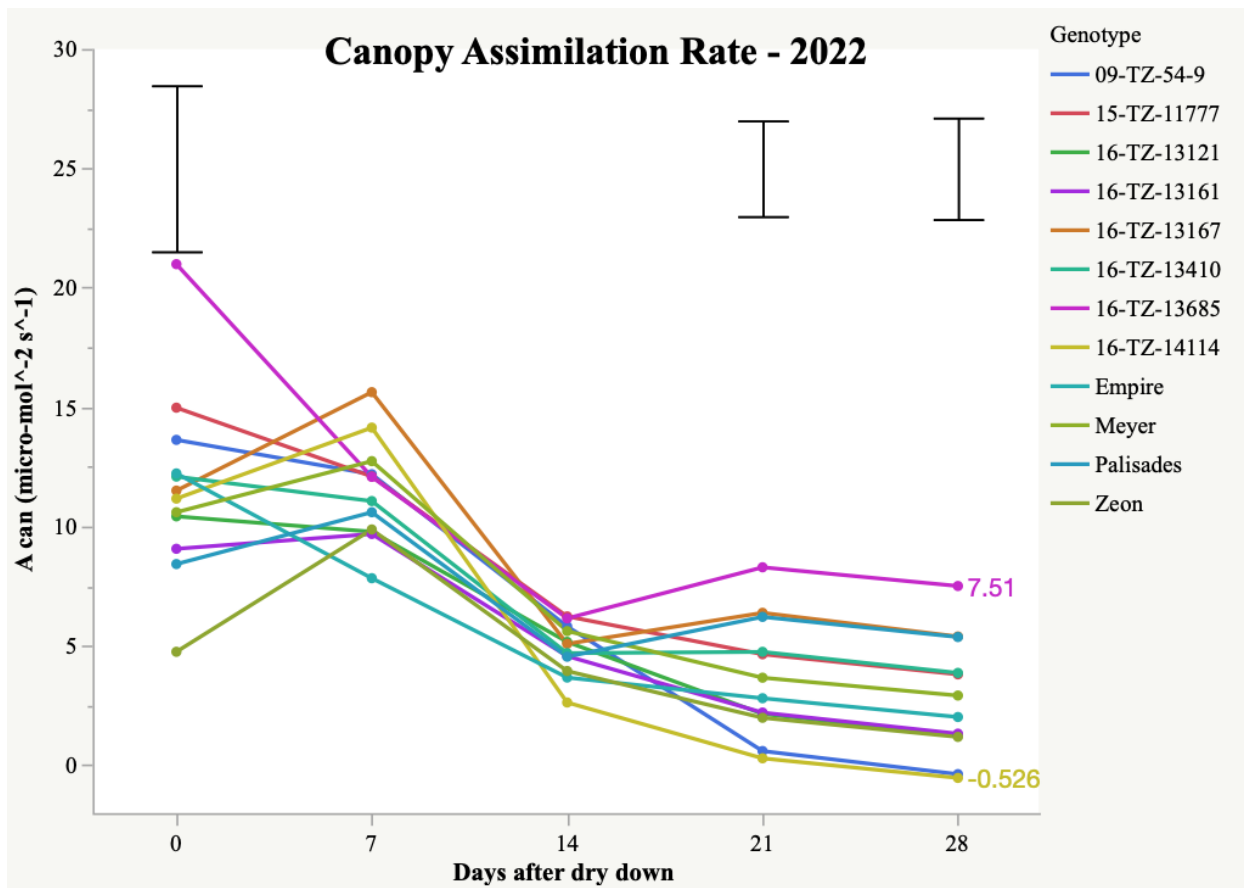


Figure 20: Change in A_{can} rates at 7, 14, 21, 28 days after dry down in 2022. Bars show LSD values ($p < 0.05$) on days when significant differences were observed among genotypes.

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CHAPTER 4

CONCLUSIONS: COMPARISON OF RESULTS BASED ON DIFFERENCES IN DROUGHT STRESS IN DIFFERENT ENVIRONMENTS

The greenhouse study tested nine zoysiagrasses and the field study involved 4 new experimental breeding lines in addition to nine genotypes tested in controlled environment. Comparison of relative drought performance for common genotypes across both studies in two different environments, highlighted few consistently better and poor performing genotypes. In the greenhouse trials, 15-TZ-11777 and 16-TZ-13161 consistently performing better, and were able to maintain better visual quality, leaf hydration levels and carbon fixation rates by efficient regulation of transpiration rates under drought stress. In field trials, 15-TZ-11777 was among the top performing lines, however 16-TZ-13161 had intermediate drought performance based on measured shoot and root responses. Palisades and Empire performed poorly under drought, likely due to the higher water use and transpiration rates along with their higher RLD, root biomass and rapid growth to produce deeper tissues, that resulted in their plant system running out of available soil water soon in the greenhouse trails. Palisades is considered to have better drought resistance under drought stress (White, Engelke et al. 1993). However, in this study, it had high levels of root growth but also greater water use rates and ultimately didn't perform well under drought stress. In the field study, no clear separation in drought performance was seen among the four standard cultivars, but Zeon and Meyer were overall poor performers based on quantified traits. Among the new breeding lines, 16-TZ-13410 and 16-TZ-13685 were consistent top performers with the best turf quality, highest leaf hydration status and better CO₂ assimilation rates under field dry down. While 16-TZ-14114 had the greatest amounts of leaf wilt, firing and poorest turf quality, and also

rated lowest among other measured traits. In the field study, the root growth pattern observed varied from what was expected, as the genotypes that were relatively doing good (16-TZ-11777, 16-TZ-13410, 16-TZ-13685) under drought stress didn't have higher root to shoot ratio as stated in previous studies but had a higher shoot to root ratio instead, and less extensive roots. Increased root to shoot ratio is generally considered most common drought effect on plants, but it is likely possible that this higher S:R ratio is due to reduced shoot growth under stress and no change in root growth as studied in ornamental perennials by (Zollinger, Kjelgren et al. 2006) or due to less restricted growth in roots when compared to shoots (McMichael and Quisenberry 1991). The increased root growth under drought conditions has been proposed to provide a greater access to soil moisture which was seen in genotypes like Palisades and Empire with root growth in present study (Huang 2000). However, there is more to drought resistance than just better root growth and development, which involves ability to maintain plant water balance under drought by regulation of transpiration. This was clearly the case with 'Palisades' poor performance in current study. Palisades had higher root to shoot ratio and greater network surface area in the field study, as in the greenhouse study, indicating similar root development responses, however its drought performance remained intermediate. Also, direct correlation of root to shoot ratio and drought tolerance has not always been observed, which can be partially explained by variations in root's water uptake capacity among different genotypes (Zhimin, Thompson et al. 2000, Matsui and Singh 2003). Despite Palisades having a larger root system, had greater transpiration rates starting from early on in dry down period and continued to have high E rates of $0.93 \text{ mmol m}^{-2} \text{ s}^{-1}$ and gs at 15 days of dry down. This can be explained by differences in growth and greater leaf area exposing larger stomatal surface to evaporative water loss under drought stress. While genotypes like 16-TZ-13161 and 15-TZ-11777 were able to better regulate the water loss as drought

progressed by reducing stomatal conductance after 5 days into dry down period and had lower g_s (12-21 $\text{mmol m}^{-2} \text{s}^{-1}$) and E rates (0.40-0.22 $\text{mmol m}^{-2} \text{s}^{-1}$) at 15 days of stress. Therefore, best performing genotypes despite having intermediate to less root growth, also lost less water via transpiration.

Variations in drought performance between greenhouse and field study was explained by different growth environment, less restricted growth in field compared to pots in greenhouse, also changes in edaphic factors, soil pH, and different temperature conditions. Both greenhouse and field study took place in relatively sandy soil media, and therefore had less water holding capacity. It may be valuable to look at these grasses also in soils that have higher clay contents, and higher water holding capacity where potential genotypes that are water spenders with large root systems won't deplete all the available water as quickly. Researchers should continue to try to identify genotypes that have robust root systems, but do not use a water spender strategy and have lower transpiration rates. Future scope of this research would be to compare the performance of the best and worst performing genotypes from this study along similar measured set of traits but also looking beyond only drought avoidance, by also understanding drought tolerance mechanisms. It would potentially involve analyzing underlying changes in protein metabolism by identifying dehydrin candidate genes and performing dehydrin expression analysis. This would likely provide substantial information about genotypic differences among these lines and how that governs drought avoidance, for future release as commercial cultivars.

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APPENDIX

Table S1: Percentage of initial green cover in nine zoysiagrass genotypes on final day of drought stress during two greenhouse experiments in 2021 and 2022

Genotype	2021	2022
09-TZ-54-9	17.74 abc	36.06 a
15-TZ-11777	39.35 a	12.86 cd
16-TZ-13121	17.78 abc	17.13 bcd
16-TZ-13161	21.22 abc	31.62 ab
16-TZ-13167	14.71 bc	20.05 abcd
Empire	0.022 c	12.63 d
Meyer	27.57 ab	31.06 abc
Palisades	6.48 bc	16.29 bcd
Zeon	40.56 a	18.56 abcd

Means connected by common letter are not significantly different at $p \leq 0.05$ level of significance

Table S2: Percentage of Initial GC of twelve zoysiagrass genotypes on final day of field dry down during 2021 and 2022

% Of Initial GC		
Genotype	Trial 2021	Trial 2022
09-TZ-54-9	30.1 bc	23.37 bcd
15-TZ-11777	47.9 ab	42.83 ab
16-TZ-13121	29.43 bc	30.18 abcd
16-TZ-13161	20.56 c	19.01 cd
16-TZ-13167	40.05 abc	35.46 abcd
16-TZ-13410	59.83 a	49.03 a
16-TZ-13685	36.41 abc	41.46 abc
16-TZ-14114	24.01 bc	14.38 d
Empire	21.44 c	17.50 d
Meyer	27.80 bc	33.95 abcd
Palisades	36.74 abc	34.45 abcd
Zeon	15.58 c	17.56 d

Means connected by common letter are not significantly different at $p \leq 0.05$ level of significance

Table S3: RWC of twelve zoysiagrass genotypes at different sampling dates during field dry down in 2021

Genotype	RWC- Field Study 2021					
	0 DAT	7 DAT	21 DAT	28 DAT	35 DAT	45 DAT
09-TZ-54-9	90.41 a	82.01 a	84.21 a	63.17 a	65.11 a	50.47 b
15-TZ-11777	90.33 a	83.36 a	88.63 a	72.81 a	78.82 a	65.11 a
16-TZ-13121	87.52 a	78.17 a	80.79 a	67.35 a	71.72 a	54.67 ab
16-TZ-13161	88.95 a	73.22 a	82.60 a	61.33 a	66.05 a	56.98 ab
16-TZ-13167	89.02 a	79.22 a	82.93 a	69.27 a	73.26 a	60.11 ab
16-TZ-13410	87.77 a	84.73 a	88.71 a	70.52 a	76.09 a	65.05 a
16-TZ-13685	88.14 a	78.32 a	85.81 a	65.53 a	81.03 a	63.76 ab
16-TZ-14114	87.63 a	70.90 a	83.03 a	69.29 a	68.16 a	61.15 ab
Empire	88.86 a	76.46 a	82.18 a	66.34 a	74.30 a	56.84 ab
Meyer	88.46 a	75.92 a	81.46 a	63.55 a	59.33 a	58.70 ab
Palisades	89.85 a	75.28 a	81.83 a	70.24 a	67.53 a	61.56 ab
Zeon	87.37 a	74.28 a	80.58 a	56.30 a	71.81 a	49.74 b

Means connected by common letter are not significantly different at $p \leq 0.05$ level of significance

Table S4: RWC in twelve zoysiagrass genotypes at different sampling dates during field dry down in 2022

RWC- Field Study 2022					
Genotype	0 DAT	7 DAT	14 DAT	21 DAT	28 DAT
09-TZ-54-9	89.17 a	75.36 a	61.09 b	45.86 b	45.34 ab
15-TZ-11777	83.18 a	78.95 a	72.69 ab	57.30 ab	56.47 ab
16-TZ-13121	80.64 a	77.45 a	69.50 ab	51.83 ab	50.89 ab
16-TZ-13161	80.62 a	79.34 a	68.17 ab	49.44 ab	49.87 ab
16-TZ-13167	75.33 a	79.66 a	70.50 ab	62.83 a	56.70 ab
16-TZ-13410	75.69 a	80.12 a	72.28 ab	57.52 ab	54.43 ab
16-TZ-13685	78.49 a	79.94 a	74.97 a	57.85 ab	59.70 a
16-TZ-14114	82.74 a	79.25 a	66.56 ab	47.78 b	45.03 ab
Empire	75.26 a	76.68 a	70.25 ab	55.02 ab	50.15 ab
Meyer	75.82 a	75.94 a	71.21 ab	54.95 ab	51.96 ab
Palisades	77.39 a	77.02 a	68.91 ab	57.13 ab	57.33 ab
Zeon	79.11 a	73.50 a	61.09 b	48.23 b	42.50 b

Means connected by common letter are not significantly different at $p \leq 0.05$ level of significance