SALINITY TOLERANCE MECHANISMS OF WARM-SEASON TURFGRASS EXPERIMENTAL LINES AND CULTIVARS

A Dissertation

by

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ABSTRACT

Growth and physiological mechanisms of select experimental lines were chosen genotypes of warm-season turfgrass species bermudagrass (*C. dactylon* sp), zoysiagrass (*Z. matrella* sp and *Z japonica* sp), St. Augustinegrass (*Stenotraphrum secundatum*), and Seashore paspalum (*Paspalum vaginatum*) were studied. Greenhouse screenings were conducted during 2014 and 2015 at Texas A&M University, College Station TX to determine relative salinity tolerance among species under four salinity levels: 0, 15, 30 and 45 dS m^{-1}. Based on these initial screening studies, in 2015, the best-performing entry of each species was examined following exposure to two levels of salinity (0 and 30 dS m^{-1}) using scanning electron microscopy and energy dispersive spectroscopy to elucidate morphological/ anatomical attributes related to salinity tolerance mechanisms.

In 2016, eight entries (2 entries per species representing the highest and lowest-performing lines for relative salinity tolerance) were advanced for further evaluation aimed at determining physiological responses to salinity. Grasses were grown in the greenhouse over 10 weeks at salinity levels of 0, 15, and 30 dS m^{-1}. Ion excretion efficiency, Na and Cl concentrations, and root and shoot tissue Na:K were evaluated to determine relationships with previously observed differences in salinity tolerance/intolerance. Collectively, the data support the notion that salinity tolerant genotypes employ one or more physiological mechanisms including salt excretion, root exclusion, limitation of Na and/or Cl transport to shoots, and maintenance of ion balance in coping with saline conditions.

DEDICATION

To my beautiful and wonderful family

“WE ARE A GREAT TEAM”

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NOMENCLATURE

EDS	Energy Dispersive Spectroscopy
dS m^{-1}	DeciSiemens per meter
µmS m^{-1}	MicroSiemens per meter
pH	Potential of Hydrogen
SEM	Scanning Electron Microscopy
mm^2	Square millimeters
NDVI	Normalized Difference Vegetation Index
µg	Micrograms
USGA	United States Golf Association
EC	Electrical Conductivity
SPSS	Statistical Package for the Social Sciences
ANOVA	Analysis of Variance
LSD	Least Significant Difference

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Potable water for landscape irrigation is becoming less available due to increasing urban populations in arid and semi-arid areas where salinity, sodicity, and drought, are constant problems due to climatic conditions (Lee et al., 2007; Marcum (2006)). Improper management of agricultural soils in arid and semi-arid regions of the world have resulted in high salinity levels, reduced crop production, and eventual abandonment (Sema, 2012). Balancing potable water use between agricultural, industrial, residential, and landscape is a major concern to local and national governments (Marcum, 2007). Some countries with arid and semi-arid regions such as Australia, Mexico, and the United States of America, now restrict the use of potable water for irrigation of recreational areas and landscapes (Rhoades et al., 1992). As a result, utilization of low-quality recycled or reclaimed wastewater, gray water or even sea water for irrigation is becoming more common at turfgrass facilities such as golf courses, municipal parks, and stadiums (Devitt et al., 2004).

According to the Golf Course Superintendents Association of America's Environmental Institute for Golf Survey (GCSAA, 2015), recycled water is now the predominant irrigation source used on golf courses in the southwestern and southeastern regions of the United States. Use of recycled water for golf course irrigation in the southern region of United States has increased by 7% and 10% from 2005 to 2013. Recycled wastewater was reportedly used by 30 and 45% of the golf courses from the southern region.

Use of low-quality recycled water can lead to elevated soil salinity levels, but also can provide additional nutrients to turf (Qian and Harivandi, 2008). Furthermore, use of recycled water has the potential to damage the foliar tissue of the plants and cause plant stress (Devitt et al., 2004). Minimizing salt application to soil can be a challenge, and therefore utmost consideration should be given to selecting salt-tolerant turfgrass species, capturing and utilizing natural rainfall to blend with or use in place of recycled water, and application of a maintenance salt leaching programs to keep root zone salinity at or below the salinity thresholds of the species/cultivars used.

Turfgrasses are generally well-suited for effluent irrigation because they function as biological filters which can remove excess salts and nutrients from saline water (Hayes et al., 1990). Furthermore, warm-season (C_4) turfgrasses are well suited for use in arid and semi-arid areas since they generally possess increased resistance to both drought and salinity stress occurring from poor water quality and/or inadequate water availability (Marcum, 2006; Uddin and Juraimi, 2013). Turfgrasses are also an important resource for rehabilitating landscapes, covering sports surfaces, stabilizing slopes, reducing soil erosion and dust, capturing carbon dioxide, releasing oxygen, aiding in groundwater capture, reducing urban heating, noise, and glare, providing health benefits to humans, and discouraging criminal activities (Beard and Green, 1994; Turgeon, 1991).

SOURCES OF SALINITY

Salinity occurs through accumulation and combination of salts due to a slope, soil type, and insufficient leaching to remove salts stored during a long period of time through natural processes like weathering of parent material or human practices such as agriculture, land clearing, and poor irrigation water quality (Carrow and Duncan, 1998). Weather conditions such as precipitation levels can also affect salt concentration in soil, as well as evaporation, which can lead to salt accumulation in soil and water. Desert zones are highly prone to elevated salinity concentrations, as the rate of evaporation is higher than the rate of precipitation (Mahajan and Tuteja, 2005).

The most frequent salinizing ions that affect soils, surface water, and ground water are calcium (Ca^{+2}), magnesium (Mg^{+2}), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), bicarbonate (HCO_3-), carbonate (CO_3^{-2}), sulfate (SO_4^{-2}), and nitrates (NO_3^{-})(Grattan, 2002). Wind and rainfall could be other sources of accumulation of salts, particularly sodium chloride, in soils. The primary minerals contained in rainfall are (Na^{+}), (Cl^{-}), (SO_4^{-2}), (Mg^{+2}), (Ca^{+2}), and (K^{+}). Rainfall in close proximity to coastal areas may have salt concentrations of 6 to 50 mg kg^{-1}, and can also be influenced by prevailing winds, decreasing with distance from the coast. Salt concentrations can also vary with soil type, accumulating to higher levels in clay compared to sandy soils (Munns and Tester, 2008).

The increasing demand for higher crop production yields in order to sustain the worlds growing population has resulted in several negative impacts on the environment, not the least of which includes salinization of land and water (Läuchli and Grattan,

2007). Furthermore, fresh water aquifers are being affected by salt water intrusion in many coastal areas as a result of increasing demand for potable water (Carrow and Duncan, 1998). Land clearing to replace native vegetation by crops may also contribute to soil and water salinity issues as a result of inappropriate and inefficient irrigation practices and excessive use of fertilizers over time (Munns and Tester, 2008; Rhoades et al., 1992).

Waterlogging is another physiological issue that can occur due to high salt levels in soil and is a complex problem related to many factors including irrigation frequency, crop/cultivars species, climatic conditions, and soil type (Rhoades et al., 1992). Salt may arise from use of brackish water, but commonly occurs from use are gray water and recycled wastewater. Gray water refers to water that has been used in the home for laundry, shower/tub, and dishwashing purposes, whereas recycled wastewater is defined as any water which was used for industrial and residential purposes and then treated through up to three processes including primary treatment (settling and screening), secondary treatment (active sludge, filtration, oxidation, chloride or UV disinfection), and tertiary treatment (clarification, coagulation, sedimentation, activated charcoal, and UV disinfection). By law in most areas of the U.S., recycled water must receive at least secondary treatment to be used for irrigating turfgrass facilities. Recycled water is commonly used in landscapes, golf courses, parks, and sport fields, however, problems with accumulation of excess salts have occurred on numerous sites due to poor water quality and/or improper irrigation management with this increasingly used water resource.

CLASSIFICATION OF SOILS AFFECTED BY SALINITY

With regard to salinity/sodicity, soils are generally classified as saline, saline-sodic, or sodic. Soils in each of these classifications have different chemical characteristics (Robbins and Gavlak, 1989). As such, a saline soil is characterized by having an EC > 4.0 dSm^{-1} , ESP < 15%, and pH < 8.5 (Carrow et al., 2001b). Saline soils contain sufficiently high amounts of total soluble salts that they can negatively affect the development of most plants (Carrow and Duncan, 1998). Saline soil is often characterized by a white surface crust due to salt accumulation on the soil surface that remains following evaporation. Although the salinity might not adversely affect soil physical properties, it clearly has negative impacts on plant growth (Provin and Pitt, 2001). Salts in soil attract water, thereby limiting water availability for plant uptake, even when the soil contains acceptable moisture levels (Kissel et al., 2012). Physiological drought, wilt, and leaf firing are common visual symptoms occurring in plants due to high soluble salts. In addition, specific ion toxicity to plants (roots and shoots) may occur from high concentrations of Na^{+}, Cl^{-}, B, HCO_3^{-}, or SO_4^{-2}.

Sodic soils are considered to be the most difficult to manage compared to those which are saline, and saline-sodic (Duncan et al., 2000). A soil that is classified as sodic presents field symptoms such as black color due to dissolution of the organic matter and it is characterized by poor structure caused from breakdown of soil aggregates, dispersion of organic and inorganic matter colloids causing plugged of pores, low permeability to air and water, and the formation of a hard crust when it is dry (Carrow et al., 2001b; Provin and Pitt, 2001). A frequent plant symptom due to sodic soil stress is a

rachitic and darked root growth. Distinctive characteristics of sodic soils are an EC < 4.0 dSm^{-1}, a high ESP $\geq$% 15, and a pH from >8.5.

Saline-sodic soils have high total salts similar to saline soils (EC > 4.0 dSm^{-1}) while also containing high levels of exchangeable Na^{+} (ESP $\geq$ 15%) and high pH (>8.5). While the high Na^{+} content leads to deterioration of the soil structure, which results in reduced soil permeability, the elevated EC in these soils actually helps to mitigate these negative affects by aiding in water permeability. Thus, these soils tend to be easier to manage than those classified as sodic (Duncan et al., 2000).

SODIUM EFFECTS ON SOIL STRUCTURE

Soils with high Na^{+} concentration and low relative Ca^{+2} and Mg^{+2} concentrations can present challenges to plant growth. Sodium is the primary mineral which destroys soil structure and leads to reduction of soil pore size, reduction of oxygen, increases potential for waterlogging, ultimately leading to higher potential run-off and soil hardness (Robbins and Gavlak, 1989).

Good soil structure allows a soil to maintain acceptable levels of permeability, allowing water and air exchange, as well as growth. Soil structure is made up of sand, silt, and clay domain particles grouped and maintained together in soil aggregates, held together by Ca^{+2} and Mg^{+2}. Clay domains are arrangements of clay platelets stacked together by attractive forces of platelet surface charges of divalent cations including Ca^{+2} (Carrow et al., 2001b).

When Na^{+} dominates cation exchange sites in relation to Ca^{+2} and Mg^{+2} , Na^{+} ions displace Ca^{+2} and Mg^{+2} between the clay domains, especially at clay platelets. With rainfall or irrigation, this is then followed by leaching of Ca^{+2} and Mg^{+2} from the soil profile (Carrow and Duncan, 1998). The attractive forces between soil aggregates begin to weaken and break down, or disperse into single platelets which lead to sealing of the soil surface. In this way, sealing and reduced permeability of sodic can increase soil and water runoff under heavy rainfall (Davis, 2003; Ghadiri et al., 2004). Conversely, sandy soils are more physically resistant to dispersion due to their large particle size and low CEC (Cisar and Snyder, 2003).

SALINITY STRESS IN PLANTS

Most plants are unable to grow in soils containing high concentration of salts because of the osmotic stress caused by root zone salinity. Specific ion content in soil and irrigation water, particularly Na^{+}, Cl^{-}, B, HCO_3^{-}, and OH^{-} at high concentrations may lead to detrimental effects in plant foliage. Water deficiency, nutrient imbalance, and ion toxicity are three primary issues resulting in plants growing under saline conditions (Grattan, 2002). The extent of negative plant responses to salinity may depend on various factors including plant growth stage, genetic tolerance, and environmental conditions such as temperature, relative humidity, and light. For example, during the spring, plants may accumulate toxic levels of some minerals, yet may not show visible symptoms of salinity stress, while during summer these same plants may express greater injury symptoms when plant metabolism is active as a result of conducive environmental

conditions (Bernstein, 1975). Where salinity is a potential concern, soil and tissue testing should be ultimately be performed to monitor and distinguish salinity problems from other biotic or abiotic stress factors that might also reduce plant quality (Ehlig and Bernstein, 1959).

Salinity in soil can induce water deficiency within plant tissue which may affect physiological processes. Salinity becomes a problem when it increases its concentration in the root zone negatively affecting the plant´s ability to take up water from the soil to maintain turgor pressure. Physiological drought due to high salinity and drought stress due to lack of available water may overlap, and both may ultimately contribute to reduced turgor pressure due to a low osmotic potential in the soil (Romero-Aranda et al., 2001).

Tissue nutrient imbalance is another physiological disorder occurring under elevated soil salinity, particularly in regard to elevated Na^+ and Cl^-. Nutrient imbalances can lead to effects on assimilation, transport, and distribution of essential mineral nutrients within the plant including Ca^{+2}, K+, and NO_3^- (Ahmad et al., 2012). Nutrient imbalances in turfgrasses under salinity stress often begin with displacement of Ca^{2+} and K^+ by excessive Na^+ and Cl^- in the soil and irrigation water (Jouyban, 2012a).. Aquaporins, which are membrane proteins facilitating transcellular symplastic transport of water and nutrients, have been shown to be largely tolerant to tolerate salt exposure. Aquaporins function in roots to select and exclude micronutrients and ion solutes. The presence of aquaporins is essential for potassium transport in plants, which function to maintain cell turgor and enzyme activity (Fricke and Peters, 2002).

Potassium is an essential nutrient known for conferring tolerance to cold, heat, and drought, as well as wear tolerance in turfgrasses (Xiong L. 2002). As a result of chemical similarities between Na^+ and K^+ ions, K^+ uptake can be inhibited by presence of elevated Na^+ in soil solutions, thus producing K^+ deficiency in plant tissues. Several reports also indicate that cell enlargement and cell division in plants can also be negatively affected due to reduced N uptake under salinity stress. Nitrate uptake is negatively affected by presence of Cl^- in soil solution, resulting in reduction of plant growth. (Ahmad et al., 2012). This is thought to occur through direct competitive effects between Cl^- and NO_3^-. Conversely, Ca^{2+} supply and concentrations in soil solution under salinity stress can aid in NO_3^- uptake due to the role of Ca in in maintaining cell membrane integrity (Miura, 2013). Calcium supply in soil may also indirectly improve K^+ uptake and transport from soil through enhancing soil structural conditions (Carrow et al., 2001b).

Ion toxicity symptoms in plants occur through continuous exposure to saline soils and/or irrigation water and accumulation in plant tissues over time. Salts are normally taken up through roots, loaded into xylem and phloem, and translocated to all plant tissues, with some plants sequestering or sequestering and/or exuding excess salts through glands or bladders (Ehlig and Bernstein, 1959; Munns, 2002). Munns (2002) found that a salt tolerant plants grown for several days under (100 mM NaCl) possessed 50 mM NaCl concentrations in the roots, 5 mM NaCl concentrations in the xylem, and 500 mM NaCl concentrations in oldest leaves. Collectively. these data suggest that salts

move through plants via both apoplastic and symplastic pathways.(Garciadeblas et al., 2003).

The method of irrigation application can also influence relative plant tolerance to saline irrigation water. For example, when saline irrigation water is applied directly to plant leaves, the tolerance to salinity can be noticeably reduced as a consequence ion accumulation on the surface of the leaves (Xiang et al., 2017). Salinity stress injury from saline irrigation has been reported from levels as low as 0.3 dS m^{-1}. Furthermore, intermittent wetting of foliage or turf leaves during the day can worsen salinity stress damage due to repeated accumulation of salt layers on the leaves through evaporation (Benes et al., 1996). For example, cotton crop yields have been shown to be reduced by 50% when receiving daytime irrigation with saline water during the daytime hours and associated high temperatures; however, no injury was reported with nighttime irrigation using the same water when the temperatures were lower (Busch and Turner Jr, 1965).

As a consequence of Na uptake and transport from roots to leaves, salt injury stress and symptoms are generally most evident in leaves, due to higher relative Na leaf concentrations (Parvaiz and Satyawati, 2008). Visual salinity stress symptoms in plants include darker green color, thicker and smaller leaves, growth reduction, margin and leaf burn, bronzing leaves, and necrotic tissue. Although many times occurring in salt-stressed plant leaves, chlorosis is not considered a direct symptom of salt injury (Bernstein, 1975). Also, while some plants may not yet express visible symptoms in mature leaves, they may be undergoing internal stress symptoms (Nable et al., 1997). For instance, plants exposed to elevated salinity may sequester Na within vacuoles,

which may cause detrimental effects in plants such as interrupted enzymatic and protein assimilation processes in the cytoplasm and reduced metabolic activity due to low photosynthetic activity and reduced leaf area. Munns (2002) summarized the sequential salinity effects in plants through the time (Table 1).

Table 1. Time-dependent effect of salinity on plant growth

Time scale	Causes	Effects
Second to minutes	Water stress	*Morphological*: Immediate reduction in root and leaf elongation rates which is sometimes are partially recoverable. *Cellular*: Shrinkage of cell volume followed by respiration due to regaining turgor
Hours	Water stress, Ca^{2+}, deficiency	*Morphological*: Permanent reduction in root and leaf elongation *Cellular*: Changes rheological behavior of cell wall
Days	Water stress, Ca^{2+} deficiency	*Morphological*: Reduction in leaf emergence, increase in root: shoot ratio *Cellular*: Inhibition cell development
Weeks	Water stress & ion toxicity	*Morphological*: Reduced branches/tiller formation, death of older leaves *Cellular*: Alteration of apical development excessive accumulation of Na^+ and Cl^-
Months	Water stress & ion toxicity	*Morphological*: Alteration in flowering time and reduced seed production. Immature death of plants *Cellular:* Alteration in the development of reproductive organs, Reduction of assimilate production.

CHAPTER II

COMPARATIVE SALINITY TOLERANCE OF WARM-SEASON TURFGRASS CULTIVARS

OVERVIEW

As population growth and demand for potable water increases, available water for irrigation has been decreasing both in quantity and quality. As a result, use of low-quality or effluent sources of irrigation is becoming more prevalent. Elevated salinity levels are a concern with use of these types of irrigation waters, and therefore, turfgrasses must increasingly possess improved resistance to stresses related to both drought and salinity. Ten cultivars of commonly used warm-season turfgrass species including bermudagrass (*Cynodon ssp.*), zoysiagrass (*Zoysia ssp.*), St Augustinegrass (*Stenotaphrum secundatum*), and seashore paspalum (*Paspalum vaginatum*) were evaluated in 12-week greenhouse experiments during 2014 and 2015. Salinity treatments included electrical conductivity levels of 2.5 (control), 15, 30, and 45 dS m^{-1}. Turf quality, percent green cover, shoot biomass reductions, root development, and recovery following salinity stress were evaluated. Results of the study demonstrated salinity tolerance differed by species, with the greatest tolerance noted within the seashore paspalum and bermudagrass cultivars. Increased growth and turf quality relative to the controls was even noted at 15 dS m^{-1} for many bermudagrass and seashore paspalum entries. St. Augustinegrass and to a lesser extent, zoysiagrass cultivars were less able to tolerate elevated salinity. More severe stress was noted during year two, which may have been related to higher average temperatures.

INTRODUCTION

Water quantity and quality are major issues of concern around the world, particularly in arid and semi-arid areas where water shortages have resulted from rapid urbanization, agriculture, and/or industry (Huang et al., 2014). Soil salinity in arid and semi-arid regions may be a consequence of poor water quality due to over use of aquifers, or due capillary rise of salts up into the root zone. In coastal areas, soil salinity problems can occur due to saltwater intrusion into aquifers as a result of excessive water removal and inadequate rates of recharge (Carrow et al., 2001a). Salts may affect plant development by damaging physiological processes through ion toxicity, ion imbalances, osmotic stress, and/or reducing soil permeability(Carrow et al., 2001a).

In the southern United States, landscape water conservation programs have been developed by municipalities and water purveyors to help alleviate pressures on potable water supplies. As a result, utilization of low-quality recycled or reclaimed wastewater, gray water, or even sea water has become common sources of irrigation at turfgrass facilities such as golf courses, municipal parks, and stadiums (Devitt et al., 2004). According to the Golf Course Superintendents Association of America's Environmental Institute for Golf Survey (GCSAA, 2015), recycled water is now the predominant irrigation source used in the southwestern and southeastern regions of the United States. Use of recycled water for golf course irrigation in the southern region of United States has increased by 7% and 10% from 2005 to 2013. Recycled wastewater was reportedly used in 30 to 45% of the golf courses from the southern region.

Turfgrasses are well suited for effluent irrigation because they function as biological filters which can remove excess salts and nutrients from saline water (Hayes et al., 1990). Warm-season turfgrasses have good adaptation to arid and semi-arid areas since they generally possess increased resistance to both drought and salinity stress occurring from poor water quality (Marcum, 2006; Uddin and Juraimi, 2013).

Relatively little is known about the physiological responses of warm-season turfgrasses bermudagrass, seashore paspalum, zoysiagrass, and St. Augustinegrass to salinity stress. These species represent some of the most widely used turfgrasses for warm-season lawns, golf courses, and athletic fields throughout the southern United States (Uddin and Juraimi, 2013). Understanding comparative salinity tolerance and physiological responses to salinity stress within these species is therefore critically important both from a practical standpoint as well as for improving and developing superior warm-season turfgrasses through breeding efforts (Abraham et al., 2008)

Given the increased need for improving both drought and salinity tolerance attributes of turfgrasses, physiologists and breeders from southeastern U.S. Universities including Texas A&M University System, University of Georgia, University of Florida, North Carolina State University, and Oklahoma State University have been partnering in recent years to more rapidly develop grasses with wide adaptation and tolerance to several abiotic stresses including salinity (Chandra, 2015).

Therefore, the objectives of this research were to evaluate comparative salinity tolerance and recovery attributes among ten warm-season turfgrass cultivars representing bermudagrass, zoysiagrass, St. Augustinegrass, and seashore paspalum.

MATERIALS AND METHODS

Growing Conditions and Plant Materials

This study was conducted in a greenhouse at Texas A&M University, College Station, TX from 1 May through 15 August 2014, with a repeat study conducted from 1 June through 15 September 2015. Ten warm-season turfgrass cultivars were used, representing bermudagrass ssp., zoysiagrass ssp., St. Augustinegrass, and seashore paspalum. Entries included 'Tifway' hybrid bermudagrass (*Cynodon dactylon x C. transvaalensis* Burt. Davy), 'Celebration' bermudagrass (*Cynodon dactylon*), 'Empire' and 'Palisades' Japanese lawngrass (*Z. japonica* Steud.), 'Zeon' manilagrass (*Zoysia matrella*), 'Raleigh', 'Floratam', and 'Palmetto' St. Augustinegrass (*Stenotaphrum secundatum*), and 'Sea Isle I' and 'Seastar' seashore paspalum (*Paspalum vaginatum*) (Table 2).

Prior to the study initiation, sod plugs (5 cm diameter x 5 cm deep) of each entry were obtained from breeder source material, washed free of soil, and roots trimmed to 5 cm before transplanting into 100 cm^2 x 10.2 cm deep pots containing medium-coarse USGA specified green sand. The washed sod plugs were allowed to fully establish into pots for 150 days before initiating salinity treatments. During establishment, grasses were irrigated daily with 0.6 cm local potable tap water and provided liquid-fertilization twice weekly using a 20-20-20 water soluble fertilizer (Peters 20-20-20, J.R. Peters, Inc., Allentown, PA 18106) to supply 1.2 g N m^{-2} wk^{-1}. Grasses were clipped weekly using scissors with clippings removed. Bermudagrass ssp., zoysiagrass ssp., and seashore

paspalum entries were maintained at a 2.5 cm while St. Augustinegrass was maintained at 5 cm height of cut during the study.

Table 2. Species, cultivar names, and origin of entries used in the Texas A&M University salinity experiments

Species	Cultivar	Origin
Cynodon dactylon x C. transvaalensis Burt.		
Davy	Tifway	University of Georgia
Cynodon dactylon	Celebration	Sod Solutions, Inc.
Z. japonica Steud	Empire	University of Florida
Zoysia matrella	Zeon	BladeRunner Farms
Z. japonica Steud	Palisades	Texas A&M University System
Stenotaphrum secundatum	Raleigh	North Carolina State University
Stenotaphrum secundatum	Floratam	Univ. Florida / Texas A&M
Stenotaphrum secundatum	Palmetto	Sod Solutions, Inc.
Paspalum vaginatum	Sea Isle I	University of Georgia
Paspalum vaginatum	Seastar	University of Georgia

Acclimation, Salinity Stress, and Recovery Period

Replicate studies consisting of four main treatments (1 m x 1 m x 5 cm deep ebb and flow benches) accommodating salinity levels of 2.5 (control), 15, 30, and 45 dS m^{-1} were used. Within each salinity main treatment, subtreatments (10 cultivars) were arranged in a completely randomized block design with 4 replicates. Prior to initiating the study, a salinity acclimation period was provided in order to gradually achieve the

desired final salinity treatment concentrations over a 5-week period. The acclimation period for study 1 was initiated on 1 May 2014 and for study 2 on 1 June 2015.

During acclimation, potted grasses were placed into ebb and flow benches and sub-irrigated daily. College Station municipal potable tap water, pH 8.1, and electrical conductivity (Devitt et al.) of <1 dS m^{-1} was used for all four salinity treatments. During sub-irrigation events, water was pumped (Universal Electric Co. TEEL 115 V) from 189 L holding tanks to completely fill ebb and flow benches for a 5-minute period, and allowing sand root zones within pots to become fully saturated. A float valve was positioned near the upper edge of each ebb and flow bench to prevent overflow and to stop pump operation once the bench was completely filled to the top.

Salinity treatments were provided by mixing tap water with Instant Ocean Sea Salt (Instant Ocean Spectrum Brands, Blacksburg, VA 24060) to achieve the respective desired EC levels (Table 3). A 13-2-13 soluble fertilizer (Miracle-Gro Professional Excel, Marysville, OH 43040) was used to produce an irrigation nutrient concentration of 300 ppm NO_3-N within the four treatments. The salinity level of each treatment was gradually increased by 10 dS m^{-1} wk^{-1} until final salt concentrations of 15, 30, and 45 dS m^{-1} were reached and maintained, at which time the 6-week experiments were initiated.

During both the acclimation period and 6-week experimental period, water level in holding tanks was measured and supplemented twice weekly to replace water lost to evaporation from the system. Pots were also overhead flushed with tap water weekly prior to sub-irrigation to prevent accumulation of salt at the soil surface in pots. After the 6-week salinity stress period, a 4-week recovery period was initiated, which began in

July 2014 and August 2015 respectively. During the recovery period, all treatments received tap water in order to evaluate recovery under non-stressed conditions.

Table 3. Saline solution analysis report

Parameter analyzed	Results	Units
Calcium (Ca)	281	ppm
Magnesium (Mg)	1171	ppm
Sodium (Na)	9631	ppm
Potassium (K)	382	ppm
Boron (B)	4.08	ppm
Carbonate (CO_3)	6	ppm
Bicarbonate (HCO_3)	119	ppm
Sulfate ($SO_{4\ -\text{calculates from total S}}$)	2338	ppm
Chloride (Cl-)	13459	ppm
Nitrate-N (NO_3-N)	0.01	ppm
Phosphorus (P)	0.04	ppm
Conductivity	45000	umhos/cm
Hardness	322	grains CaCO3/gallon
Hardness	5524	ppm CaCO3
Alkalinity	108	ppm CaCO3
Total Dissolved Salts (TDS)	37800	ppm
SAR	56.3	

Throughout the acclimation, salinity stress, and recovery period, salinity concentrations and nitrate concentrations of irrigation water were monitored twice weekly using a portable EC meter (EC 110 Meter Field Scout, Spectrum Technologies,

Inc., Aurora, IL, 60504) and a compact NO_3-nitrate ion meter (LAQUA Twin Nitrate Meter, Spectrum Technologies, Aurora, IL, 60504). Fertilizer was added to compensate for nutrient depletion from the system and to maintain a target concentration of 300 ppm NO_3-N within each treatment.

Environmental Conditions in the Greenhouse

Environmental conditions in the greenhouse were monitored during the study period using a weather station (WatchDog 2000 Weather Station, Spectrum Technologies, Inc., Aurora, IL 60504). Solar radiation, relative humidity, and temperature data were recorded and averaged for each phase of the study (acclimation, salinity stress, and recovery) both years (Table 4)

Table 4. Environmental conditions including solar radiation, relative humidity, and temperatures in the greenhouse during the Texas A&M salinity experiments.

	Solar Radiation W m^{-2}	Relative Humidity %	Temperature °C		
			High	Low	Mean
Year 1 (2014)†					
Acclimation period ‡	160	85	30.5	23.3	26.6
Salinity stress§	173	87	32.7	22.7	28.3
Recovery period ¶	148	87	35.0	24.4	28.3
Year 2 (2015)†					
Acclimation period ‡	160	87	36.6	23.8	28.3
Salinity stress §	176	79	34.4	26.6	30.0
Recovery period ¶	138	78	34.4	26.1	30.0

†May 1 to September 1
‡Acclimation period was weeks 1 to 5
§Salinity stress period was weeks 6 to 10
¶Recovery period was weeks 11 to 14

Measurements and Data Collection

To evaluate turfgrass response to salinity stress, turfgrass visual quality (Morris and Shearman, 1998), percent green cover, normalized difference vegetation index (NDVI), percent shoot biomass reduction, and final root biomass were measured during the study. Grasses were visually rated for turfgrass quality using a 0-9 scale weekly, adapted from Morris and Shearman (1998), where 0= completely brown turf, 6 = minimum acceptable, and 9= perfect green turf quality. Turfgrass quality measurements were taken from weeks 1 through 10, as well as at the conclusion of the week 11-14 recovery period.

Percent green cover of grasses was determined by digital image analysis of light box images taken at weeks 1, 5, 10 (end of salinity stress period), and 14 (end of recovery period) using a Canon 4x optical zoom digital camera (Canon Co.) mounted on a 30 cm diameter x 50 cm height light-box. The light box cancelled out outside light and created uniform light within the box via 8 LED bulbs. Camera settings were as follows: image type (JPEG Image), dimensions (969 x 1049), color (sRGB), no flash, focal length (7 mm), F-stop (F 4.2), exposure time (1/2 sec), ISO-800, white balance auto (Karcher and Richardson, 2005). Digital images were analyzed for percent green cover using digital image analysis software (Sigma Scan Pro, Image Analysis Version 5.0) and the Turf Analysis Macro (Richardson et al., 2001) for determining percent green cover within each pot.

Weekly shoot growth rates within each treatment were also determined from weeks 1 through 10 by clipping grasses to a height of 2.5 cm (bermudagrass, seashore

paspalum, and zoysiagrass) and 5 cm (St Augustinegrass). All clippings were oven dried (VWR Gravity Convention Oven) for 72 h at 65°C with dry weights determined using a digital scale (Denver Instrument P-403 Digital Balance/Scale).

At the end of the salinity stress period (week 10) clipping dry weights were evaluated between salinity and control treatments in order to calculate percent reduction in shoot biomass caused by each salinity level. The following formula was used to determine percent biomass reduction:

$$\% \text{ Biomass Reduction} = (1 - (a/b)) \times 100$$

a= Clipping dry weight for a given entry within a given cultivar and salinity treatment

b= Clipping dry weight average of all entries for the same cultivar in the control treatment

Prior to initiating the freshwater recovery period at week 11, turfgrasses were also evaluated using NDVI (Field Scout TCM 500 NDVI, Turf Color Meter Spectrum) in order to determine relative differences in photosynthetic efficiency.

Statistical Analysis

The experiment was arranged as a completely randomized block design with 4 replicates. Data were subjected to analysis of variance using the general linear model, univariate test procedure using SPSS ver. 21.0 (IBM Corp, Armonk, NY) to determine statistical significance of the results. Mean separation procedures were performed using Fisher´s LSD at the $P \leq 0.05$ level.

RESULTS

Environmental Conditions

During the 2014 study, maximum daily temperatures during the three experimental phases (acclimation period, salinity stress, and recovery period) averaged 30.5, 32.7, and 35.0 °C, respectively, while minimum temperatures averaged 23.3, 22.7, and 24.4, respectively.

During the 2015 study, maximum temperatures for the three phases averaged 36.6, 34.4, and 34.4 °C, respectively, while minimum temperatures averaged 23.8, 26.6, and 26.1, respectively. Relative humidity during the three 2014 experimental phases averaged 85, 87 and 87%, respectively. For the 2015 study, relative humidity for the three experimental phases averaged 87, 79, and 78%, respectively (Table 4).

Visual Turfgrass Quality

ANOVA revealed significant cultivar main effects for turfgrass quality at all salinity levels and within both studies. A cultivar x year interaction was also detected for the 45 dS m^{-1} treatment. (Table 5).

Table 5. Analysis of variance for parameters measured at the final week (week 6) of saliniity stress exposure for control, 15, 30, and 45 dS m^{-1} salinity levels.

	Quality	% Green Cover	Final NDVI	% Biomass-Reduction	Post-Recovery % Green Cover	Final Root Biomass
Control						
Cultivar	**	***	***		***	***
Year	***	***	ns		***	ns
Cultivar x Year	ns	ns	ns		ns	ns
15 dS m^{-1}						
Cultivar	***	***	***	*	***	***
Year	**	***	**	***	***	**
Cultivar x Year	ns	**	ns	**	**	**
30 dS m^{-1}						
Cultivar	**	**	***	ns	***	***
Year	ns	***	*	ns	***	ns
Cultivar x Year	ns	ns	ns	ns	ns	ns
45 dS m^{-1}						
Cultivar	***	***	***	***	***	***
Year	***	***	*	***	***	ns
Cultivar x Year	ns	*	ns	**	ns	ns

* Significant at the 0.05 probability level
**Significant at the 0.01 probability level
*** Significant at the 0.001 probability level
ns = Not significant

ANOVA detected a significant cultivar effect for turf quality, but there was no cultivar x year interaction (Table 5). In general, turf quality, as noted by % change between pre- and post-salinity stress, was reduced in all cultivars as salinity concentration was increased (Table 6). When moving from control to 15 dS m^{-1} treatments, only Celebration responded favorably to the 15 dS m^{-1} treatment, even increasing in turf quality after ten weeks of salinity exposure. The majority of cultivars declined in turf quality following exposure to 15dS m^{-1} salinity. Although it showed a

slight decrease in quality from control to 15 dS m^{-1}, Seastar exhibited the highest turf quality at 15 dS m^{-1}, outperforming all other entries with 7.1 turf quality rating. Celebration, Sea Isle I, and Seastar each were able to maintain acceptable turf quality (> 6) at 15 dS m^{-1}.

At 30 dS m^{-1} salinity levels, Celebration, Sea Isle 1, and Seastar had the highest turf quality (6.6, 6.1, and 6.2 respectively), while all other cultivars were unable to maintain acceptable turf quality (Table 6). At 45 dS m^{-1} salinity stress, no cultivars were able to maintain acceptable quality, and all cultivars suffered greater than 75% growth declines. While Sea Isle I, Celebration, and Seastar were the top performers, they were only able to maintain turf quality levels of 1.8, 1.5, and 1.3, respectively.

ANOVA also detected a significant year effect on turf quality (Table 5). As such, mean turf quality, when pooling across cultivars, was significantly reduced from year 1 (5.2) to year 2 (3.7) studies (data not shown). It is likely that this was related to the higher temperatures occurring during year two, which were ~2 and 4°C higher than in year one (daily high and low temperatures, respectively) (Table 4).

Table 6. Cultivar main effect on turfgrass quality after five weeks of salinity stress. Data are pooled across both studies. Positive values denote an increase in quality, while negative values denote a decline in quality.

	Control			15 dS m^{-1}		30 dS m^{-1}		45 dS m^{-1}	
	Pre	Post	Change		Change		Change		Change
Cultivars	†	‡	%	Post‡	%	Post‡	%	Post‡	%
Tifway	6.8	5.8	-15	2.5	-63	2.7	-60	0.5	-93
Celebration	6.1	8.8	44	6.9	13	6.6	8	1.5	-75
Empire	6.3	7.6	21	4.4	-30	4.1	-35	1.3	-79
Zeon	6.8	4.9	-28	4.1	-40	1.6	-76	0.5	-93
Palisades	6.8	8.5	25	4.8	-29	4.8	-29	1.0	-85
Raleigh	6.1	4.4	-28	2.8	-54	1.6	-74	0.3	-95
Floratam	6.9	7.3	6	2.4	-65	2.7	-61	0.5	-93
Palmetto	6.2	4.1	-34	1.6	-74	2.1	-66	0.5	-92
Sea Isle I	8.0	8.8	10	6.4	-20	6.1	-24	1.8	-78
Seastar	7.5	8.8	17	7.1	-5	6.2	-17	1.3	-83
LSD	1.3	2.4	36	2.5	39	3.1	53	1.3	20

†Pre is the mean turf quality of each cultivar at week 0 prior to salinity treatment
‡Post is the average of each treatment after salinity stress period

Digital Image Analysis of Final Percent Green Cover

ANOVA detected significant cultivar main effects at all salinity levels as well as cultivar by year interactions at 15 and 45 dS m^{-1} for percent green cover (% GC) (Table 5). In the control treatment, Empire, Palisades, Sea Isle I, and Seastar showed the highest % GC (70, 72, 76 and 77%, respectively) (Table 7).

At 15 dS m^{-1} in year 1, lower % GC was observed among all entries, while in year 2 almost all entries responded more favorably to salinity, particularly Celebration, Palisades, and Seastar, which all exhibited the highest % GC (Table 7). At 30 dS m^{-1}percent green cover Celebration, Palisades, and Seastar again maintained the highest % GC at 30 dS m^{-1}salinity. At 45 dS m^{-1}, Celebration and Seastar were the top

performing lines in terms of green cover (45.5 and 40.3% GC, respectively) for year 1, while in year 2, all bermudagrass, zoysia spp., and seashore paspalum entries performed relatively similarly (~40-50 % GC), and significantly better than that of St. Augustinegrass entries (~20 % GC).

Table 7. Final % green cover after five weeks of salinity stress

Cultivars	Control	15 dS m^{-1}		30 dS m^{-1}	45 dS m^{-1}	
		Year 1	Year 2		Year 1	Year 2
Tifway	43.4	40.0	40.0	31.0	32.5	40.3
Celebration	56.8	34.5	73.8	50.4	45.5	52.8
Empire	70.3	33.5	65.5	48.4	21.5	48.0
Zeon	51.9	27.5	65.0	43.9	23.3	46.3
Palisades	72.0	45.0	77.3	53.3	27.5	49.5
Raleigh	36.6	10.0	53.0	39.4	20.3	19.3
Floratam	56.9	24.8	66.0	47.0	20.0	20.0
Palmetto	35.5	13.3	46.5	36.1	9.8	20.3
Sea Isle I	76.6	48.3	69.8	48.9	31.3	43.3
Seastar	77.0	49.0	88.0	55.9	40.3	44.0
LSD	20.3	13.3	27.5	18.7	18.6	19.8

Final NDVI

There were significant cultivar and year main effects for final NDVI at all salinity levels, however there were no significant cultivar x year interactions at any salinity level (Table 5). A decline in NDVI was observed as salinity concentration was increased in all cultivars (Table 8). Celebration (0.68 to 0.55), Sea Isle I (0.73 to 0.55), and Seastar (0.74 to 0.56) maintained the highest NDVI values when taking into account all cultivars and salinity levels.

Table 8. Final NDVI after five weeks of salinity stress for control, 15, 30, and 45 dS m^{-1} salinity levels. Data are pooled across years.

Cultivars	Control	15 dS m^{-1}	30 dS m^{-1}	45 dS m^{-1}
Tifway	0.64	0.62	0.52	0.47
Celebration	0.68	0.68	0.63	0.55
Empire	0.68	0.61	0.55	0.48
Zeon	0.58	0.54	0.50	0.38
Palisades	0.71	0.60	0.62	0.48
Raleigh	0.46	0.42	0.42	0.34
Floratam	0.55	0.50	0.46	0.37
Palmetto	0.43	0.40	0.51	0.35
Sea Isle I	0.73	0.67	0.63	0.55
Seastar	0.74	0.71	0.66	0.56
LSD	0.10	0.10	0.16	0.14

Clipping Biomass Reductions

ANOVA detected significant cultivar x year interactions at both 15 and 45 dS m^{-1} salinity levels; however, there were no significant effects or interactions noted for NDVI at 30 dS m^{-1} (Table 5). Biomass reductions occurred with increasing salinity levels (Table 9). The most vigorous turfgrass under mild and moderate levels of salinity in year 1 was Sea Isle I, which actually increased biomass production (97% and 23% increased growth relative to controls) at 15 and 30 dS m^{-1} salinity levels in (Table 9). Tifway, Empire, Palisades, Floratam, and Palmetto all showed increased growth under 15 dS m^{-1} salinity levels in year 1, but exhibited reduced growth at these same EC levels during year 2. Increased biomass production (less severe biomass reductions) under salinity occurred in year 1, during which average temperatures were 26.6 and 28.3°C at acclimation and salinity stress periods, respectively (Table 4). By comparison, in

addition to lower relative humidity, higher average temperatures occurred in year 2 (28.3 and 30° C during acclimation and salinity stress periods, respectively). The combination of higher temperatures and lower relative humidity in year 2 may have contributed to these observed differences.

At 45 dS m^{-1} salinity, St. Augustinegrass entries experienced the greatest biomass reduction (86.5 to 100% reductions in year 1 and 80.3 to 90.4% reductions in year 2) of any species. By comparison, bermudagrass and seashore paspalum entries exhibited the least reduction in clipping biomass (~50-70% reductions) during year 1. In year 2, seashore paspalum entries sustained significantly less biomass reduction than all other species at 45 dS m^{-1}.

Table 9. Clipping biomass reductions (relative to control treatment) at 15, 30, and 45 dS m^{-1} salinity levels. Cultivar x year interaction was significant at 15 and 45 dS m^{-1} salinity levels, so data are split by year.

	15 dS m^{-1}		30 dS m^{-1}		45 dS m^{-1}	
Cultivars	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2
Tifway	49.4	-22.4	-41.2	-42.3	-52.6	-82.6
Celebration	-2.0	-48.0	-52.1	-44.0	-72.4	-86.1
Empire	22.8	-66.0	-12.5	-60.1	-55.7	-87.5
Zeon	-23.8	-34.4	-39.0	-33.7	-61.8	-100.0
Palisades	35.0	-57.1	-12.1	-58.3	-74.0	-86.8
Raleigh	-16.5	-35.7	-46.1	-41.9	-98.0	-80.3
Floratam	41.1	-74.0	-43.5	-76.6	-86.5	-93.4
Palmetto	6.0	-39.2	-21.4	-40.2	-100.0	-90.5
Sea Isle I	97.0	-29.9	23.0	-32.3	-49.0	-74.8
Seastar	-7.5	-8.4	-47.8	-13.5	-69.3	-62.3
LSD	68.0	ns	ns	ns	33.6	25.8

† Positive values denote growth
‡ Negative values denote decline in growth

Post Recovery Period % Green Cover

ANOVA detected a significant cultivar x year interaction at the 15 dS m^{-1} salinity level, and also significant cultivar main effects at all salinity levels for post-recovery % GC (Table 5). After the four-week recovery period, % GC increased among most entries, with the exception of St. Augustinegrass cultivars, which never recovered to greater than ~20% GC (Table 10). In general, percent green cover was similar or improved following recovery at 15 dS m^{-1} compared to both controls as well as higher salinity concentrations.

Table 10. Final % Green Cover after the four week of recovery period for control, 15, 30, and 45 dS m^{-1} salinity levels.

Cultivars	Control	15 dS m^{-1}		30 dS m^{-1}	45 dS m^{-1}
		Year 1	Year 2		
Tifway	46.8	42.0	42.0	35.8	53.5
Celebration	50.5	51.0	62.0	55.9	59.5
Empire	54.5	38.8	68.5	64.3	53.6
Zeon	34.0	41.5	65.8	56.3	44.3
Palisades	60.5	52.3	79.3	59.9	50.3
Raleigh	17.8	12.0	51.5	37.9	22.5
Floratam	24.3	24.8	67.3	60.6	19.9
Palmetto	9.8	5.0	39.3	35.1	19.1
Sea Isle I	60.3	64.8	65.0	73.3	46.8
Seastar	49.3	63.5	79.8	64.5	48.8
LSD	34.9	18.6	29.6	23.4	16.4

Final Root Dry Weight

ANOVA detected significant cultivar x year interaction for final root dry weight at the 15 dS m^{-1} salinity level, as well as significant cultivar main effects for final root dry weights at all salinity levels (Table 5). Therefore, root data are pooled between years for all but the 15 dS m^{-1} salinity level (Table 11). Root dry weights under control conditions differed significantly among the ten cultivars, with seashore paspalum entries producing the greatest root (2.6 and 2.5 g DW respectively, for SeaIsle I and Seastar). At 15 dS m^{-1}, SeaIsle I and Seastar exhibited the greatest amounts of root growth (3.7 and 3.2 g DW, respectively) among all cultivas in year 1. However, Palmetto and Zeon exhibited increased root biomass root production (170 and 124%, respectively) at the 15 dS m^{-1} salinity level. In year 2, seashore paspalum cultivars (Sea Isle I and Seastar) again had the highest root growth among cultivars (2.3 and 2.5 g DW, respectively) at 15 dS m^{-1}.

Again in year 2, root growth was stimulated at 15 dS m^{-1} salinity in about half of the entries, with an increase of 113% root growth increase observed in Raleigh St. Augustinegrass. At 30 dS m^{-1}, Sea Isle 1 and Seastar again had the highest root dry weights (2.5 and 2.8 g, respectively). Also, significant differences were not detected between cultivars for % biomass reduction at 30 dS m^{-1}, Palisades and Palmetto showed the greatest increases (60 and 52% increases) while Empire and Celebration (21 and 14% decrease) had the greatest decrease in root growth (Table 11). At 45 dS m^{-1} salinity, significant differences were no detected, root growth was again greatest in Sea Isle 1 and Seastar (2.3 and 2.1 g, respectively), while the least root growth was observed in Zeon and Raleigh (0.5 and 0.7 g, respectively). At 45 dS m^{-1}, all entries suffered reduced root growth, with the exception of Palmetto and Palisades (20 and 4% increases).

Table 11. Final root dry weight and % root biomass-change after six weeks of salinity stress for control, 15, 30, and 45 dS m^{-1} salinity levels.

Cultivars	Control	----------15 dS m^{-1}----------		---------30 dS m^{-1}--------		----------45 dS m^{-1}---------	
	Root Dry Weight (g)	Root Dry Weight (g)	% Biomass change	Root Dry Weight (g)	% Biomass change	Root Dry Weight (g)	% Biomass change
		----------Yr 1----------		----------Yr 2----------			
Tifway	1.1	2.4	46	0.8	59	1.3	23	0.9	-8
Celebration	1.8	2.5	45	2.5	29	1.5	-14	1.3	-26
Empire	1.3	1.1	5	1.5	-8	1.0	-21	1.0	-23
Zeon	0.9	1.2	124	0.8	-32	0.8	10	0.5	-24
Palisades	1.1	0.9	-33	1.1	27	1.6	60	1.1	4
Raleigh	1.3	1.5	-6	2.0	113	1.3	9	0.7	-42
Floratam	1.9	1.5	-5	1.6	-30	2.1	12	1.1	-38
Palmetto	1.2	2.9	170	0.6	-52	1.7	52	1.4	20
Sea Isle I	2.6	3.7	24	2.3	1	2.5	-6	2.3	-12
Seastar	2.5	3.2	8	2.5	24	2.8	17	2.1	-14
LSD	1.1	1.5	139	1.4	136	1.3	ns	1.0	ns

† Positive values denote growth
‡ Negative values denote decline in growth

DISCUSSIONS

The response of turfgrass to abiotic stress such as drought or salinity is commonly measured through visual turf quality ratings and/or shoot and root biomass reduction (Steinke et al., 2009). Turf quality has been shown to be affected differently based on the species being exposed to salinity stress. For example, *Zoysia matrella* cultivars have been shown to exhibit improved turf quality compared to *Zoysia japonica* cultivars previously (Marcum et al., 1998). In our study, seashore paspalum and bermudagrass cultivars generally outperformed zoysia ssp. and St. Augustinegrass cultivars. Celebration bermudagrass exhibited superior turf quality, and maintained acceptable levels of quality under both 15 and 30 dS m^{-1} salinity treatments. Seashore paspalum exhibited the best turf quality at 15 dS m^{-1} salinity while also maintaining minimum acceptable quality at 30 dS m^{-1}.

The remaining cultivars experienced severe decline in turf quality with increased salinity concentration (Table 6). Based on our results, the relative salinity tolerance observed at 15 dS m^{-1} salinity concentration occurred in the following order, from highest to lowest observed tolerance: Seashore paspalum > bermudagrass ssp. > zoysiagrass ssp. > St. Augustinegrass. Marcum and Murdoch (1994) also found that seashore paspalum maintained the best quality (5.3) at 40 dS m^{-1} salinity, followed by zoysia matrella (4.7), St Augustinegrass (4.0), and bermudagrass (2.0).

Shoot growth stimulation at low levels of salinity has also previously been reported in salt tolerant grasses including seashore paspalum and St. Augustinegrass under 15 dS m^{-1} (Marcum and Murdoch, 1994). In fact, seashore paspalum ecotypes as

well as ‘Tifgreen’, Tifway, and ‘Tifeagle’ bermudagrass all were shown to exhibit shoot growth stimulation at 24 dS m^{-1} compared to control level (Greenway and Munns, 1980; Lee et al., 2004).

In our study, clipping biomass reductions (as a % of the control) was significantly affected by a cultivar x year interaction, and there were no significant effects detected at 30 dS m^{-1} (Table 5). Interestingly, differences were also noted between years, with the maximum biomass production of most turfgrass species occurring in year 1 (2014). Using 50% biomass reduction as a threshold, the most vigorous and salinity tolerant turfgrass was Sea Isle I, which showed stimulated biomass production (97% and 23% increases at 15 and 30 dS m^{-1}, respectively, in 2014) (Table 9). Seashore paspalum clearly exhibits adaptive mechanisms conferring superior salinity tolerance. These mechanisms may include osmotic adjustment and ion selection by aquaporins (Bhardwaj et al., 2013). Other cultivars showing less vigor under 15 dS m^{-1} salinity, but still maintaining intermediate salinity tolerance were Tifway, Empire, Palisades, Floratam, and Palmetto. While not to the same extent as seashore paspalum, these cultivars all showed some degree of shoot growth stimulation at salinity levels of 15 dS m^{-1} in 2014.

Higher temperatures during the year 2 (2015) appeared to compound salinity stress effects, as noticeably greater biomass reductions were observed in year 2 at each of the salinity levels. Empire, Palisades, and Floratam all experienced the greatest biomass reduction declines from year 1 to year 2, possibly indicating relatively greater sensitivity of these cultivars to combined salinity and heat stress.

Duncan and Carrow (2001) report that turfgrasses that possess inherently vigorous root growth characteristics are often those that are better adapted for growth in or recovery from adverse abiotic stresses such as sodic and saline soils, extreme temperatures, ion toxicities, and nutrient imbalances. Furthermore, root growth stimulation under saline conditions has previously been reported in bermudagrass (Carrow et al., 2001b), seashore paspalum (Lee et al., 2004), and St Augustinegrass (Meyer et al., 1989; Peacock et al., 1993). In our study, root growth appeared to be less affected by salinity treatments than shoot growth. Root growth stimulation varied by cultivar in both years, from -33% (decrease) to 170% (increase) at 15 dS m^{-1} in year 1 to -52% (decrease) to 113% (increase) in year 2. At 30 dS m^{-1}, root growth varied somewhat less, from -23% (decrease) to 45% (increase) when pooled across years. Remarkably, at 30 dS m^{-1} salinity, Palisades and Palmetto each showed increased root growth (45 and 42%, respectively), followed by Tifway and Seastar (18 and 12%, respectively).

The observation of maintained or even increased root growth by St. Augustinegrass cultivars under increasing salinity is interesting, considering the poor performance of the species with regard to turf quality and clipping production at these same levels. Based on our results, a number of the warm-season species and cultivars tested exhibit halophytic attributes based on their increased shoot and root biomass production under low salinity levels (< 20 dS m^{-1}). Such responses to salinity stress may occur through adaptive mechanisms to prevent ion toxicity, osmotic stress, and/or ion

imbalance, resulting in more efficient water and nutrient uptake under salinity (Gorham et al., 1985).

CONCLUSIONS

Turfgrass responses to salinity stress are complex, involving several physiological mechanisms and depend on salinity concentration, length of exposure, and effects of other compounding stresses such as mineral nutrition imbalance, osmotic stress, and stomatal closure. The warm-season turfgrasses evaluated in this study demonstrated a wide range of salinity tolerance, and responses varied between cultivars in terms of shoot and root production, turf quality, and percent green cover, and photosynthetic efficiency. The results should also be taken in the context of a sub-irrigated system, where only belowground tissues, and not foliage were exposed to salinity stress. While it is difficult to assign precise salinity thresholds to each cultivar due to variability between studies, as well as inconsistency between biomass reduction and turf quality data, relative salinity tolerance ranges of these cultivars based on maintenance of acceptable turf quality ranged from 30 to 45 dS m^{-1} (Sea Isle 1, Seastar, and Celebration), to between 2.5 and 15 dS m^{-1} (all other cultivars). Based on 50% clipping biomass reduction thresholds, Empire, Palisades, and Floratam tolerated less than 15 dS m^{-1}, Celebration tolerated between 15 and 30 dS m^{-1}, and all other cultivars tolerated between 30 and 45 dS m^{-1} salinity. While these levels may to some extent exceed those of practical significance in the field, they do provide excellent comparative

data on the relative salinity tolerance of the warm-season turf cultivars tested, as well as their ability to recuperate from periodic salt stress injury.

CHAPTER III

LEAF ANATOMICAL ANALYSIS OF SALT TOLERANT TURFGRASS EXPERIMENTAL LINES AND CULTIVARS USING SCANNING ELECTRON MICROSCOPY AND ELECTRON DISPERSIVE SPECTROSCOPY

OVERVIEW

As population growth and demands for potable water increase, use of low-quality or effluent sources of irrigation will become more prevalent. Elevated salinity levels are a concern with these types of irrigation waters. Therefore, turfgrasses must increasingly possess high levels of tolerance to both drought and salinity. Experimental lines and cultivars of 4 turfgrass species including bermudagrass (*Cynodon ssp.*), zoysiagrass (*Zoysia ssp.*), St. Augustinegrass (*Stenotaphrum secundatum* Walt. Kuntze), and seashore paspalum (*Paspalum vaginatum*), which had all demonstrated superior drought tolerance in prior multi-location field drought screening, were evaluated for salinity tolerance in a greenhouse study.

The objectives of this research were to utilize Scanning Electron Microscopy (Sema) combined with Energy Dispersive X-ray Spectroscopy (EDS) to explore and characterize anatomical and physiological responses of these species to two levels of salinity stress (control = 2.5 and 30 dS m^{-1}) through examination of both adaxial and cross-sectional internal leaf features including salt glands and elemental composition of salts on and within the leaves. . Results demonstrated unique differences among the 4 species. St. Augustinegrass showed no anatomical differences when exposed to elevated salinity. Bermudagrass lacked salt glands at the 2.5 dS m^{-1} salinity level, while

zoysiagrass possessed constitutive salt gland development which noticeably increased in number in response to elevated salinity. Seashore paspalum appears to possess bladders, in which were detected high levels of Na. This information could provide breeders and physiologists with a better understanding of the mechanisms involved in salinity tolerance among various warm-season turf species.

INTRODUCTION

Utilization of low-quality recycled or reclaimed wastewater, and to some extent, even ocean water for irrigation is becoming more common at turfgrass facilities such as golf courses, municipal parks, and stadiums (Devitt et al., 2004). According to the Golf Course Superintendents Association of America's Environmental Institute for Golf Survey (GCSAA, 2015), recycled water is now the predominant irrigation source used on golf courses in the southwestern and southeastern regions of the United States, increasing in these regions by 7% and 10%, respectively, from 2005 to 2013. According to this same survey, recycled wastewater is currently used in 30 to 45% of the golf courses in the southern region.

Use of low-quality irrigation water can provide additional nutrients to turf, but also contributes to elevated soil salinity levels(Qian and Harivandi, 2008). Turfgrasses are well suited for effluent irrigation because they function as biological filters which can remove excess salts and nutrients from saline water (Hayes et al., 1990). However, turfgrass species and cultivars vary in their relative ability to tolerate salinity, both with regard to soil salinity as well as foliar tissue injury (Devitt et al., 2004). Minimizing salt

application to soil can be a challenge, and therefore consideration should be given to selecting salt-tolerant turfgrass species and cultivars for these situations.

Avoiding ion toxic effects appears to be an important mechanism for survival under saline environments. As such, halophytic plant species have developed mechanisms for tolerating salinity stress including specialized mechanisms such as bladders, salt glands, and/or trichomes that regulate internal salt load for ion excretion or accumulation on the leaf surface and sub-surface (Wahid, 2003). For example, *Attriplex* sp. possesses bladder cells, which are specialized structures into which ions are sequestered under salt stress, and which eventually die or fall off the leaf (Prasad, 1997) Some warm-season turfgrasses such as bermudagrass, zoysiagrass, buffalograss, and saltgrass have been reported to exhibit salt glands which could be capable of storing or excreting ions from shoot tissue (Marcum and Murdoch, 1994).

Previous studies have also suggested an association between turfgrass salinity tolerance and salt gland density and activity (Marcum et al., 2003). Although there is limited published information on warm-season turfgrass species structural and functional adaptations for coping with salinity tolerance, there is currently a lack of information pertaining to salinity tolerance responses in St. Augustinegrass, as well as in many of the newer improved bermudagrass, zoysiagrass, and Seashore paspalum cultivars which have shown high levels of drought and/or salinity tolerance in the field. Therefore, the objective of this study was to utilize scanning electron microscopy and energy dispersive spectroscopy to characterize leaf surface and cross-sectional anatomy, salt exudation characteristics, and elemental composition under increasing salinity using the most salt-

tolerant lines of four warm-season turfgrasses, as determined from prior salinity screenings.

MATERIALS AND METHODS

Growing Conditions and Plant Material

This study was conducted in a greenhouse at Texas A&M University, College Station, TX from 1 Aug. through 1 Sept. 2015, with a repeat study conducted from 26 Sept through 26 Oct. 2015. Four warm-season turfgrass cultivars were used in this experiment, representing 4 species. Entries included 'Celebration' bermudagrass (*Cynodon dactylon*), 'DALZ1313' zoysiagrass (*Zoysia matrella* x *Z. japonica* Steud), 'Floratam' St. Augustinegrass (*Stenotaphrum secundatum*), and 'UGP3' seashore paspalum (*Paspalum vaginatum*) (Table 12). Each was identified through prior screenings to possess superior salinity tolerance compared to other genotypes of the same species.

Table 12. Species, cultivar names, and origin of entries used

Species	Previously Tested Salinity Tolerance	Cultivar	Origin
Cynodon dactylon	High	Celebration	Sod solutions, Inc.
Z. matrella x Z. japonica	High	DALZ1313	Texas A&M University System
Stenotaphrum secundatum	High	Floratam	Univ. Florida /Texas A&M
Paspalum vaginatum	High	UGP3	University of Georgia

Prior to the study initiation, sod plugs (5 cm diameter x 5 cm deep) of each entry were obtained from breeder source material, washed free of soil, and roots trimmed to 5 cm before transplanting into 100 cm^2 x 10.2 cm deep pots containing medium-coarse USGA specified green sand. The washed sod plugs were allowed to fully establish into pots for 150-days before initiating salinity treatments. During establishment, grasses were irrigated daily with 0.6 cm of potable tap water and provided liquid-fertilization twice weekly using a 20-20-20 water soluble fertilizer (Peters 20-20-20, J.R. Peters, Inc., Allentown, PA 18106) to supply 1.2 g N m^{-2} wk^{-1}. Grasses were clipped weekly using scissors with clippings removed. Bermudagrasses, zoysiagrass, and seashore paspalum entries were maintained at a 2.5 cm while St. Augustinegrass was maintained at 5 cm height of cut.

Environmental conditions including solar radiation, temperature, and relative humidity in the greenhouse were monitored during the study period using a weather station (WatchDog 2000 Weather Station, Spectrum Technologies, Inc., Aurora, IL 60504).

Acclimation & Salinity Exposure Phase

Replicate studies consisting of two main treatments (1 m x 1 m x 5 cm deep ebb and flow benches) accommodating salinity levels of 2.5 (control) and 30 dS m^{-1} were used. Within each salinity level, the four species were arranged in a completely randomized design with 4 replicates pots per species. Prior to initiating the study, an acclimation period was gradually imposed to achieve the final salinity concentration by

increasing electrical conductivity concentrations by 10 dS m^{-1} per week a 2-week period. The acclimation period for study 1 was initiated on 11 July 2015 and for study 2 on 5 Sept 2015.

During acclimation, potted grasses were placed into ebb and flow benches and sub-irrigated daily. Municipal potable tap water, with pH 8.1, and electrical conductivity of <1 dS m^{-1} was used for two salinity treatments. During sub-irrigation events, water was pumped (Universal Electric Co. TEEL 115 V) daily from 189 L holding tanks to completely fill ebb and flow benches for a 5-minute period, and allowing sand root zones within pots to become fully saturated. A float valve was positioned near the upper edge of each ebb and flow bench to prevent overflow and to stop pump operation once the bench was completely filled to the top.

Salinity treatments were provided by mixing tap water with Instant Ocean Sea Salt (Instant Ocean Spectrum Brands, Blacksburg, VA 24060) to achieve the respective desired EC levels (Table 3). A 13-2-13 soluble fertilizer (Miracle-Gro Professional Excel, Marysville, OH 43040) was used to produce an irrigation nutrient concentration of 300 ppm NO_3-N within the two treatments. Following the aforementioned salinity acclimation period, the 2-week experiments were initiated.

During both the acclimation period and experimental period, the water level in the holding tanks was measured and supplemented twice weekly to replace water lost to evaporation from the system. Pots were also overhead flushed with tap water weekly prior to sub-irrigation to prevent accumulation of salt at the soil surface in pots.

Throughout the acclimation and salinity exposure phase, salinity concentrations and nitrate concentrations of irrigation water were monitored twice weekly using a portable EC meter (EC 110 Meter Field Scout, Spectrum Technologies, Inc., Aurora, IL, 60504) and a compact NO_3-nitrate ion meter (LAQUA Twin Nitrate Meter, Spectrum Technologies, Aurora, IL, 60504). Fertilizer was added to compensate for nutrient depletion from the system and to maintain a target concentration of 300 ppm NO_3-N within each treatment.

Environmental Conditions in the Greenhouse

Greenhouse environmental conditions were monitored over the acclimation and salinity stress periods for both experiments using a weather station (WatchDog 2000, Spectrum Technologies, Inc.). During the first study, mean maximum temperatures during the two experimental periods (acclimation period and salinity stress) were 37.5 and 35.7 °C, respectively, while average minimum temperatures were 22.5 and 23.8 °C, respectively. During the repeat study, average maximum temperatures for the two phases were 36.2 and 37.0 °C, respectively, while average minimum temperatures were 23.1 and 21.1 °C, respectively, for acclimation and salinity stress periods. Average relative humidity during the first study was 84 and 76%, for acclimation and salinity stress periods. For the repeat study, average relative humidity was 78 and 75%, for acclimation and salinity stress periods, respectively (Table 13).

Table 13. Environmental conditions including solar radiation, relative humidity, and temperatures in the greenhouse during the Texas A&M salinity experiments.

	Solar Radiation W m^{-2}	Relative Humidity %	Temperature °C		
			High	Low	Average
Study 1 †					
Acclimation ††	178	84	37.5	22.3	29.1
Salinity Stress §	158	76	35.7	23.8	30.1
Study 2 ‡					
Acclimation ††	138	78	36.2	23.1	30.0
Salinity Stress §	135	75	37.0	21.1	28.0

†July 11 to September 1
‡Sept 5 to October 26
††Acclimation period was weeks 1 and 2
§ Salinity stress periods were weeks 3 through 6

Laboratory Examination of Leaves Via SEM and EDS

Following the salinity stress exposure phase, grasses were transferred to the TAMU Microscopy Imaging Center for SEM examination, where leaves were fixed for analyzing in two phases. The first phase involved analysis of adaxial leaf surface anatomy, and occurred in Sept 2015. The second phase involved leaf cross section analysis, and occurred in Nov 2015.

In preparation for leaf analysis, the second oldest fully expanded leaf of a randomly selected plant within each pot was carefully removed using a pair of forceps. One leaf per rep was harvested and immediately submerged into liquid nitrogen for 1 min followed by a 30 sec. submersion in methanol and a 1 min. submersion in hexamethyl disilazane. For leaf surface anatomy, leaves were mounted on SEM cylinder

specimen mounts (Cambridge, Leica, EISS/LEO, FEI/Philips, CamScan, Tescan, aluminum, grooved edge, Ø32mm) and for leaf cross section analysis, leaves were mounted on Low Profile 45° / 90° SEM mount (Ø12.7mm, pin leg fits into Cambridge, Leica, ZEISS, LEO, FEI, Philips, CamScan, Tescan, ETEC, and Amray SEM's Aluminum, grooved edge, 9.5mm pin height) using double-sided carbon tape. Leaves were oriented so that the adaxial surface could be examined. Mounted leaves were coated using Cressington Coating System 308 R for 30 nm of carbon. A Tescan Vega 6 scanning electron microscope was used to examine leaf surface anatomy, salt glands, and salt crystals characteristics on the adaxial leaf surfaces and examine leaf cross section (epidermis on the adaxial side, vascular tissue, and epidermis on the abaxial side) of each species at high 30 kV, working distance ranging from 10.5 to 12.4 mm, magnification range from 126 to 642x, and using both secondary (Tescan High Vacuum) and EDS detectors (Oxford Aztec). EDS was used in combination with SEM to determine elemental composition and weight percent of salts and other compounds associated with both the adaxial leaf surface, as well as cross-sectional zones composed of upper (adaxial) and lower (abaxial) epidermis, mesophyll, and vascular bundle regions within leaves. Particular emphasis was placed on detection and location of Na and Cl ions. The EDS technique offered a resolution of 5% error.

Statistical Analysis

The experiment was arranged as a complete random design with 4 replicates pots (leaves) per species. At the conclusion of the study, data were subjected to analysis of

variance using the general linear model, univariate test procedure using SPSS ver. 21.0 (IBM Corp, Armonk, NY) to determine statistical significance of the results. Mean separation procedures were performed using Fisher´s LSD at the $P \leq 0.05$ level.

RESULTS

Scanning Electron Microscopy Examination of Leaf Surfaces

Microscopic examination revealed distinctive differences between the leaf surfaces of the four species, both in and between the control and 30 dS m^{-1} salinity treatments. Bermudagrass and zoysiagrass showed presence of parallel arrangement of vascular bundles on the adaxial surface. Salt glands were detected emerging from the epidermis along the vascular bundles in zoysiagrass under both control and 30 dS m^{-1} treatments; however, in bermudagrass salt glands were only detected under 30 dS m^{-1} salinity, with no salt glands detected under the 2.5 dS m^2 control salinity level (Figure 1). Seashore paspalum exhibited bladders under both control and 30 dS m^{-1} salinity, which developed along the vascular bundles on the adaxial leaf surface (Figure 2). St Augustinegrass lacked any detectable salt glands or bladders along the adaxial leaf surface under both treatments (Figure 2).

The salt gland anatomy also differed between zoysiagrass and bermudagrass. As such, salt glands of zoysiagrass appeared more swollen and rounded than salt glands of bermudagrass, which were narrower and more pointed in nature (Figure 1).

Zoysiagrass also appears to possess constitutive salt gland development in the absence of salinity stress, with gland density increasing from ~50 glands mm^{-2} under

control levels to ~70 glands mm^{-2} under 30 ds m^{-1} salinity stress. Conversely, bermudagrass lacked any detectable salt glands under control treatments, and developed a much lower density of salt glands in response to salinity stress (~8 glands mm^{-2}) (Figure 3). Salt crystals were detected only in zoysiagrass and bermudagrass, adjacent to salt glands on the adaxial surface. This also confirmed that the salt crystals observed on the leaf surface in zoysiagrass and bermudagrass were likely being excreted via the corresponding salt glands. Energy dispersive spectroscopy was subsequently utilized to determine chemical composition of both leaf surfaces as well as excreted salt crystals.

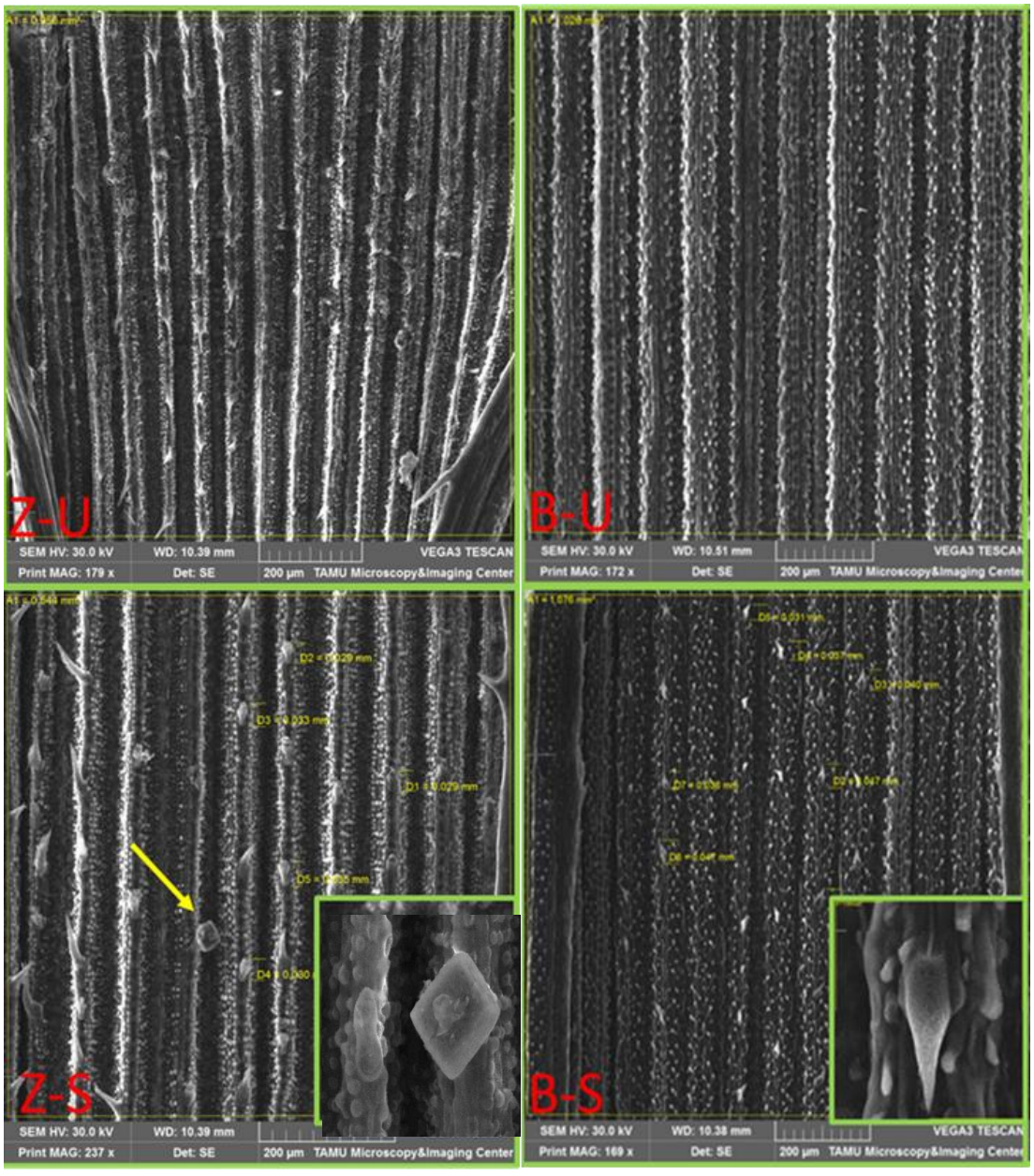

Figure 1. SEM images of adaxial leaf surfaces in zoysiagrass (Z) and bermudagrass (B). Yellow arrow refers to a salt crystal found on zoysiagrass. Upper images (U) are controls, while lower images were exposed to 30 dS m^{-1} salinity (S) for 2 weeks. Scale bar = 200 µm

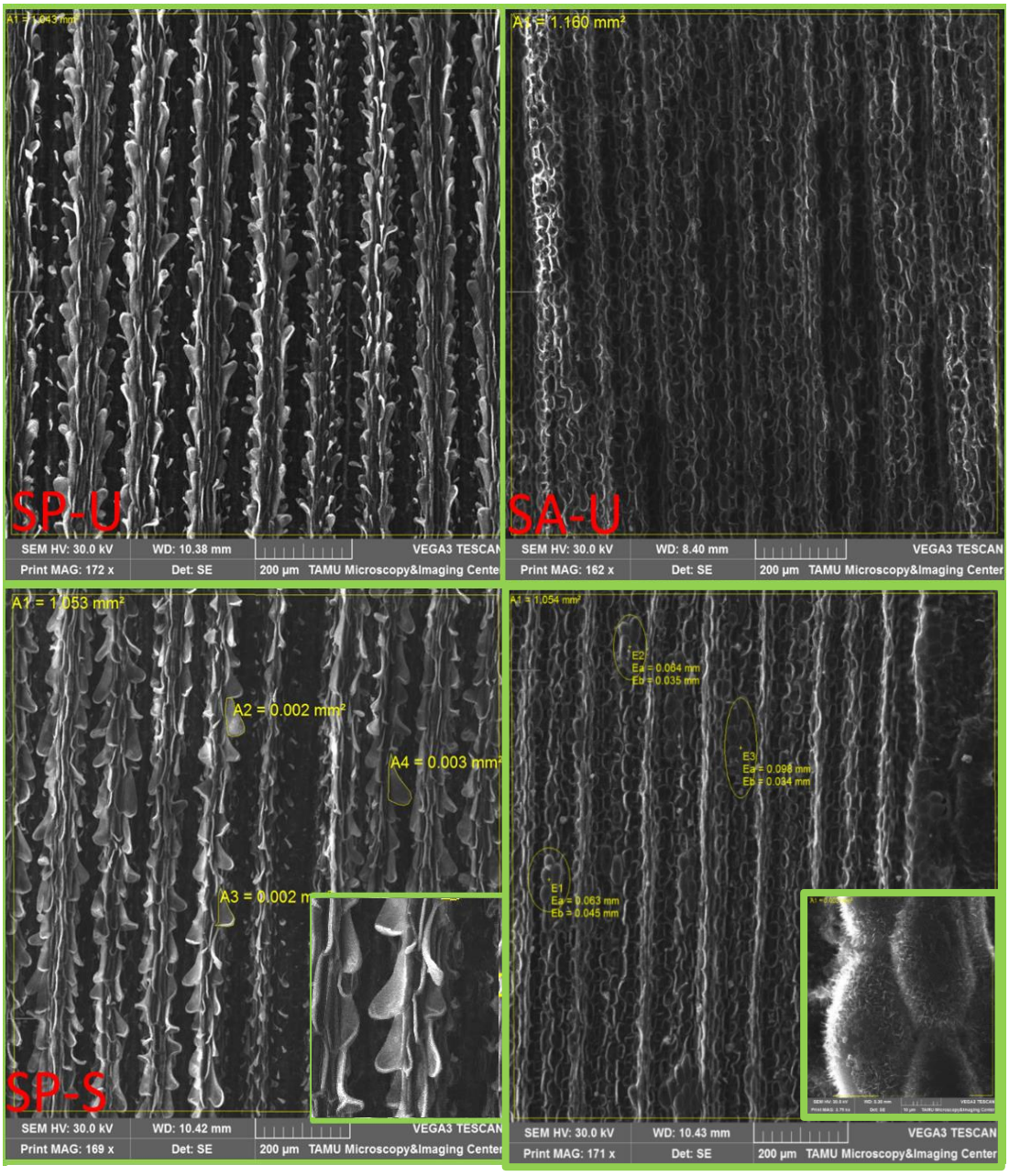

Figure 2. SEM images of adaxial leaf surface of seashore paspalum (SP) and St. Augustinegrass (SA). Upper images (U) are controls, while lower images were grown under 30 dS m^{-1} salinity (S) for 2 weeks. Scale bar = 200 µm

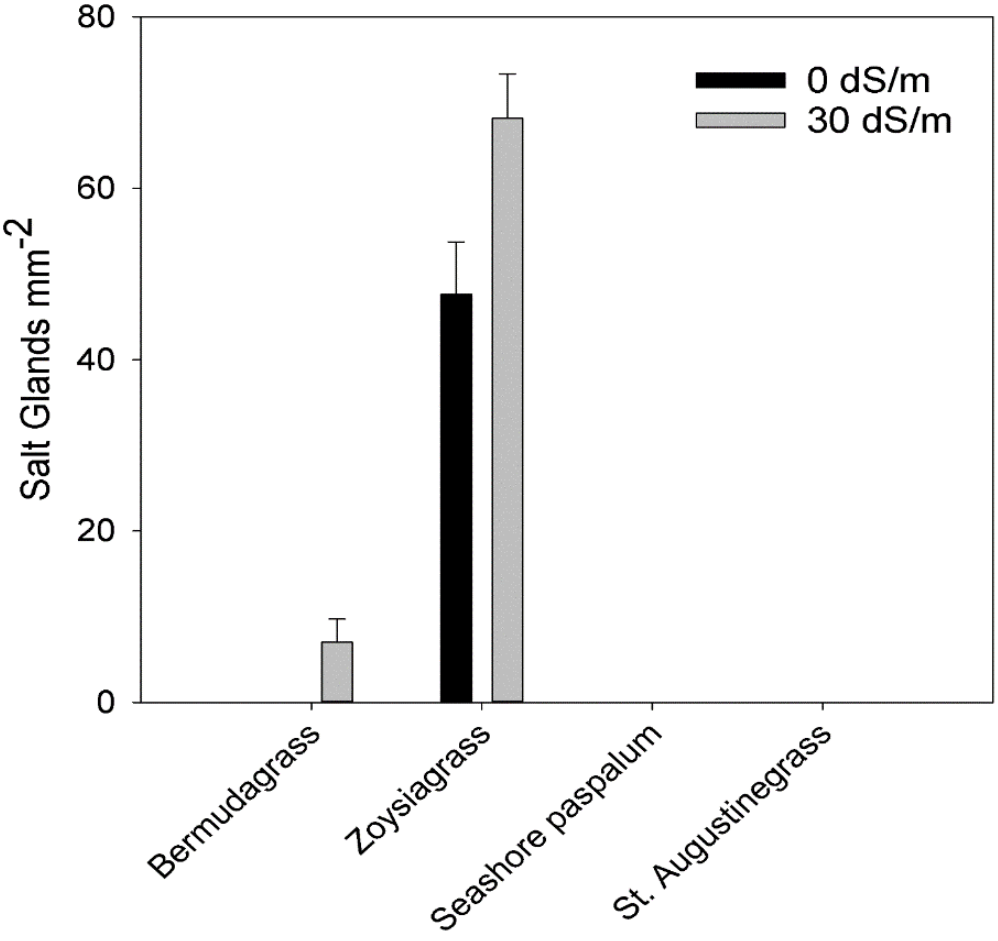

Figure 3. Salt gland counts for the 4 species under control =2.5 and 30 dS m^{-2} salinity stress. Counts were made on a 1 mm^2 section of adaxial leaf surface at the center of the second-oldest expanded leaf. Error bars denote standard error (n=4)

Energy Dispersive Spectroscopy Analysis of Leaf Surface

EDS (X-ray spectra) confirmed that salt gland ion excretions under the 30 dS m^{-1} salinity treatment in zoysiagrass and bermudagrass were composed predominantly of Na and Cl ions. However, no salt crystals were visible on leaf surfaces for either species when grown under the control treatment. Although no salt crystals were evident in either treatments for St. Augustinegrass and seashore paspalum, EDS detected a higher weight % of Na and Cl across the leaf surface areas of these species relative to zoyisagrass and bermudagrass, suggesting that these elements may be accumulating at or just below the leaf surface in epidermal cells or bladders (Table 14).

St. Augustinegrass and seashore paspalum possessed the highest weight percent of Na (1.79 and 1.54%, respectively) and Cl (1.30 and 1.84%, respectively), followed by zoysiagrass (1.05 and 1.23%, respectively), and bermudagrass (0.58 and 0.99%, respectively). Sodium appeared to be accumulating within stomatal guard cells of St. Augustinegrass leaves (Table 14). Energy dispersive spectroscopy analysis also indicated the highest K:Na (based on weight % detection levels) in bermudagrass and seashore paspalum (2.2:1.0 and 1.4:1.0, respectively), while St. Augustinegrass K:Na was lowest at 0.58:1.0 under 30 dS m^{-1} salinity levels (Table 13). Interestingly, the highest Si levels were detected in zoysiagrass, and was detected predominantly along vascular bundles in (Table 14).

Table 14. Energy dispersive spectroscopy analysis of major chemical elements detected on leaf surface and subsurface.

Cultivar	Treatments	C	O	Na	Mg	Al	Si	P	S	Cl	K	Ca	Fe
		----------Weight Total %----------											
Celebration	Control	66.26	31.32	0.04	0.09	0.01	0.28	0.33	0.22	0.22	0.82	0.44	0.01
	30 dS m^{-1}	69.93	25.79	0.58	0.10	0.02	0.21	0.31	0.33	0.99	1.26	0.48	0.00
UGP3	Control	64.31	29.74	0.61	0.26	0.01	0.62	0.32	0.79	1.02	2.02	0.30	0.01
	30 dS m^{-1}	65.22	27.81	1.30	0.18	0.02	0.38	0.15	0.41	1.84	1.76	0.18	0.01
Floratam	Control	68.19	29.38	0.34	0.13	0.02	0.06	0.19	0.17	0.32	1.06	0.15	0.02
	30 dS m^{-1}	67.10	29.08	1.79	0.24	0.02	0.20	0.12	0.17	1.54	0.57	0.14	0.02
DALZ1313	Control	67.71	29.82	0.25	0.10	0.02	0.42	0.12	0.17	0.30	0.99	0.11	0.02
	30 dS m^{-1}	75.33	20.32	1.05	0.17	0.1	0.72	0.04	0.36	1.23	0.61	0.16	0.03
LDS		4.29	4.78	0.56	0.17	0.03	0.44	0.20	0.44	1.02	0.81	0.24	0.04
Effects		----------Probability > F----------											
Species		***	**	***	*	ns	**	***	**	*	***	***	ns
Salinity		**	***	***	ns	ns	ns	*	ns	***	ns	ns	ns
Species & Salinity		**	**	*	ns	ns	ns	ns	ns	ns	ns	ns	ns

Energy Dispersive Spectroscopy Analysis of Leaf Cross-Sections

ANOVA revealed a significant cultivar x salinity x location interaction for weight total percent of Na, Cl, and K (Table 15).

Table 15. Analysis of variance of chemical elements found by EDS as affected by Cultivar, Salinity, and Plant Tissue Location main effects and interactions.

	Na	Cl	K
	-----Probability > F-----		
Cultivars	***	***	***
Salinity	***	***	ns
Location	***	***	***
Cultivars x Salinity	***	***	***
Cultivars x Location	***	***	***
Salinity x Location	***	***	**
Cultivars x Salinity x Location	***	***	**

ǂ Location refers cross-sectional leaf zone examined through EDS
* Significant at the 0.05 probability level
**Significant at the 0.01 probability level
*** Significant at the 0.001 probability level

Energy dispersive spectroscopy (X-ray spectra) confirmed weight percent of both Na and Cl increased under salinity stress (30 dS m^{-1}) within the three locations (adaxial epidermis, vascular tissue, and abaxial epidermis) of the leaf cross section in all species (Tables 16 and 17). However, St. Augustinegrass exhibited the highest weight % of Na and Cl across all locations of the leaf cross section, supporting the theory that St. Augustinegrass may lack the ability to both exclude these ions at the roots and/or sequester ions into epidermal cells. Under salinity stress, bermudagrass and zoysiagrass showed presence of higher weight percent of Na (0.43 and 0.57 %, respectively) and Cl

(0.79 and 1.35 % respectively) at the adaxial epidermis compared to the vascular tissue and abaxial epidermis locations. This observation is consistent with the observed presence of salt glands and associated exudation of salt crystals from salt glands in this location of the leaf (Tables 16 and 17). Among all species, seashore paspalum and St. Augustinegrass possessed the highest weight% of Na (0.89 and 1.31%, respectively) in vascular tissues, while Seahore paspalum, zoysiagrass, and St. Augustinegrass all showed relatively higher weight % of Cl (1.33, 1.18, and 1.33 Wt%, respectively) compared to bermudagrass (0.4 Wt %) within vascular tissue.

Table 16. Cultivar x Salinity Level x Location interaction on Weight Total % of sodium (Na) detected within three regions of the leaf cross section through EDS.

Cultivars	Control				30 dS m^{-1}			
	Total	E. Ad*	V. T.**	E. Ab***	Total	E. Ad*	V. T.**	E. Ab***
	----------Weight Total %----------							
Celebration	0.057	0.035	0.032	0.013	0.890	0.438	0.267	0.205
UGP3	0.157	0.060	0.048	0.063	2.213	0.600	0.887	0.253
DALZ1313	0.197	0.045	0.018	0.013	2.213	0.797	0.653	0.267
Floratam	0.260	0.327	0.312	0.352	2.973	1.483	1.310	1.410
LSD	0.188	0.08	0.079	0.101	0.800	0.321	0.302	0.220

*Epidermis at the adaxial side
**Vascular Tissue
***Epidermis at the abaxial side

Table 17. Weight Total % of chloride detected within three regions of the leaf cross section through EDS.

Cultivars	Control				30 dS m^{-1}			
	Total	E. Ad*	V. T.**	E. Ab***	Total	E. Ad*	V. T.**	E. Ab***
	----------Weight Total %----------							
Celebration	0.177	0.077	0.073	0.068	1.160	0.577	0.407	0.375
UGP3	0.217	0.260	0.425	0.340	2.130	0.965	1.335	0.612
DALZ1313	0.127	0.020	0.027	0.012	2.477	1.358	1.182	1.133
Floratam	0.280	0.379	0.332	0.327	2.000	1.507	1.333	1.413
LSD	0.267	0.129	0.146	0.140	1.164	0.452	0.450	0.466

*Epidermis at the adaxial side
**Vascular Tissue
***Epidermis at the abaxial side

EDS analysis of the leaf cross section indicated that bermudagrass and seashore paspalum possessed the highest weight percent of K under both control and salinity stress treatments within each of the three leaf locations; however, whereas weight percent of K in bermudagrass slightly increased when moving to high salinity, it decreased from 2.03 to 1.60 Wt. % when moving from control to high salinity in seashore paspalum (Table 18). Bermudagrass and seashore paspalum also maintained low Na:K, which may suggest that these two species were able to maintain higher root specificity for K and/or selective transport capacity from roots to shoots under salinity stress.

In contrast, zoysiagrass and St. Augustinegrass decreased weight percent of K within the three different leaf locations under high salinity (Table 18). This may indicatethese two turfgrasses possess lower K uptake selectivity and/or selective transport from roots to shoots. Furthermore, zoysiagrass and St Augustinegrass

appeared to provide little to no regulation of Na transport from roots to shoots. However, Na excretion by salt glands on zoysiagrass leaf surfaces is likely to aid in mitigating Na accumulation in this species.

Table 18. Weight Total % of potassium detected within three regions of the leaf cross section through EDS.

Cultivars	Control				30 dS m^{-1}			
	Total	E. Ad*	V. T.**	E. Ab***	Total	E. Ad*	V. T.**	E. Ab***
	----------Weight Total %----------							
Celebration	1.457	1.325	1.332	1.285	1.570	1.828	1.777	1.557
UGP3	2.030	1.598	1.663	1.423	1.607	1.017	1.295	0.987
DALZ1313	1.330	0.452	0.502	0.358	0.597	0.290	0.353	0.267
Floratam	1.078	0.830	0.935	0.755	0.993	0.493	0.438	0.437
LSD	1.348	0.378	0.503	0.614	1.112	0.441	0.322	0.212

*Epidermis at the adaxial side
**Vascular Tissue
***Epidermis at the abaxial side

DISCUSSIONS

Leaf anatomical characteristics and internal leaf elemental compartmentalization aspects were examined in this study to elucidate relative salinity tolerance mechanisms in these species, which had all been shown to have superior salinity tolerance relative to other genotypes of the same species in prior screenings. Results showed that bermudagrass (cultivar Celebration) and Zoysiagrass (experimental line DALZ1313) each developed salt glands on the adaxial leaf surface in response to elevated salinity. Salt gland density also increased with increasing salinity, but differences were observed in salt gland density (Fig 1). The salt gland density of DALZ1313 was significantly

different than that of Celebration in response to increased salinity concentration. This observation suggests that increased salt gland density could be one of several responses contributing to enhanced salinity tolerance (Fig 3). Salt crystal presence on leaf surfaces, indicative of ion secretion, was observed through SEM and confirmed with EDS in both bermudagrass and zoysiagrass. This observation is consistent with previous reports on the presence of salt glands in zoysiagrass (*Zoysia japonica* and *Z. matrella*), which were noted on both leaf surfaces (Marcum, 1990; Rao, 2008).

The warm-season turfgrasses in this study exhibited anatomical leaf surface differences that were consistent with their measured relative salinity tolerances. Intraspecific differences in salinity tolerance were related to a combination of salt gland presence, salt gland density, and apparent ion translocation from root to shoot, as detected through EDS. As such, chemical analysis of plant tissues would be important in future studies of this type for corroborating EDS elemental detection data (Angeles-Chavez et al., 2012). The EDS was used to detect ion location and weight percent of ions on or just within the adaxial leaf surface, as well as cross-sectional locations internal to the leaf.

Observations made with SEM and EDS technology confirmed the presence of Na and Cl in salt crystals located adjacent to salt glands in Celebration and DALZ1313, however seashore paspalum did not exhibit ion secretion activity under salinity. This information is consistent with previous studies in zoyisagrass and bermudagrass under salinity stress (Chen et al., 2009; Marcum and Murdoch, 1994). Our work also showed that seashore paspalum and St Augustinegrass species lack salt glands and ability to

excrete ions. Bermudagrass and zoysiagrass have been studied and classified into the sub-family Chloridoideae (Gould, 1983). However, the ability or inability of seashore paspalum to excrete salt is not well understood. Some specialists have classified seashore paspalum as a recretohalophyte which has specialized salt-secreting mechanism on the leaf surface, and also into the sub-family Panicoideae, many of which have been shown to possess salt glands which secrete ions or bladders which are distensible sac that can hold fluid with ions (Liphschitz, 1982: Kefu, 2002). But Marcum (1994), reported that seashore paspalum species lacked the ability to excrete ions under salinity stress. This, combined with our observations through SEM and EDS suggest the possibility that although seashore paspalum do not have salt glands, they may possess bladders inside which salt ions may be sequestered and subsequently removed through leaf senescence or mowing.

EDS also detected increased Na and Cl concentration just within the subsurface of all four species through cross-section analysis of plants grown at 30 dS m^{-1} salinity (Tables 14, 16 and 17). Similarly, elevated K in proportion to Na was detected in both Celebration and UGP3 at high salinity levels. These results support the idea that bermdagrass and seashore paspalum, may utilize two one or more different mechanisms for coping with salinity stress, including ion secretion via salt glands (bermudagrass) as well as specialized ion selectivity and/or translocation of K over Na (Tables 14 and 18).

CONCLUSIONS

This study utilized SEM and EDS to examine leaf surface and cross sectional characteristics of four turfgrass species to salinity stress. The observations highlight the fact that turfgrass response to salinity stress is complex, involving one or more physiological mechanisms including ion secretion via salt glands, increasing salt gland density, and distribution/sequestration of Na and Cl in the plant. While showing a high degree of salinity tolerance in prior screenings, the four turfgrass species in this study differed with respect to leaf surface anatomy. In bermudagrass and zoysiagrass cultivars was salinity tolerance was partially related to salt gland presence and salt gland density. Also, relative salinity tolerance among these turfgrass species could be related to Na:K. Physiological mechanisms, which might include selective K uptake or translocation affinity over Na may help to limit sodium uptake and accumulation in plant tissues. In this study, species that demonstrated the highest salinity tolerance in prior screenings (seashore paspalum and bermudagrass) showed a capacity to maintain relatively higher K in proportion to Na and Cl relative to K in leaf tissues. Collectively, these results offer insight on anatomical responses and mechanisms employed by these warm-season turfgrasses in coping with elevated salinity, information that could be useful to physiologists and breeders.

CHAPTER IV

PHYSIOLOGICAL RESPONSES TO SALT STRESS IN WARM-SEASON TURFGRASSES WITH CONTRASTING SALINITY TOLERANCE

OVERVIEW

There is an increased need to understand physiological mechanisms of halophytic turfgrass species for potential use in salt-affected soils due to increased use of recycled water for irrigation in arid and semi-arid regions. Greenhouse screenings were conducted during 2014 and 2015 at Texas A&M University, College Station TX to determine relative salinity tolerance among 45 experimental entries and cultivars representing four warm-season turf species under salinity levels ranging from 2.5 to 45 dS m^{-1}. In 2016, eight entries (two entries representing the highest and lowest relative salinity tolerance from each species) were advanced for additional evaluations aimed at determining physiological responses to salinity.Entries included 'Celebration' and 'UGB79' bermudagrass (*C. dactylon* and *C. dactylon x C.transvaalensis,* respectively), 'DALZ1313' and Zeon' zoysiagrass (*Zoysia matrella x Z. japonica* and *Z. matrella,* respectively), 'UGP3' and 'UGP38' seashore paspalum (*Paspalum vaginatum*), and 'Floratam' and 'Palmetto' St. Augustinegrass (*Stenotaphrum secundatum*). Grasses were grown in the greenhouse for ten weeks at salinity levels of 0, 15, and 30 dS m^{-1} to evaluate responses to increasing salinity. Physiological parameters including EC_{50}, ion excretion rate, root/shoot tissue Na and Cl concentrations, and Na:K of root/ shoot tissues were determined. Results indicated that all grasses adjusted osmotically under increasing salinity levels. Differences in the relative increase in Na:K were noted among

species, with bermudagrass and seashore paspalum entries maintaining proportionally higher K under salinity stress. Differences in total ion excretion as well as tissue Na and Cl concentrations appear to contribute to the previously observed differences in salinity tolerance between species.

INTRODUCTION

According to the Golf Course Superintendents Association of America's Environmental Institute for Golf Survey (GCSAA, 2015), recycled water is now the predominant irrigation source used in the southwestern and southeastern regions of the United States. Use of recycled water for golf course irrigation increased in these regions by 7 and 10%, respectively, from 2005 to 2013. Recycled water often contains elevated levels of salts, and thus, can lead to salinity stress in the turf root zone (Qian and Harivandi, 2008). Recycled water also has the potential to cause direct foliar injury to turfgrasses (Devitt et al., 2004).

Turfgrasses are well suited for recycled water because they function as biological filters which can remove excess salts and nutrients from saline water (Hayes et al., 1990). However, minimizing salt application to soil can be a challenge, and therefore consideration should be given to selecting salt-tolerant turfgrass species, capturing and utilizing natural rainfall, minimizing irrigation application, and application of a maintenance salt leaching program. Warm-season turfgrasses are best-suited for low quality water use, especially in arid and semi-arid areas of the world, since they

generally possess increased resistance to both drought and salinity stress (Marcum, 2006; Uddin and Juraimi, 2013).

Nutrient imbalance in plants commonly occurs under salinity stress. While not an essential plant nutrient, excess sodium uptake by root systems disrupts plant potassium nutrition due to the similar chemical nature between Na and K (Lee et al., 2007). The potassium ion is essential for many cellular functions such as maintaining cell turgor and enzyme activities (Zhu, 2001). Aquaporin cells (water channels) in the root system have also been shown to have greater affinity for sodium than potassium. Aquaporin cells use symplastic and apoplastic pathways for potassium uptake, however sodium moves cell to cell passively, via the symplastic pathway. This reduced potassium/sodium selectivity may ultimately contribute to growth inhibition in plants arising from potassium imbalance (Bhardwaj et al., 2013)

Warm-season turfgrasses are generally well adapted to drought and salinity conditions, because they have developed mechanisms to translocate (xylem and phloem), compartmentalize, accumulate (vacuole), and excrete (salt glands) ions within or from cells (NaCl) in order to regulate water content within plant tissues.

Salinity effects vary between and within turfgrass species, but a common effect of salinity is reduced biomass production. For example, several turfgrass species which were exposed to nine months of salinity stress from 5 to 41 dS m^{-1} NaCl, exhibited 50% shoot and root growth reductions (Alshammary et al., 2004). Plant tolerance to salinity has been thought to be related to maintenance of low Na:K of shoots and roots. This has been suggested to be partially related to conference of salinity tolerance in both common

bermudagrass (*Cynodon dactylon* (L.) Merr) and seashore paspalum (*Paspalum vaginatum* Swartz) cultivars (Marcum and Murdoch, 1990).

Salt excretion is another reported mechanism utilized by some warm season grasses when coping with salinity (Marcum, 2002). The relationship between salt excretion capacity and relative salinity tolerance, especially between genotypes of the same species that show contrasting salinity tolerance is another question for which little to no published data are available.

Therefore, the objectives of this study were to determine comparative salt excretion capacities and root/shoot tissue accumulation profiles under increasing salinity levels for previously tested turfgrass genotypes demonstrating contrasting salinity tolerance.

MATERIALS AND METHODS

Growing Conditions and Plant Materials

This study was conducted in a greenhouse at Texas A&M University, College Station, TX from 1 January through 11 July 2016, with a repeat study conducted from 1 January through 30 September 2016. Eight warm-season turfgrass cultivars (two entries representing the highest and lowest relative salinity tolerance from each species) were used in this experiment. Each was identified through previous screenings to possess the highest and lowest salinity tolerance compared to other genotypes of the same species. Entries included Celebration' and 'UGB79' bermudagrass (*C. dactylon* and *C. dactylon x C. transvaalensis,* respectively), 'DALZ1313' and Zeon' manilagrass (*Zoysia matrella x Z. japonica and Z. matrella, respectively*), 'UGP3' and 'UGP38' seashore paspalum (*Paspalum vaginatum*), and 'Floratam' and 'Palmetto' St. Augustinegrass (*Stenotaphrum Secundatum*) (Table 19).

Table 19. Species, cultivar names or experimental designations, and origin of entries used.

Species	Tested Salinity Tolerance	Cultivars	Origin
Cynodon dactylon	High†	Celebration	Sod solutions
C. transvaalensis x C dactylon = (3x)	Low‡	UGB79	University of Georgia
Z. matrella x Z. japónica	High†	DALZ1313	Texas A&M University System
Zoysia matrella	Low‡	Zeon	BladeRunner Farms
Stenotaphrum secundatum	High‡‡	Floratam	Univ. Florida/ Texas A&M
Stenotaphrum secundatum	Low‡‡	Palmetto	Sod Solutions
Paspalum vaginatum	High††	UGP3	University of Georgia
Paspalum vaginatum	Low†	UGP38	University of Georgia

† 30 < 15 dS m^{-1}
‡ < 15 dS m^{-1}
†† > 30 dS m^{-1}
‡‡ < 10 dS m^{-1}

Prior to the study initiation, sod plugs (5 cm diameter x 5 cm deep) of each entry were washed free of soil, with roots trimmed to 5 cm before transplanting into 100 cm^2 x 10.2 cm deep pots containing medium-coarse USGA specified green sand. The washed sod plugs were allowed to fully establish into pots for 150-days before initiating salinity treatments. During establishment, grasses were irrigated daily with 0.6 cm tap water and provided liquid-fertilization twice weekly using a 20-20-20 water soluble fertilizer (Peters 20-20-20, J.R. Peters, Inc., Allentown, PA 18106) to supply 1.2 g N m^{-2} wk^{-1}. Grasses were clipped weekly using scissors with clippings removed. Bermudagrasses, zoysiagrass, and seashore paspalum entries were maintained at a 2.5 cm while St. Augustinegrass was maintained at 5 cm height of cut.

Environmental conditions in the greenhouse were monitored during the study period using a weather station (WatchDog 2000 Weather Station, Spectrum Technologies, Inc., Aurora, IL 60504). Daily high, low, and mean temperature, relative humidity, and solar radiation were recorded for the acclimation and study period (Table 20).

Acclimation & Salinity Exposure Phases

Repeated studies consisting of four main treatments (1 m x 1 m x 5 cm deep ebb and flow benches) accommodating salinity levels of 2.5 (control), 15, and 30 dS m^{-1} were used. Within each salinity level eight cultivars/experimental lines were arranged in a completely randomized design with 6 replicates pots per genotype. Prior to initiating the study, a salinity acclimation period was gradually imposed in which the salinity level was increased by 10 dS m^{-1} wk^{-1} until a final concentration 30 dS m^{-1} wk^{-1} was reached on day 14, at which time the 3-week experiments were initiated. The acclimation period for study 1 began on 10 June 2016 and for study 2 began on 5 August 2016.

During acclimation, potted grasses were placed into ebb and flow benches and sub-irrigated daily. Potable tap water, with pH 8.1, and electrical conductivity of <1 dS m^{-1} was used for all 3 salinity treatments. During sub-irrigation events, water was pumped from 189 L holding tanks to completely fill ebb and flow benches for a 5-minute period, and allowing sand root zones within pots to become fully saturated. A float valve was positioned near the upper edge of each ebb and flow bench to prevent overflow and to stop pump operation once the bench was completely filled to the top. Salinity treatments were provided by mixing tap water with Instant Ocean Sea Salt

(Instant Ocean Spectrum Brands, Blacksburg, VA 24060) to achieve the respective desired EC levels (Table 3). A 13-2-13 soluble fertilizer (Miracle-Gro Professional Excel, Marysville, OH 43040) was used to produce an irrigation nutrient concentration of 300 ppm NO_3-N within the four treatments.

During both the acclimation and experimental periods, the water level in holding tanks was measured and supplemented twice weekly to replace water lost to evaporation from the system. Pots were also overhead flushed with tap water weekly prior to sub-irrigation to prevent accumulation of salt at the soil surface in pots. Throughout the acclimation and salinity exposure phase, salinity concentrations and nitrate concentrations of irrigation water were monitored twice weekly using a portable EC meter (EC 110 Meter Field Scout, Spectrum Technologies, Inc., Aurora, IL, 60504) and a compact NO_3-nitrate ion meter (LAQUA Twin Nitrate Meter, Spectrum Technologies, Aurora, IL). Fertilizer was added to compensate for nutrient depletion from the system and to maintain a target concentration of 300 ppm NO_3-N within each treatment.

Measurements and Data Collection

To evaluate turfgrass response to salinity stress, turfgrass visual quality (Morris and Shearman, 1998), percent green cover, percent shoot biomass reduction, and final root biomass were measured during the study. Grasses were visually rated for turfgrass quality using a 0-9 scale weekly (Morris and Shearman, 1998), where 0= completely brown turf, 6 = minimum acceptable, and 9= perfect green turf quality. Turfgrass quality measurements were taken from weeks 3 through 7. Percent green cover of grasses was

determined by digital image analysis of light box images taken at weeks 3 & 7 (end of salinity stress period), using a Canon 4x optical zoom digital camera (Canon Co.) mounted on a 30 cm diameter x 50 cm height light-box. The light box cancelled out outside light and created uniform light within the box via 8 LED bulbs. Camera settings were as follows: image type (JPEG Image), dimensions (969 x 1049), color (sRGB), no flash, focal length (7 mm), F-stop (F 4.2), exposure time (1/2 sec), ISO-800, white balance auto (Karcher and Richardson, 2005). The digital images were analyzed using digital image analysis (Sigma Scan Pro, Image Analysis Version 5.0) and the Turf Analysis macro (Richardson et al., 2001) for determining percent green cover within each pot.

Weekly shoot growth rates within each treatment were also determined from weeks 3 through 7 by clipping grasses to a height of 2.5 cm (bermudagrass, seashore paspalum, and zoysiagrass) or 5 cm (St Augustinegrass). All clippings were oven dried (VWR Gravity Convention Oven) for 72 h at 65°C with dry weights determined using a digital scale (Denver Instrument P-403 Digital Balance/Scale). At the end of the salinity exposure phase (week 7) clipping dry weights were evaluated between salinity and control treatments in order to calculate percent reduction in shoot biomass caused by each salinity level. The following formula was used to determine percent clipping biomass reduction:

$$\% \text{ Clipping Biomass Reduction} = (1 - (a/b)) \times 100$$

a= Clipping dry weight for a given entry within a given cultivar and salinity treatment

b= Clipping dry weight average of all entries for the same cultivar in the control treatment

Laboratory Examination of Leaves

Salt excretion capacity and tissue ion content were determined every 7 days for 4 weeks. For determining salt excretion capacity, three replicates of each genotype were thoroughly rinsed using distilled water prior to harvesting leaf tissues, while three replicate pots remained unrinsed. In this way, the comparison of these two sets (rinsed vs. unrinsed) leaves allowed for distinguishing salt excretion capacity from exudation of solutes from cut leaf ends. Within each salinity level, 10 mature leaves were randomly selected from each replicate pot, excised, and placed into scintillation vials. Ten ml of distilled water was then added to each vial, sealed, and shaken for 10 seconds in the laboratory before measuring electrical conductivity using an electrical conductivity meter. Leaves were removed, dried at 65°C for 48 h and weighed.

For determination of tissue ion content in plant tissue, clippings were obtained over the 4 weeks. After 4 weeks at the target salt concentrations, grasses were harvested and washed free of sand and oven dried at 65°C for 48 h to determine total dry weights for above and below ground tissues. Above ground (clippings + verdure) and below ground (roots and rhizomes) tissues were then submitted to the Texas A&M AgriLife Soil, Water, and Tissue Testing Laboratory for elemental analysis. Plant minerals were determined by ICP analysis of a nitric acid digest (Havlin et at., 1989)

Statistical Analysis

The experiment was arranged as a completely randomized design with six replicates pots per species. Data where subjected to analysis of variance using the general linear model, univariate test procedure of SPSS ver. 21.0 (IBM Corp, Armonk, NY) to determine statistical significance of the results. Mean separation procedures were performed using Fisher´s LSD at the $P \leq 0.05$ level.

RESULTS

Environmental Conditions

Greenhouse environmental conditions were monitored over the 6-week study periods in both experiments. During the first study, mean maximum temperatures during the two experimental phases (acclimation and salinity exposure phase) were 36 and 37°C, respectively, while mean minimum temperatures were 27 and 27°C, respectively. During the repeat study, mean maximum temperatures for the two phases were 35 and 34°C, respectively, while mean minimum temperatures for the two phases were 25 and 26°C, respectively. Relative humidity during the two phases of the first study averaged 80 and 75%, respectively. For the second study, mean relative humidity was 82 and 73%, respectively, for the acclimation and salinity exposure phases (Table 20).

Table 20. Environmental Conditions in the greenhouse during the two studies.

	Solar Radiation	Daylight hr d^{-1}			Relative Humidity	Temperature °C		
	W m^{-2}	Max	Min	Average	%	High	Low	Average
Study 1 (2016)*								
Acclimation period **	180	13.6	13.4	14.3	80	36	27	31
Salinity stress***	166	13.3	12.4	13.0	75	37	27	31
Study 2 (2016)****								
Acclimation period **	97	13.1	12.4	12.3	82	35	25	29
Salinity stress***	126	12.4	11.5	12.1	73	34	26	29

*Months from Jul 10th to August 30th
**Acclimation period was weeks 1 to 3
***Salinity stress periods was weeks 4 to 7
****Months from August 5th to Sept 30th

ANOVA revealed significant cultivar main effects for turfgrass quality at all salinity levels during both studies. A cultivar x study interaction was also detected at the 30 dS m^{-1} treatment (Table 21).

Table 21. Analysis of variance for the Texas A&M greenhouse salinity experiment

	Salt Exretion	Final Quality	% Final Green Cover	% Biomass-Reduction	Final Root Biomass
Control					
Cultivar	***	***	***		**
Study	***	***	***		ns
Cultivar x Study	***	***	***		ns
15 dS m^{-1}					
Cultivar	***	***	***	***	***
Study	ns	ns	ns	***	ns
Cultivar x Study	***	***	***	***	**
30 dS m^{-1}					
Cultivar	***	***	***	***	***
Study	ns	***	***	***	***
Cultivar x Study	ns	***	***	***	**

*Significant at the 0.05 probability level
**Significant at the 0.01 probability level
***Significant at the 0.001 probability level
ns = Not Significant

Salt Excretion Capacity

Salt excretion capacity is expressed as the difference in ion secretion between unrinsed and rinsed leaves under both 15 and 30 dS m^{-1} salinity levels (Table 22). Salt excretion increased significantly in Celebration, DALZ1313, and Zeon when salinity increased from 2.5 (control) to 15 dS m^{-1} to 30 dS m^{-1}. DALZ1313 and Zeon zoysiagrasses showed the highest excretion capacity of all cultivars (1926, 1454, and 3686 µS cm^{-1} g^{-1} for DALZ1313 and 715, 1437, and 1860 µS cm^{-1} g^{-1} for Zeon at 2.5, 15, and 30 dS m^{-1} salinity levels, respectively). Celebration exhibited lower levels of salt excretion than zoysiagrass at both 15 and 30 dS m^{-1} salinity levels. In Seashore paspalum (UGP3 and UGP38) and St Augustinegrass (Floratam and Palmetto), little to no salt excretion activity was detected across all salinity treatments (Table 22).

Table 22. Mean salt excretion for the turfgrass cultivars/ experimental lines over the four weeks of salt secretion (µS cm^{-1} g^{-1} per 7 days). Positive % Change values denote increased excretion relative to control, while negative values indicate lower excretion relative to controls.

Cultivars	Control		15 dS m^{-1}				30 dS m^{-1}	
	Study 1	Study 2	Study 1		Study 2			
	Salt Excretion Rate	Salt Excretion Rate	Salt Excretion Rate	Change %	Salt Excretion Rate	Change %	Salt Excretion Rate	Change %
Celebration	419.0	558.4	673.5	61	960.0	72	1391.3	186
UGB79	256.0	237.2	314.9	23	277.1	17	349.58	42
DALZ1313	457.8	646.3	1926.5	321	1454.9	125	3686.3	568
Zeon	454.9	669.0	715.5	57	1437.0	115	1860.8	231
UGP3	147.7	161.8	160.8	9	166.1	3	177.0	14
UGP38	151.2	162.5	152.8	1	186.9	15	172.4	10
Floratam	421.5	382.1	448.4	6	411.1	8	397.7	-1
Palmetto	311.6	343.6	306.8	-2	359.8	5	365.3	12
LSD	130.4	154.0	401.7	80	197.7	48	825.5	63

Final Turf Quality

In general, turf quality, as noted by % change between pre- and post-salinity stress, was reduced in all cultivars as salinity concentration was increased in both studies (Table 23). In study one, when moving from control to 15 dS m^{-1} treatments, bermudagrass (Celebration and UGB79) and seashore paspalum (UGP3 and UGP38) suffered the least injury following four weeks of exposure to the 15 dS m^{-1} salinity level. Only Celebration, UGP3, UGP38, and Palmetto exhibited turf quality (8, 7.6, 7.3, and 6.0, respectively) above acceptable levels. All cultivars, when grown at 30 dS m^{-1} salinity, declined in turf quality relative to lower salinity levels; however, Celebration maintained the highest turf quality (7.6) followed of DALZ1313, UGP3 and UGP38 (6.5, 6.5 and 6.5, respectively). St. Augustinegrass (Floratam and Palmetto) turf quality was reduced 50% or more below acceptable levels at 30 dS m^{-1} salinity. Overall, Celebration was the best performing entry in terms of turf quality at 15 and 30 dS m^{-1} salinity levels.

In study two, turf quality of Celebration, DALZ1313, UGP3, and UGP38 was maintained above acceptable levels (7.1, 8, 8, and 7.1, respectively) throughout the whole experimental period under 15 dS m^{-1}. Celebration, DALZ1313 and UGP3 were the only entries to respond favorably to in turf quality when salinity level was increased from 2.5 (control) to 15 dS m^{-1} after four weeks of salinity exposure. DALZ1313 even maintained acceptable turf quality under 30 dS m^{-1} salinity concentration. Turf quality in St Augustinegrass cultivars Floratam and Palmetto decreased sharply below acceptable levels following exposure to both 15 and 30 dS m^{-1} treatments.

Table 23. Final turf quality of the 10 turfgrass entries after 30 days of salinity stress exposure. Positive values denote growth and negative values denote decline in growth

	Study 1							Study 2						
	Control			15 dS m^{-1}		30 dS m^{-1}		Control			15 dS m^{-1}		30 dS m^{-1}	
	Pre	Post	Change	Post	Change	Post	Change	Pre	Post	Change	Post	Change	Post	Change
Cultivars			%		%		%			%		%		%
Celebration	8.2	8.0	-2	8.0	-2	7.6	-7	6.8	8.8	29	7.1	4	2.0	-71
UGB79	5.4	3.6	-33	5.0	-7	3.8	-30	3.8	6.8	77	3.6	-6	0.2	-96
DALZ1313	7.4	6.3	-15	5.5	-26	6.5	-12	7.8	8.5	9	8.0	3	7.5	-4
Zeon	7.4	6.1	-17	5.0	-32	5.1	-31	6.7	7.8	16	6.0	-11	1.2	-83
UGP3	8.2	8.0	-2	7.6	-7	6.5	-20	7.7	9	16	8.0	3	5.6	-28
UGP38	8.0	8.0	0	7.3	-8	6.5	-18	7.2	8.8	22	7.1	-1	4.0	-44
Floratam	7.2	5.6	-22	5.8	-19	2.3	-68	7.0	5.6	-20	4.8	-31	0.8	-88
Palmetto	7.0	8.1	15	6.6	-6	3.5	-50	5.2	8.1	55	4.1	-22	0.8	-84
LSD	1.7	1.6	23	1.7	25	2.4	31	1.1	1.0	16	2.0	40	2.3	36

Digital image analysis for percent green cover after salinity stress

Significant cultivar main effects and cultivar x study interactions occurred for percent green cover (% GC) in both studies (Table 21). In study one, in the control treatment, Celebration, UGP3, UGP38, and Palmetto showed the highest % GC (87, 84, 90, and 94 %, respectively). In study two in the control treatment, UGB79 was the only cultivar that did not exceed 80% GC (Table 24).

At 15 dS m^{-1} salinity, lower % GC was observed among all cultivars when compared with control treatment, however, only Celebration and UGP3 maintained % GC rates above 80% in both studies. At 30 dS m^{-1}, % GC was negatively affected by the increased salinity concentration. Also at the 30 dS m^{-1} salinity level, Celebration, DALZ1313, UGP3, and UGP38 were able to maintain the highest % GC in study one, while DALZ1313 and UGP3 exhibited the highest % GC within 30 dS m^{-1} salinity level in study two.

Table 24. Final % green cover as affected by cultivar and salinity level after four weeks of salinity stress.

	Study 1			Study 2		
Cultivars	Control	15 dS m^{-1}	30 dS m^{-1}	Control	15 dS m^{-1}	30 dS m^{-1}
Celebration	87	84	79	91	81	30
UGB79	40	33	40	72	43	12
DALZ1313	67	56	66	91	86	83
Zeon	67	55	55	83	65	13
UGP3	84	81	70	93	89	60
UGP38	90	89	72	91	78	29
Floratam	65	63	24	88	55	19
Palmetto	94	75	39	83	56	8
LSD	16	18	25	12	20	29

% Clipping Biomass Reduction

ANOVA revealed a significant cultivar x study interaction for % biomass reduction at both 15 and 30 dS m^{-1} salinity levels ($P<0.001$) (Tables 21). Biomass reductions generally increased with increasing salinity concentration. The most vigorous-growing turfgrasses were UGB79, Zeon and UGP3, which each increased biomass production by 40, 18.9, and 14.97% at 15 dS m^{-1} salinity in study one (Table 25).

Although 50% biomass reduction was not reached for any cultivar in study one, it was reached at the 30 dS m^{-1} salinity level in study two for UGB79, Zeon, and Palmetto. Higher relative biomass reductions among of these turfgrass species occurred in study two compared to study one, which may have been related to lower levels of solar radiation, which was reduced from166 to 126 W m^{-2} between studies one and two. Environmental conditions such as lower solar radiation, lower temperatures, or even

shorter photoperiod may have differentially influenced biomass reductions between the studies (Table 20).

Table 25. % shoot biomass reduction as affected by cultivar and salinity level. Positive values denote growth and negative values denote decline in growth.

	Study 1		Study 2	
Cultivars	15 dS m^{-1}	30 dS m^{-1}	15 dS m^{-1}	30 dS m^{-1}
Celebration	1.30	2.97	-28.07	-47.51
UGB79	18.90	7.93	-48.36	-55.46
DALZ1313	7.87	-26.32	-0.44	-17.11
Zeon	40.00	-20.18	-31.44	-57.13
UGP3	14.97	0.69	-10.50	-21.62
UGP38	6.49	12.89	-18.34	-29.94
Floratam	-7.43	-37.21	-46.98	-46.27
Palmetto	-4.97	-32.49	-74.69	-78.80
LSD	29.30	27.70	18.70	23.50

Final Root Dry Weight

ANOVA showed a cultivar main effect for final root dry weight within the control, 15, and 30 dS m^{-1} salinity treatments; however, a significant cultivar x study interaction also occurred within 15 and 30 dS m^{-1} treatments. Therefore, comparisons have been made among cultivars by study (Table 21). Root growth was significantly different among the eight entries. UGP3 and UGP38 exhibited the greatest root growth (2.1 and 1.7 g DW, respectively) among all entries at 15 dS m^{-1}, however, UGB79 and Zeon had the highest % root biomass increases (13 and 67 %) among cultivars at this same salinity level. In study two, seashore paspalums (UGP3 and UGP38) again had the greatest root growth among cultivars (2.9 and 3.2 g DW, respectively) but Celebration,

Zeon, UGP38, and Palmetto exhibited the highest increases in root biomass production (19, 67, 39, and 17%, respectively) when salinity increased from control to 15 dS m^{-1} levels. Root growth was reduced even further in species when salinity concentration increased to 30 dS m^{-1} in both studies. However, in study one, some cultivars including DALZ1313, UGP3, and Floratam (-56, -50, and -59 %, respectively) exhibited 50% or greater root biomass reductions at 30 dS m^{-1} levels, while in study two, Zeon, UGP38, Floratam, and Palmetto showed increased root biomass under these same salinity levels. Both St. Augustinegrass cultivars and Zeon zoysiagrass maintained or increased their root biomass with increased salinity concentration in both studies, which is interesting considering their relatively poor salinity tolerance as measured through other parameters in the study.

Table 26. Cultivar x salinity level interaction for final root mass (g) and % root biomass reduction after four weeks of salinity stress. Positive values denote growth and negative values denote decline in growth.

		Study 1				Study 2			
	Control (g)	15 dS m^{-1}		30 dS m^{-1}		15 dS m^{-1}		30 dS m^{-1}	
Cultivars		(g)	Change %	(g)	Change %	(g)	Change %	(g)	Change %
Celebration	2.1	2.0	-5	1.9	-10	2.5	19	1.6	-24
UGB79	0.8	0.9	13	0.8	0	0.7	-13	0.6	-25
DALZ1313	0.9	0.8	-11	0.4	-56	0.8	-11	0.8	-11
Zeon	0.6	1.0	67	0.6	0	1.0	67	0.7	17
UGP3	3.0	2.1	-30	1.5	-50	2.9	-3	2.5	-17
UGP38	2.3	1.7	-26	2.2	-4	3.2	39	2.5	9
Floratam	1.7	1.6	-6	0.7	-59	1.5	-12	2.1	24
Palmetto	1.2	1.2	0	1.0	-17	1.4	17	1.5	25
LSD	0.8	1.1	80	0.8	61	1.2	104	0.9	36

Elemental Analysis of Above and Belowground Tissues

ANOVA revealed significant cultivar main effects for all chemical elements analyzed (N, Na, Cl, and K) in plant tissues. A cultivar x plant tissue interaction was also detected for both salinity treatments, indicating that cultivars differed with regard to the relative proportion of chemical elements detected between above and belowground tissues (Table 27).

Table 27. ANOVA for study, cultivar, and plant tissue main effects and interactions on N, Na, Cl, and K levels in plant tissues (above vs. below ground) after 30 days of salinity stress.

	Absolute				Concentration			
	N	Na	Cl	K	N	Na	Cl	K
Control (2.5 dS m^{-1})								
Study	ns	ns	ns	ns	ns	***	***	ns
Cultivar	***	***	***	***	***	***	***	***
Plant tissue	***	***	***	***	***	***	***	***
Study x Cultivar	***	***	***	***	ns	ns	ns	ns
Study x Plant tissue	ns	ns	ns	ns	ns	ns	ns	ns
Cultivar x Plant tissue	***	***	***	***	***	***	***	***
Study x Cultivar x Plant tissue	**	**	**	**	ns	ns	ns	ns
15 dS m^{-1}								
Study	***	ns	***	***	**	**	***	ns
Cultivar	***	***	***	***	***	***	***	***
Plant tissue	***	***	***	***	***	***	***	***
Study x Cultivar	***	***	***	***	ns	ns	**	**
Study x Plant tissue	***	**	***	***	ns	ns	ns	ns
Cultivar x Plant tissue	***	***	***	***	***	***	***	***
Study x Cultivar x Plant tissue	***	***	***	***	ns	ns	ns	ns

* Significant at the 0.05 probability level
**Significant at the 0.01 probability level
*** Significant at the 0.001 probability level
ns = Not significant

Plant Tissue Ion Accumulation

Absolute N content (g) in shoot tissues exceeded that found in roots for Celebration, DALZ1313, UGP3, and UGP38 (0.10, 0.7, 0.13, and 0.11 total g N, respectively) under 2.5 dS m^{-1} (control) salinity level. Similarly, these same cultivars exhibited the highest shoot: root ratio (1.1:1, 1.2:1, 1.4:1, and 1.6:1, respectively) (Table 28).

All cultivars showed higher N concentration in shoots compared to roots under control level, however, Celebration, UGP3, and UGP38 exhibited more than 50% higher nitrogen concentration shoots than in roots (2.7:1, 3.0:1, and 4.0:1, respectively) (Table 28). Overall, Celebration, UGP3, and UGP38 appeared to exhibit the most favorable N relations among all cultivars, both in terms of absolute N and N concentrations in plant tissue at the control (2.5 dS m^{-1}) salinity level.

Table 28. Presence of nitrogen in plant tissue as influenced by cultivar, presented in both absolute terms as well as on concentration basis after 30 days of salinity stress at 2.5 dS m^{-1}(control level).

Cultivar	Absolute N (g)				Concentration (%)		
	Shoots	Roots	Total	Ratio	Shoots	Roots	Ratio
Celebration	0.10	0.09	0.19	1.1:1	3.5	1.3	2.7:1
UGB79	0.04	0.05	0.10	0.8:1	2.4	1.2	2.0:1
DALZ1313	0.07	0.06	0.14	1.2:1	2.5	1.4	1.7:1
Zeon	0.04	0.06	0.10	0.7:1	2.0	1.4	1.4:1
UGP3	0.13	0.09	0.23	1.4:1	3.6	1.2	3.0:1
UGP38	0.11	0.07	0.19	1.6:1	3.7	0.9	4.0:1
Floratam	0.08	0.14	0.23	0.6:1	3.8	2.1	1.8:1
Palmetto	0.06	0.11	0.17	0.5:1	3.1	1.7	1.8:1
LSD	0.04	0.05	0.18		0.54	0.36	

Absolute N contents decreased sharply with increased salinity concentration (15 dS m^{-1}), both within shoot and root tissues in study one (Table 28). Cultivars also exhibited marked differences in their N responses under increased salinity when comparing between studies one and two, which may have been related to differences in solar radiation levels or photoperiod between studies (Table 20). Interestingly, in study two, at 15 dS m^{-1} salinity, all cultivars exhibited much higher absolute shoot N content than compared to that detected at 2.5 dS m^{-1} (control) salinity levels (Table 28), or when compared to that noted for study one (Table 29). In comparison to control levels, all cultivars also maintained higher shoot:root N concentration ratios under 15 dS m^{-1}, with Celebration, UGB79, UGP3, and UGP38 exhibiting the highest ratio among cultivars in study one and (Table 29).

Table 29 Presence of Nitrogen in plant tissue as influenced by cultivar, presented in terms of both absolute N (g) and on concentration basis (ppm) after 30 days of salinity stress at 15 dS m^{-1}.

	Absolute N (g)										
	Study 1				Study 2				Concentration (%)		
Cultivars	Shoot	Root	Total	Ratio	Shoot	Root	Total	Ratio	Shoot	Root	Ratio
Celebration	0.072	0.096	0.168	0.8:1	0.165	0.097	0.262	1.7:1	3.150	1.368	2.3:1
UGB79	0.032	0.076	0.108	0.4:1	0.056	0.041	0.097	1.3:1	2.400	1.104	2.1:1
DALZ1313	0.039	0.060	0.099	0.7:1	0.122	0.062	0.184	1.9:1	2.175	1.481	1.4:1
Zeon	0.051	0.073	0.124	0.7:1	0.045	0.074	0.119	0.6:1	1.858	1.326	1.3:1
UGP3	0.080	0.074	0.154	1.1:1	0.186	0.092	0.278	2.0:1	3.267	1.288	2.6:1
UGP38	0.061	0.091	0.152	0.7:1	0.127	0.077	0.204	1.6:1	3.320	0.969	3.5:1
Floratam	0.032	0.097	0.129	0.3:1	0.081	0.082	0.163	1:1	3.033	1.605	1.8:1
Palmetto	0.040	0.195	0.235	0.2:1	0.053	0.025	0.078	2.1:1	2.925	1.740	1.7:1
LSD	0.037	0.053	0.082		0.050	0.041	0.165		0.544	0.201	

Sodium accumulated to a greater extent in root tissues under 2.5 dS m^{-1} (control) salinity levels for all cultivars (Table 30). Floratam and Palmetto had higher amounts of Na (86561 and 70332 μg, respectively) in root tissue compared to other cultivars. All cultivars showed higher shoot than root sodium, when presented in terms of concentration (ppm) under control level.

All cultivars increased Na concentration in both shoot and root tissue when salinity was increased from 2.5 dS m^{-1} (control level) to 15 dS m^{-1}, however Celebration, UGP3, Floratam, and Palmetto increased sodium root concentrations by greater than 100% when comparing controls (7119, 7566, 12721, and 10638 ppm, respectively) to 15 dS m^{-1} (14403, 15744, 25963, and 22497 ppm, respectively) treatments. DALZ1313 and UGP3 were somewhat unique in their responses to increased salinity, exhibiting higher root compared to shoot sodium concentrations (0.9:1 for both) at 15 dS m^{-1} (Table 31). This is an interesting observation, considering that these were two of the top performing entries in this and prior salinity screenings.

Table 30. Presence of sodium in plant tissue as influenced by cultivar, presented both in absolute terms (µg) as well on concentration (ppm) basis after 30 days of salinity stress at 2.5 dS m^{-1}(control level).

Cultivars	Absolute (µg)				Concentration (ppm)		
	Shoots	Roots	Total	Ratio	Shoots	Roots	Ratio
Celebration	27949	48715	76664	0.6:1	9727	7119	1.4:1
UGB79	19686	30102	49788	0.7:1	11249	6708	1.7:1
DALZ1313	22729	26642	49371	0.9:1	7234	5593	1.3:1
Zeon	17129	28941	46070	0.6:1	8250	6481	1.3:1
UGP3	40144	62421	102565	0.6:1	11134	7566	1.5:1
UGP38	35343	51423	86766	0.7:1	11650	6791	1.7:1
Floratam	41499	86561	128060	0.5:1	19842	12721	1.6:1
Palmetto	44776	70332	115108	0.6:1	22404	10638	2.1:1
LSD	19985	24816	88475		1774	1750	

In both studies and for most cultivars, Na contents in shoot and root tissues increased as salinity was increased, with a greater relative proportion was maintained in root compared to shoot tissues (Table 31). However, in study one, the difference between root and shoot Na content was more pronounced than that in study two, when environmental conditions such as solar radiation, photoperiod, and temperature were reduced (study one solar radiation of 166 wat m^{-2}, max, min, and mean air temperatures of 37, 27, and 31°C, respectively vs. study two solar radiation of 126 wat m^{-2}, max, min, and mean temperatures of 34, 26, and 29°C, respectively). Differences were also noted in percent biomass reductions, with greater reductions noted in study two vs. study one at the 15 dS m^{-1} salinity concentration (Table 25).

Table 31. Presence of sodium both in terms of absolute amounts (µg) as well as on concentration basis (ppm) in plant tissue as influenced by cultivar after 30 days of salinity stress at 15 dS m^{-1}.

	Absolute (µg)								Concentration (ppm)		
	Study 1				Study 2						
Cultivars	Shoot	Root	Total	Ratio	Shoot	Root	Total	Ratio	Shoot	Root	Ratio
Celebration	33951	99400	133351	0.3:1	76223	101913	178136	0.7:1	14743	14403	1:1
UGB79	18552	70068	88620	0.3:1	29631	40662	70293	0.7:1	13022	10550	1.2:1
DALZ1313	17264	43129	60393	0.4:1	55572	43623	99195	1.3:1	9909	10516	0.9:1
Zeon	42089	72666	114755	0.6:1	37808	67178	104986	0.6:1	15244	12695	1.2:1
UGP3	27530	111939	139469	0.2:1	84564	110860	195424	0.8:1	15291	15744	0.9:1
UGP38	38860	100402	139262	0.4:1	67606	103483	171089	0.7:1	16893	13035	1.3:1
Floratam	34937	151379	186316	0.2:1	73303	137897	211200	0.5:1	29404	25963	1.1:1
Palmetto	42807	255847	298654	0.2:1	50932	91636	142568	0.6:1	30283	22497	1.3:1
LSD	30975	67366	160240		32871	61306	148458		4763	3254	

Chloride accumulated mainly in the shoot tissue among all cultivars under 2.5 dS m^{-1} (control) salinity level, although UGB79, Floratam, and Palmetto had higher chloride content in root compared to shoot tissue with a ratio of 0.9:1, 0.6:1, and 0.6:1, respectively (Table 32). All cultivars exhibited higher shoot compared to root Cl when presented in terms of concentration basis, with ratios ranging from 2.1:1 to 4.3:1 under 2.5 dS m^{-1} (control) salinity level.

All cultivars increased Cl concentration in both shoot and root tissue when salinity was increased from 2.5 dS m^{-1} (control level) to 15 dS m^{-1}. Entries also maintained higher Cl concentrations in shoot compared to root tissue at elevated salinity, ratios ranging from 1.1:1 to 2.7:1 (Table 33). Celebration, UGP3, and UGP38 exhibited the highest difference in Cl concentration between shoots and roots at both salinity levels, with a ratio of 3.1:1, 3.5:1, and 4.3:1, respectively in 2.5 dS m^{-1}(control level) and a ratio and 1.2:1, 1.1:1, and 1.6:1, respectively in 15 dS m^{-1} (Tables 32 and 33).

In both studies, Cl content (absolute Cl) among cultivars in shoot and root tissue increased as salinity was increased, with a greater proportion of Cl detected in root tissue compared to shoot tissue (Table 33). However, in study one, at 15 dS m^{-1}, the difference between root and shoot Cl content was more pronounced than the difference between root and shoot Na content had been in study two, when biomass reduction was more severe (Table 25).

Table 32. Presence of chloride both in terms of absolute amounts (µg) as well as on concentration basis (ppm) in plant tissue as influenced by cultivar after 30 days of salinity stress in 2.5 dS m^{-1} (control level).

	Absolute (µg)				Concentration (ppm)		
Cultivars	Shoots	Roots	Total	Ratio	Shoots	Roots	Ratio
Celebration	30815	24594	55409	1.3:1	11443	3601	3.1:1
UGB79	8049	8865	16914	0.9:1	4436	1995	2.2:1
DALZ1313	27242	15649	42891	1.7:1	8640	3281	2.6:1
Zeon	22168	21417	43585	1.0:1	10449	4773	2.1:1
UGP3	41336	26044	67380	1.6:1	11197	3179	3.5:1
UGP38	40867	23122	63989	1.8:1	13123	3010	4.3:1
Floratam	25691	39741	65432	0.6:1	12439	5852	2.1:1
Palmetto	25978	42317	68295	0.6:1	13656	6437	2.1:1
LSD	12662	13212	52217		1567	1015	

Table 33. Presence of chloride both in terms of absolute amounts (µg) as well as on concentration basis (ppm) in plant tissue as influenced by cultivar after 30 days of salinity stress at 15 dS m^{-1}.

	Absolute (µg)								Concentration (ppm)		
	Study 1				Study 2						
Cultivars	Shoot	Root	Total	Ratio	Shoot	Root	Total	Ratio	Shoot	Root	Ratio
Celebration	33189	82596	115785	0.4:1	89990	110027	200017	0.8:1	15828	13585	1.2:1
UGB79	15202	61875	77077	0.2:1	28387	41697	70084	0.7:1	11408	9831	1.2:1
DALZ1313	19584	37149	56733	0.5:1	73697	47580	121277	1.5:1	12178	10164	1.2:1
Zeon	42128	64070	106198	0.7:1	46970	71966	118936	0.7:1	17210	12305	1.4:1
UGP3	23976	91130	115106	0.3:1	111133	116174	227307	1.0:1	16437	14451	1.1:1
UGP38	39067	76380	115447	0.5:1	76325	97038	173363	0.8:1	18019	11003	1.6:1
Floratam	30459	131761	162220	0.2:1	71790	163454	235244	0.4:1	27107	10164	2.7:1
Palmetto	37438	202570	240008	0.2:1	46268	140139	183407	0.3:1	26775	21362	1.3:1
LSD	28530	49842	139136		33268	69368	158047		4977	3426	

Potassium accumulated mainly in the shoot tissue with Celebration, DALZ1313, UGP3, and UGP38, which showed shoot:root ratios (in terms of absolute K) of 1.0:1, 1.3:1, 1.3:1, and 1.4:1, respectively, at 2.5 dS m^{-1} (control level) salinity (Table 34). All cultivars exhibited a greater K concentration in shoot compared to root tissues, with ratios ranging from 1.7:1 (Zeon) to 3.5:1 (UGP 38) under 2.5 dS m^{-1} (control) salinity level (Table 34).

Table 34. Presence of potassium both in terms of absolute amounts (µg) as well as on concentration basis (ppm) in plant tissue as influenced by cultivar after 30 days of salinity stress at 2.5 dS m^{-1} (control level).

	Absolute (µg)				Concentration (ppm)		
Cultivars	Shoots	Roots	Total	Ratio	Shoots	Roots	Ratio
Celebration	86652	82438	169090	1.0:1	30253	11725	2.6:1
UGB79	20976	24404	45380	0.9:1	11885	5525	2.2:1
DALZ1313	45964	34159	80123	1.3:1	14746	7188	2.1:1
Zeon	34630	43203	77833	0.8:1	16126	9649	1.7:1
UGP3	113734	88160	201894	1.3:1	31031	10681	2.9:1
UGP38	98779	69852	168631	1.4:1	32216	9233	3.5:1
Floratam	62334	92228	154562	0.7:1	29152	13543	2.2:1
Palmetto	47881	70278	118159	0.7:1	24822	10571	2.3:1
LSD	29188	31744	118159		2015	2080	

In study one, all cultivars decreased potassium content in both shoot and root tissue with increasing salinity from 2.5 dS m^{-1} (control level) to 15 dS m^{-1}. Also, six of the eight entries showed higher absolute K content in root compared to shoot tissue, with ratios ranging from 0.9:1 (DALZ1313) to 0.2:1 (Palmetto). The exception to this was Celebration and UGP38, which both exhibited similar potassium content between shoot and root tissue, with a ratio of 1.0:1 (Table 35)

In study two, at 15 dS m^{-1}salinity, all cultivars showed higher K content in shoot compared to root tissue, with ratios ranging from 1.1:1 to 2.7:1, not including Zeon which exhibited a ratio of 0.8:1 (Table 34). Celebration, UGB79, DALZ1313, UGP3, and UGP38 all increased K content in shoot compared to root tissue under increased salinity. Also, all cultivars decreased K concentration in both shoot and root tissues under increased salinity; with higher K concentrations detected in shoots compared to roots and final ratios ranging from 1.8:1 to 3.6:1 (Table 35).

Table 35. Presence of potassium both in terms of absolute amounts (µg) as well as on concentration basis (ppm) in plant tissue as influenced by cultivar after 30 days of salinity stress at 15 dS m^{-1}.

	Absolute (µg)								Concentration (ppm)		
	Study 1				Study 2						
Cultivars	Shoot	Root	Total	Ratio	Shoot	Root	Total	Ratio	Shoot	Root	Ratio
Celebration	65984	68170	134154	0.9:1	138961	75415	214376	1.8:1	27758	10220	2.7:1
UGB79	12122	21739	33861	0.6:1	25374	13031	38405	1.9:1	9875	3382	2.9:1
DALZ1313	20687	22757	43444	0.9:1	64051	23957	88008	2.7:1	11459	5616	2.0:1
Zeon	26856	33074	59930	0.8:1	27747	33313	61060	0.8:1	10613	5999	1.8:1
UGP3	54931	76070	131001	0.7:1	162456	75998	238454	2.1:1	28861	10729	2.7:1
UGP38	61678	59641	121319	1.0:1	114505	63655	178160	1.8:1	27811	7830	3.6:1
Floratam	19957	47877	67834	0.4:1	50373	44061	94434	1.1:1	18736	8219	2.3:1
Palmetto	21182	86449	107631	0.2:1	30668	15123	45791	2.0:1	16416	7676	2.1:1
LSD	27971	28902	95200		35912	21056	100041		3325	1381	

Sodium to Potassium (Na:K) Ratios

All cultivars showed lower Na:K ratios (on a concentration basis) in both shoot and root tissues at the control compared to the elevated 15 dS m^{-1} level (Tables 36 and 37). However, UGB79 and Palmetto (both the lesser salinity tolerant for their respective species) exhibited higher Na concentrations in root tissue under control levels, with ratios of 0.9:1 both respectively (Table 36). Celebration, UGP3, and UGP38 exhibited the lowest Na:K ratio in shoot tissue among cultivars, with ratios of 0.3:1, 0.4:1, and 0.4:1, respectively.

Table 36. Shoot and root Na:K concentration ratios under 2.5 dS m^{-1} (control level).

	Na			K				
	Concentration (ppm)			Concentration (ppm)			Ratio Na / K	
Cultivars	Shoot	Root	Ratio	Shoot	Root	Ratio	Shoot	Root
Celebration	9727	7119	1.4:1	30253	11725	2.6:1	0.3:1	0.6:1
UGB79	11249	6708	1.7:1	11885	5525	2.2:1	0.9:1	1.2:1
DALZ1313	7234	5593	1.3:1	14746	7188	2.1:1	0.5:1	0.8:1
Zeon	8250	6481	1.3:1	16126	9649	1.7:1	0.5:1	0.7:1
UGP3	11134	7566	1.5:1	31031	10681	2.9:1	0.4:1	0.7:1
UGP38	11650	6791	1.7:1	32216	9233	3.5:1	0.4:1	0.7:1
Floratam	19842	12721	1.6:1	29152	13543	2.2:1	0.7:1	0.9:1
Palmetto	22404	10638	2.1:1	24822	10571	2.3:1	0.9:1	1.0:1
LSD	1774	1750		2015	2080			

Increased Na:K concentration ratios were detected in both shoot and root tissue with increased salinity concentration (Table 37). Nevertheless, Celebration, DALZ1313, UGP3, and UGP38 each maintained lower Na:K concentration ratios in shoot tissue among all cultivars at the increased salinity concentration (Table 37). These same cultivars also maintained even lower Na:K in root tissues, suggesting selective uptake for K may be occurring at the roots, but also additional regulation is occurring during cellular transport of elements from roots to shoots.

Table 37. Shoot and root Na:K concentration ratios under 15 dS m^{-1}.

	Na			K				
	Concentration (ppm)			Concentration (ppm)			Ratio Na / K	
Cultivar	Shoot	Root	Ratio	Shoot	Root	Ratio	Shoot	Root
Celebration	14743	14403	1.0:1	27758	10220	2.7:1	0.5:1	1.4:1
UGB79	13022	10550	1.2:1	9875	3382	2.9:1	1.3:1	3.1:1
DALZ1313	9909	10516	0.9:1	11459	5616	2.0:1	0.9:1	1.9:1
Zeon	15244	12695	1.2:1	10613	5999	1.8:1	1.4:1	2.1:1
UGP3	15291	15744	1.0:1	28861	10729	2.7:1	0.5:1	1.5:1
UGP38	16893	13035	1.3:1	27811	7830	3.6:1	0.6:1	1.7:1
Floratam	29404	25963	1.1:1	18736	8219	2.3:1	1.6:1	3.2:1
Palmetto	30283	22497	1.3:1	16416	7676	2.1:1	1.8:1	2.9:1
LSD	4763	3254		3325	1381			

DISCUSSIONS

Leaf salt excretion rates were correlated with salinity tolerance parameters such as turf quality, % green cover, and biomass production. These findings are consistent with those of previous reports (Marcum et al., 1998). The development of glandular structures which are specialized mechanisms aiding in salinity tolerance in zoysiagrass and bermudagrass species also led to higher levels of detected ion excretion in these entries during this study. This demonstrates that leaf salt excretion rate efficiency is an important process of ion regulation which contributes to salinity tolerance (Naz et al., 2009). Based on our ion excretion data, DALZ1313, Zeon, and Celebration exhibited increased ion excretion rates when salinity concentration was increased (Table 21). DALZ1313 exhibited the highest ion excretion at 15 dS m^{-1} salinity concentration. Na excretion through salt glands in warm-season turfgrasses has been reported by Marcum (1998), Marcum and Pessarakli (2006), and Naz (2009).

Salt excretion intensity and salt glad density were also correlated, when comparing this with earlier work with the same entries. As such, as salinity concentration increased, DALZ1313 exhibited salt excretion rate three times higher than that of Celebration (Table 21). DALZ1313 had in previous work, also shown 1.4 times higher salt gland density under 30 dS m^{-1} compared to 2.5 dS m^{-1} salinity (Figure 3). However, Seashore paspalum did not exhibit ion excretion activity under increasing salinity concentrations, also consistent with earlier reports (Chen et al., 2009; Marcum and Murdoch, 1994). This experiment confirmed that seashore paspalum and St Augustinegrass species lack the ability to excrete ions (Table 21). Bermudagrass and

zoysiagrass have been studied and classified into the sub-family Chloridoideae (Gould, 1983). Although seashore paspalum's ion excretion mechanism is still not well understood, some specialists have classified it into the recretohalophyte group or into the sub-family Panicoideae, which have salt glands or bladders (Liphschitz, 1982: Kefu, 2002). However, Marcum (1994) has reported that seashore paspalum species do not excrete ions under salinity stress. Thus, we propose that although seashore paspalum species do not have detectable salt glands, they do appear to possess bladders on their adaxial leaf surfaces, in which salt ions may be sequestered and which may eventually be removed either through senescence or mowing removal of leaf tissue.

Elevated shoot growth under salinity stress is another indicator of salinity tolerance. However, in study one, turf quality and % green cover data did not relate well to biomass production (Tables 22, 23, and 24, respectively) data under 15 and 30 dS m^{-1} salinity concentration. Overall, the entries reduced turf quality and % green cover, however the cultivars increased biomass production under both saline treatments, not including St Augustinegrass species (Table 24). Nevertheless, Celebration, UGP3, and UGP38 were the top performing entries among cultivars, maintaining positive biomass production, good turf quality, and high % green cover, which are important attributes for survival under salinity. O' Leary (1995) reported that halophytes were capable of increasing biomass production under saline conditions due to their ability to either tolerate osmotic stress, excrete Na throught salt glands, and/or compartmentalize sodium and chloride within cells.

However, in study two, biomass reduction was more evident and it did not increase among all cultivars under both salinity treatments compared to study one. According to the weather station data, solar radiation and day-length (photoperiod) were each reduced during study 2, which is not surprising given that study one was conducted during the summer and study two was conducted in early fall (Table 19). Warm-season turfgrasses produce optimal biomass with high levels of solar radiation and day lengths exceeding 13 hours per day, and when these conditions are not met, biomass production is reduced through development of narrow elongated leaves, thin upright stems, elongated internodes, and weak rhizomes and stolons (McCarty, 2011). In our study, shoot biomass production, turf quality, and % green cover were closely associated with salinity tolerance of the cultivars tested (Table 22, 23, and 24). Salinity stress combined with lower amounts of full sunlight may have combined to produce greater impacts on photosynthetic efficiency in study two. Biomass production in our study could have been affected by photosynthesis responses to multiple stresses such as increased sodium and chloride concentration, reduced potassium concentration in leaf tissue, and reduced full sunlight which make interpreting the data somewhat complex. Photosynthesis can be affected by reducing CO_2 availability and reduction of leaf turgor pressure, both of which are directly affected by stomata closure occurring under salinity stress and reduced sun-light (Chaves et al., 2009).

Under salinity stress, particularly increased concentration of sodium and chloride, mineral nutrition of plants is affected. Elevated Na effects may result in biomass reduction, leaf damage, and an increased sodium concentration in shoot and

root tissue (Blumwald et al., 2000). Salinity stress alters Na^+/K^+ ratio in shoot and root tissue due to the influx of sodium through pathways which also function in the uptake of K. However, increased biomass production under salinity stress might explained by several physiological mechanisms including exclusion at the root level, excretion of toxic ions via salt glands, and enhanced maintenance of K:Na ratios in shoot tissue (Jouyban, 2012b).

Increased sodium and chloride concentration and reduced potassium concentration in leaf tissue with increasing salinity level in some cultivars was evident in both studies. However, plant growth was stimulated in study one and plant growth was reduced in study two under the same salinity levels. The entries expressed different mechanisms for tolerating and avoiding salinity stress. Cultivars such as UGB79, Zeon, Floratam, and Palmetto, which were predominantly the lesser salinity tolerant based on prior testing) accumulated high Na^+ to K^+ ratios in shoot and root tissue under 15 dS m^{-1} in both studies. Despite this, their biomass production was stimulated in study one and their turf quality and percentage green color were on the verge of minimally acceptable. Thus, these aforementioned entries showed limited salinity tolerance at 15 dS m^{-1} salinity concentration. St Augustinegrass species had the highest Na:K ratio in shoot and root tissues, which indicates limited root exclusion and/or transport regulation of Na from root to shoots where Na accumulated to relatively high levels.

Higher shoot:root ratios of K (based on concentration) were present under the control treatment, and root K concentration decreased in almost all cultivars with increasing salinity concentration (not including UGP3 which increased potassium

concentration in root tissue). While Na can substitute for K in some processes, K deficiency can lead to shoot biomass reductions since it is an essential element for maintaining cell turgor pressure and some enzyme activities (Jouyban, 2012b). However, Celebration, DALZ1313, UGP3, and UGP38 showed the highest K uptake over Na in shoot tissue, and the lower Na:K ratios in root tissue. Taken together, this suggests that salinity tolerance in Celebration and DALZ1313 may be linked to greater ion selectivity at the roots and/or toxic ion excretion via salt glands, while for UGP3 and UGP38, ion selectivity at the roots and preference for K during intercellular transport may be significant mechanisms contributing to salinity tolerance. These results are consistent with those of Chen (2009), Marcum (1990), and Marcum (1994). High K:Na ratio in plant tissue appears to be highly correlated with salinity tolerance in turfgrass and plant species (Lee et al., 2007)

CONCLUSIONS

Turfgrass response to salinity stress is because a complex phenomenon involving several physiological mechanisms such as ion secretion via salt glands, salt gland density, distribution of Na and Cl in the plant, and regulation of uptake and transport of toxic ions via aquaporins in the root system. The turfgrass entries selected for inclusion this study had previously expressed contrasting tolerance to salinity, and again demonstrated a variation in responses to increased salinity in terms of shoot and root production, turf quality, and percent green cover, between salinity treatments and between and within species. Salinity tolerance in this study was determined primarily on

the basis of turf quality and electrical conductivity at which 50 percent shoot biomass reductions occurred. These ranged from 15 dS m^{-1} (UGB79, Floratam, and Palmetto) to ~30 dS m^{-1} (Celebration and Zeon). Celebration bermudagrass and seashore paspalum cultivars UGP3 and UGP38 maintained the highest shoot growth rates suggesting they have better mechanisms for tolerating salinity stress. Salinity tolerance within bermudagrass and zoysiagrass cultivars was also also directly related to excretion of salt ions via salt glands. According to our results, sodium concentration increased in shoot tissue with increased salinity level. Differences in Na distribution between salt-sensitive and salt-tolerant entries also played a clear role in conferring salinity tolerance.

Physiological mechanisms, which might include K^+ selection and translocation affinity over Na^+, may reduce sodium uptake via root system. Turfgrass cultivars which had shown the least salinity tolerance in prior studies acquired both Na and Cl, translocating it from roots to leaves, where toxic ions were accumulated, leading to poor turf quality. However, cultivars which had shown the higher salinity tolerance employed mechanisms to maintain lower Na and Cl, relative to K concentrations.

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CHAPTER V

SUMMARY

In these series of experiments, salinity tolerance of 45 genotypes of warm-season turfgrass species bermudagrass (*C. dactylon* sp), zoysiagrass (*Z. matrella* sp and *Z japonica* sp), St. Augustinegrass (*Stenotraphrum secundatum*), and Seashore paspalum (*Paspalum vaginatum*) were examined in through physiological and anatomical investigations at salinity levels ranging from 2.5 to 45 dS m^{-1}. Based on our results, salinity tolerances of these four species studied were as follows: Seashore paspalum sp, bermudagrass sp, zoysiagrass sp, and St. Augustinegrass sp.

Based on initial screening studies conducted in 2015, the top-performing entry of each species was examined following exposure to two levels of salinity (0 and 30 dS m^{-1}) using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) to elucidate morphological/ anatomical attributes related to salinity tolerance mechanisms. In this study, salt sequestration and excretion mechanisms on leaf surfaces were studied and differences were found that appear to contribute to observed differences in salt tolerance of these species. When comparing these four top performing entries (Celebration, UGP3, DALZ1313, and Floratam), it became apparent that DALZ1313, not only had a higher adaxial salt gland density than Celebration, but also showed a further increase in salt gland density following exposure to elevated salinity.

In order to more accurately determine whether these species also exhibited Na secretion or other morphological differences, the leaf surfaces were then examined with

EDS. Salt gland structures of Celebration and DALZ1313 were somewhat similar, and few differences were observed between these species. The results obtained from this study could also be used for future studies aimed at turfgrass improvement. Celebration and DALZ1313 each showed an induction in salt gland density in response to salt, and therefore may have utility as potential lines for isolation of candidate genes involved in induction of salt glands. These genes might be transformed into other commercial genotypes of *Zoysia* that are tolerant to other abiotic stresses, making them well better adapted for growth and maintenance under conditions involving low quality water.

In 2016, eight entries (2 entries per species representing the highest and lowest-performing lines relative salinity tolerance) were advanced for further evaluation aimed at determining physiological responses to salinity. Grasses were grown in the greenhouse over 10 weeks at salinity levels of 0, 15, and 30 dS m^{-1}. Ion excretion efficiency, Na and Cl concentrations, and root and shoot tissue Na:K were evaluated to determine relationships with previously observed differences in salinity tolerance/intolerance. Collectively, the findings from this study support the observation that salinity tolerant genotypes employ one or more physiological mechanisms including salt excretion, root exclusion, limitation of Na and/or Cl transport to shoots, and maintenance of ion balance in coping with saline conditions. Salinity tolerant turfgrasses genotypes identified through our research were capable of maintaining normal metabolism and continued growth. Conversely, those genotypes lacking salinity tolerance eventually declined in quality and died due to inability to maintain favorable ion balance and/or osmotic potential.

Increasing salinity concentration allowed for detection of sensitive and tolerant plants. Seashore paspalum sp, bermudagrass sp, and zoysiagrass sp generally performed well when irrigated with saline water from 10 to 30 dS m^{-1} under controlled conditions. Seashore paspalum maintained acceptable turf quality even as salinity approached 30 to 45 dS m^{-1} levels. The overall implication is that a complex assortment of physiological mechanisms was found to contribute to salinity tolerance in the warm-season turfgrasses used in this study. These included salt gland development and ion secretion, maintenance of ion selectivity ion balance in terms of Na:K ratio and concentrations in shoot and root tissue, as well osmotic adjustment and deep root systems.

These results will be useful both from a practical perspective in selecting the most salinity tolerant turf species for salt-affected sites, but also in terms of the contributions it provides in terms of basic knowledge that will allow physiologists and breeders to make marked improvements toward enhanced salinity tolerance traits in the turfgrasses of the future.

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