SALINITY TOLERANCE OF TALL FESCUE (*FESTUCA ARUNDINACEA* SCHREB.)

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Yang Gao

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Gao, Yang, M.S.; Department of Plant Sciences, College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, April 2011. Salinity Tolerance of Tall Fescue (*Festuca arundinacea* Schreb.). Major Professor: Dr. Deying Li.

Tall fescue (*Festuca arundinacea* Schreb.) is one of the commonly used cool-season turfgrass species. Despite the many advantages of tall fescue, there are some properties that restrict its use as turfgrass. Recent efforts on selection of turf-type tall fescue focus on fine leaf texture, darker green color, seedling turgor, overall density, tolerance to extreme temperatures, and other stresses. The objectives of this research were to develop techniques in screening salinity tolerant tall fescue and to investigate the leaf senescence process, growth and nutrient uptake of tall fescue under salinity stress induced by different types of salt. Two tall fescue cultivars, Tar Heel II (salt tolerant) and Wolfpack (salt sensitive) were grown in silica sand as growth medium and fertilized with Hoagland solution. Salt treatments were added to the medium along with Hoagland solution. At least three indices calculated based on single leaf spectrum showed promise as sensitive means to differentiate salinity stress from untreated plants. Those indices also are strongly correlated to many physiological parameters that have been shown to be reliable measures of salinity tolerance in many plant species including tall fescue. Tall fescue leaf senescence was accelerated by salinity stress which was different from the aging process under shade. Tall fescue had several adaptation strategies in order to conserve water under salinity stress, while the plants under shade had adaptation mechanisms centered on light harvesting. Tall fescue accelerated old leaf senescence, whereas under shade conditions, tall fescue showed slowing down in the new leaf development as well as mature leaf aging. Tall fescue growth responded to salinity stress differently from shade stress, a fact that has to be taken into consideration when selecting for stress tolerant traits, such as leaf length, root to shoot ratio. Leaf appearance rate may be different with stress tolerance levels and mediate the tiller number and shoot density. By comparing and contrasting the effects on nutrients uptake, it was shown that K, Ca, Mg may be very important in the ion balance and salinity stress tolerance. Tall fescue leaf firing was mainly caused by an alkaline condition with pH higher than 9 as in Na_2CO_3, or moderate pH combined with high salinity (high EC) as in $CaCl_2$. Plant growth was more affected by sodicity which was high in Na_2CO_3 and Na_2SO_4. Osmotic adjustment also played an important role in tall fescue salinity stress in chlorides. Chloride effects were closely related to RWC of the leaves. The combination of different salts along with the variation of their physical and chemical properties, such as EC, pH, and osmotic potential, made the differentiation of their influence on tall fescue stress rather difficult. Multiple properties, such as growth, morphological, and physiological, should be measured to better understand the effects of different salts.

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GENERAL INTRODUCTION

Tall fescue (*Festuca arundinacea* Schreb.) is one of the commonly used cool-season turfgrass species. It was introduced to the United States as a forage species and has been used for soil conservation because of its deep root system and rapid growth characteristics (Murray and Powell, 1979). Tall fescue has a relatively recent use as a turfgrass through improvement efforts started in 1962 (Samples et al., 2009). In the past 40 years, much has changed that it is now widely accepted as a desirable turfgrass, especially under unfavorable conditions because of its tolerance to wear, drought, high temperature, shade, and salinity. By the 1980s, it was used for home lawns, play grounds, roughs and fairways on golf courses, as well as roadsides in 42 of 50 states of the United States (Murray and Powell, 1979).

Tall fescue has a wide variation of phenotypes and genotypes. Breeders classify tall fescue into forage and turf types based on functions (Hopkins et al., 2009). Despite the many advantages of tall fescue, there are some properties that restrict its use as turfgrass. Recent efforts on selection of turf-type tall fescue focus on fine leaf texture, darker green color, seedling turgor, overall density, tolerance to extreme temperatures, and disease (rust, brown patch) resistance (Hopkins et al., 2009; Samples et al., 2009).

In addition to elite cultivars, turf quality of tall fescue is affected by cultural practices which rely on the understanding of its physiological responses to adverse environmental conditions (Carrow and Duncan, 2003). One of the challenges in tall fescue management is irrigation with recycled water in an existing stressful environment. Previous research on salinity tolerance of tall fescue focused on quality or yield reduction when irrigated with saline water (Leskys et al., 1999; McKenzie et al., 1994). Limited research has been conducted to understand the physiological responses of tall fescue to salinity.

Turfgrass breeders will benefit from a better understanding of tall fescue's adaptations to stress and effective selection tools for screening large amounts of germplasm. Turfgrass managers can also adjust their management programs to improve tall fescue tolerance to stresses and reduce input and environmental effects. The objectives of this research were 1) to evaluate traditional physiological parameters and remote sensing techniques in detecting salinity stress in tall fescue, 2) to investigate the leaf senescence process induced by salinity as compared to natural aging and accelerated senescence caused by shade in order to understand contrasting adaptive strategies, 3) to study the growth and nutrient uptake of tall fescue under salinity stress, and 4) to compare the physiological responses of tall fescue to different salt types.

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CHAPTER 1. LITERATURE REVIEW

Water Shortage and Salinity Problems in Turfgrass Management

Turfgrass is an indispensable part of our life because of its functional, recreational, and ornamental benefits. However, turfgrass consumes large amounts of water and mismanagement of chemical applications on turfgrass may result in environmental pollution. Milesi et al. (2005) estimated that lawn coverage in the continental United States was about 20.2 million hectares. The total turf area is expected to further increase with rapid urbanization in America. In arid and semiarid regions, irrigation of turfgrass can sometimes account for 75% of the total household water consumption (Mayer et al., 1999). Golf course water bills as much as $1 million per year are quite common (Dean et al., 1996).

The increasing water shortage has forced turfgrass managers to look into alternative water sources. Lower prices and a consistent supply make recycled waters very practical alternatives for irrigation. Recycled water, such as reclaimed municipal effluent and brackish drainage water, may provide some essential nutrients to turfgrass, and typically contains more than 1000 mg L^{-1} salts (Miyamoto and Chacon, 2006). However, the undesirable chemicals contained in recycled waters may adversely affect turf quality. Salinity problems in turf also can happen in salt-affected soils, areas with salt water intrusion (McCarty and Dudeck, 1993; Murdoch, 1987), and roadsides where salts are used for snow and ice removal (Greub et al., 1985). One of the solutions to salinity problems in turfgrass is to use salt tolerant species.

Tall fescue is a cool-season grass with good wear and heat tolerance, and can grow in soils with a pH range of 4.7 to 9.5 (Barnes, 1990). Tall fescue has better tolerance of alkalinity and salinity than many other cool-season turfgrasses (Beard, 1973; Marcum, 2006; Wu et al., 1988). The species has been widely used as turfgrass in 42 out of 50 states of the U.S. (Murray and Powell, 1979). Although seed germination was not affected by moderate salinity (Johnson, et al., 2007), salt crust inhibited tall fescue seedling penetration (Frelich et al., 1973). Horst and Beadle (1984) reported large variations of germination rates and seedling performance within 16 tall fescue cultivars evaluated at 7 500 to 15 000 mg L^{-1} of NaCl and $CaCl_2$. Wipff and Rose-Fricker (2003) screened 59 commercial and experimental cultivars at the mature stage using 17 000 and 24 000 mg L^{-1} of NaCl, and reported that 'Tar Heel II' and 'Pure Gold' had the highest survival rate and Wolfpack had the lowest survival rate. Previous research on salt tolerance focused on quality or yield reduction when irrigated with saline water (Leskys et al., 1999; McKenzie et al., 1994). Limited research has been conducted to understand the physiological responses of tall fescue to salinity.

Salinity Tolerance in Turfgrass

Salt tolerance can be evaluated at morphological, anatomical, and physiological levels. Salt glands are reported in over 30 species within the tribes Chlorideae, Eragrosteae, Aeluropodeae, and Pappophoreae (Liphshchitz and Waisel, 1974; Taleisnik and Anton, 1988; Amarasinghe and Watson, 1989), and all members in the subfamily Chloridoideae (Gould and Shaw, 1983) of the grass family. Examples of chloridoid species are *Distichlis* spp., *Cynodon* spp., *Zoysia* spp., *Buchloe* spp., *Bouteloua* spp., and *Sporobolus* spp.. Most of the reported research focused on plant growth, such as shoot weight (Chen et al., 2009; Leskys et al., 1999; Zhao et al., 2007), shoot weight reduction (Dudeck and Peacock, 1985a and 1985b), root weight or length (Kik, 1989; Wu et al., 1988; Zhao et al., 2007), shoot/leaf length (Horst and Beadle, 1984), visual shoot injury (Greub et al., 1985; Dean et al., 1996; Dean-Knox et al., 1998), plant survival (Ahti et al., 1980), and seed germination (Marcar, 1987; Johnson et al., 2007). Even when using the same method, results often are difficult to compare because of the interactions among genotypes and edaphic conditions. For example, the salinity concentration to reduce shoot weight by 50% in 'Tifway' bermudagrass (*Cynodon dactylon* L. × *C. transvaalensis* L.) has been reported as 33 dS m^{-1} (Dudeck and Peacock, 1985d), 27 dS m^{-1} (Marcum and Murdoch, 1994), 18.6 dS m^{-1} (Dudeck et al., 1983), and 12 dS m^{-1} (Smith et al., 1993). Salt tolerance also is different at different stages of growth and development (Hughes et al., 1975; Rose-Fricker and Wipff, 2001).

Osmotic adjustment to avoid physiological drought has been considered as one of the strategies of salt tolerance in plants (Bernstein and Hayward, 1958). Currently, saline ion exclusion from shoots, along with minimal yet adequate osmotic adjustment, is considered to play a central role in salt tolerance in many plant species (Hu et al., 1998; Fricke and Peters, 2002). Saline ion exclusion has been reported in many species (Akita and Cabuslay, 1990; Khan and Marshall, 1981; Rogers et al., 1997) and confirmed in tall fescue by transforming a vacuolar Na^+/H^+ antiporter gene from *Arabidopsis thaliana* L. (Zhao et al., 2007).

Effects of Salinity Stress on Turfgrass Growth

The detrimental effects of salinity can be attributed to toxicity of excessive Na^+, Cl^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-, and BO_3^-, as well as nutrient imbalance and deficiency (Bowman et al., 2006). High levels of salinity disrupt ion homeostasis in plants by inhibiting the uptake of essential nutrients like K^+, Ca^{2+}, and NO_3^- and excessive accumulation of Na^+ and Cl^-

(Lacerda et al., 2003; Marschner, 1995; Zhu, 2001). As a consequence, cell division and elongation decline or stop completely (Hasegawa et al., 2000). Salinity reduced the uptake and partitioning of N to leaves and increased the retention of N in the roots of tall fescue (Bowman et al., 2006). A study on physiological responses to salinity shock in tall fescue, Kentucky bluegrass (*Poa pratensis* L.), and perennial ryegrass (*Lolium perenne* L.) revealed that one of the visible symptoms was accelerated leaf senescence (Wang et al., 2009).

Leaf senescence has been commonly defined as a series of degenerative changes that lead to death (Nooden, 1980), which is a complex succession of physiological and molecular events, and usually involves three stages: initiation, degeneration, and termination (Yoshida, 2003). Leaf senescence is considered to be age-dependent, but also can be induced by shading, pathogens, and environmental stresses (salt stress, water, temperature). Information on leaf senescence comes from different conditions of the leaves: intact, freshly detached from the plant, and decomposing deciduous leaves. Molecular biology is at the forefront of senescence research, usually at the gene, sub cellular and cellular levels (Chen et al., 2008; Godiard et al., 1998; Hayden and Christopher, 2004). All plant hormones reportedly are involved in the senescence process (Abreu and Munne-Bosch, 2008; Carimi et al., 2004; Dugardeyn and Straeten, 2008; Ella et al., 2003; Hogan et al., 2006; Hung and Kao, 2003; Rakwal and Komatsu, 2001; Yu et al., 2008). The levels of many reactive oxygen species are increased at certain stages of senescence (Niewiadomska et al., 2009; Orendi et al., 2001). Ion detoxification (Murgia et al., 2007), pH adjustment (Bao et al., 2009; Berkowitz and Gibbs, 1983), and water conductivity (Azaizeh and Steudle, 1991) have been studied in the process of salt-induced senescence. In general, the leaf senescence process includes disassembling of chloroplasts and other organelles (Benjamin et al., 1999) and hydroxylation of chlorophyll, proteins, lipids, nucleic acids, and carbohydrates (Aliferis et al., 2006; Nooden et al., 1997; Quirino et al., 2000; Rajcan et al., 1999; Roca et al., 2004; Stirpe et al., 1996).

The nutrients released by hydrolysis in senescing tissues are often transported to growing and storage tissues (Nooden et al., 1997; Quirino et al., 2000). This recycling process is selective; elements such as Cl and Na are retained in the dead leaves, while P and K are mobilized (Lin and Wang, 2001). Nutrient mobilization under salinity stress therefore is considered as an adaptation mechanism (Hasegawa et al., 2000). Essentially, the whole process of senescence and death of leaves caused by salinity stress may be an avoidance mechanism that minimizes water loss (Herralde et al., 1998). Nutrient mobilization during senescence was reported in *Arabidopsis* (Himelblau and Amasino, 2001), barley (*Hordeum vulgare* L.) (Wiedemuth et al., 2005), and French bean (*Phaseolus vulgaris* L.) (Yordanov et al., 2008).

Reflectance Spectrum for Stress Detection

Remote sensing technology with visible or infrared spectroscopy has emerged as an effective methodology to detect plant responses to biotic and abiotic stress, and nutrient sufficient levels (Adams et al., 1999; Pinter et al., 2003). Crop spectral reflectance in the range 400-2500 nm has been found to be closely related to plant characteristics (Leone et al., 2007), such as leaf water content (Carter, 1991), leaf water potential (Cohen, 1991; Naumann et al., 2008), and chlorophyll content (Ciganda et al., 2009). Significant correlation also was found between spectral properties and plant biomass, and leaf area index in eggplant (*Solanum melongena* L.) (Leone et al., 2007) and seashore paspalum

(*Paspalum vaginatum* Swartz) (Lee et al., 2004) grown in saline soils. Normalized vegetation index (NDVI) and reflectance ratio were reported to be strongly correlated with photosynthetic capacity and stomatal conductance (Carter, 1998). Red-edge position, which was defined as the point of maximum slope on the reflectance spectrum of a plant leaf between red and near-infrared wavelengths (Baranoski and Rokne, 2005) was strongly correlated with chlorophyll content (Buschman and Nagel, 1993).

Many indices have been developed from the reflectance spectrum to detect plant response to different stresses. Some of them are first derivatives from the spectrum, such as simple ratio vegetation index (SR), NDVI, stress index (SI), and water index (WI). Others are second derivatives, such as yellow index (YI) (Adams et al., 1999). Different wavelengths have been used in construction of those indices and in certain cases modifications have been made by introducing a new wave length in the algorithm (Sims et al., 2002). A thorough review can be found in Volterrani et al. (2005) and in Jiang and Carrow (2007).

Salinity Stress in Turfgrass Induced by Different Salt Ions

It has long been reported that sulfate salinity and chloride salinity are different (Hanson-Porath and Poljakoff-Mayber, 1970; Meiri et al., 1971). Chloride salt suppressed P uptake and shoot and root growth more than sulfate salt in a study with wheat (*Triticum aestivum* L.) (Zahoor et al., 2007). With salinity levels at the same electrolyte concentration, sulfate had less reduction impact than chloride on the yield of wheat and rice (*Oryza sativa* L.) (Minhas et al., 2007). Research on French beans (Bhivare and Nimbalkar, 1984) and corn (*Zea mays* L.) (Kostandi and Soliman, 1998) also showed that sulfate was less detrimental to yield. Colmer et al. (1996) reported that external sulfate concentration ranging from 0.1 to 100 mol m^{-3} had no effect on the leaf glycine betaine, proline, or asparagine in *Spartina alterniflora* Loisel. In a trial with tomato (*Lycopersicon esculentum* Mill.), K_2SO_4 corrected salinity problems and K deficiency caused by treatment with 60 m*M* NaCl (Kaya et al., 2002).

In an iso-osmotic study with pepper (*Capsicum annuum* L.), Navarro et al. (2003) found that sulfate salt resulted in more growth reduction than chloride salt due to the higher concentration of sodium and less osmotic adjustment in sulfate. However, the same author reported more yield and quality reduction from sulfate than chloride (Navarro et al., 2002). Sulfate was reported more detrimental to barley yield than chloride (Datta et al., 1994, 1995). Germination of sunflower (*Helianthus annuus* L.) seeds was inhibited by carbonate, followed by sulfate and chloride when the seeds were treated with 10, 20, and 50 m*M* NaCl and Na_2SO_4, and 5, 10, and 15 m*M* Na_2CO_3 (Manivannan et al., 2008). In a study with pea (*Pisum sativum* L.), Na_2CO_3 caused the most reduction in osmotic pressure of leaves, followed by Na_2SO_4 and NaCl, while the shoot and root dry weight reduction was greater with NaCl, followed by Na_2CO_3 and Na_2SO_4 (Hamdia and Shaddad, 1996).

Clearly, there is a disagreement on both the research results and the hypotheses accounting for the responses of plants to different salts. Elucidating the underlying mechanisms of physiological responses to different salts in tall fescue is important for turfgrass management and also may cast light on the understanding of salinity stress in plants.

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CHAPTER 2. DETECTING PHYSIOLOGICAL RESPONSES OF TALL FESCUE TO SALINITY STRESS BASED ON SINGLE LEAF SPECTRUM

Abstract

Salinity problems are becoming more severe in turfgrass management with the increasing use of recycled water for irrigation. Recycled waters, such as reclaimed municipal effluent and brackish drainage water, usually have high levels of salts, which can cause physiological stress to turfgrass and deteriorate soil structure. Tall fescue (*Festuca arundinacea* Schreb.) is a cool-season turfgrass with moderate to high tolerance to salinity. The objectives of this study were to develop a practical methodology for screening salt-tolerant turfgrass plants from a large population in the breeding process. Two tall fescue cultivars, Tar Heel II (salt tolerant) and Wolfpack (salt sensitive) were seeded in plastic tubes filled with washed silica sand and were fertilized with half-strength Hoagland solution until the 4-leaf stage. Salinity treatments were applied by adding NaCl and $CaCl_2$ (1:1 w/w) to the Hoagland solution in 16 000 mg L^{-1} with an electrical conductivity (EC) of approximately 25 dS m^{-1} at the 4-leaf stage. A control was included with plants receiving Hoagland solution only. This study showed that salinity stress in tall fescue may be reflected by parameters at different levels of plant physiological processes. At least three indices calculated based on single leaf spectrum showed promise as sensitive means to differentiate salinity stress from untreated plants. Those indices also are strongly correlated to many physiological parameters that have been shown to be reliable measures of salinity tolerance in many plant species including tall fescue.

Introduction

Salinity problems are becoming more severe in turfgrass management with the increasing use of recycled water for irrigation. Recycled waters, such as reclaimed municipal effluent and brackish drainage water, usually have high levels of salts, which can cause physiological stress to turfgrass and deteriorate soil structure (Carrow and Duncan, 1998). One of the solutions to this problem is to use salt-tolerant species or cultivars. Tall fescue (*Festuca arundinacea* Schreb.) is a cool-season turfgrass with moderate to high tolerance to salinity (Beard, 1973; Marcum, 2006; Wu et al., 1988). It also has a wide range of adaptation to heat, drought, and soil pH, and therefore a great potential to be used in salt affected soils (Barnes, 1990).

The adverse effects of salinity on turfgrass health are attributed to toxicity of excessive ions, nutrient imbalance and deficiency, as well as physiological drought (Bowman et al., 2006; Lacerda et al., 2003; Marschner, 1995; Zhu, 2001). Physiological responses of tall fescue to salinity stress have been the focus of many previous studies. As a result, great progress has been made toward a better understanding of the mechanism of salinity tolerance. Currently, saline ion exclusion from shoots, along with minimal yet adequate osmotic adjustment, is considered to play a central role in salt tolerance of many plant species (Hu et al., 1998; Fricke and Peters, 2002) and was confirmed in tall fescue by transforming a vacuolar Na^+/H^+ antiporter gene from *Arabidopsis thaliana* L. (Zhao et al., 2007). Scavenging of reactive oxygen species (Niewiadomska et al., 2009; Orendi et al., 2001), ion detoxification (Murgia et al., 2007), and pH adjustment (Bao et al., 2009; Berkowitz and Gibbs, 1983) have all been considered contributing to the mechanism of salt tolerance. Many physiological parameters have been used as indicators of stress levels due to heat and drought in tall fescue, such as net photosynthesis rate, transpiration rate (Tr), stomatal conductance (g_s), relative water content (RWC), photochemical efficiency (F_v/F_m), leaf appearance rate, specific leaf area, relative growth rate, biomass and water use efficiency (Huang and Gao, 1999; Swarthout et al., 2009; Wang et al., 2009). Similar parameters should be applicable to salinity stress in tall fescue (Dean-Knox et al., 1998). However, most of the methods are time consuming and require destructive sampling. Quick and simple screening methods can help turfgrass breeders evaluate large amounts of plants in a more timely and economic manner, which yet needs to be developed.

Remote sensing technology with visible or infrared spectroscopy has emerged as an effective methodology to detect plant responses to biotic and abiotic stress, and nutrient sufficient levels (Adams et al., 1999; Pinter et al., 2003). Many vegetation indices have been developed using leaf reflectance spectrum. Reflectance spectrum has been found indicative of leaf water content (Carter, 1991) or leaf water potential (Cohen, 1991; Naumann et al., 2008), and chlorophyll content (Ciganda et al., 2009). A significant correlation was found between spectral properties and plant biomass, leaf area index in eggplant (*Solanum melongena* L.) (Leone et al., 2007) and seashore paspalum (*Paspalum vaginatum* Swartz) (Lee et al., 2004) grown in saline soils. Normalized difference vegetation index (NDVI) and reflectance simple ratio vegetation index (SR) also were correlated strongly with photosynthetic capacity and stomatal conductance (Carter, 1998). Red-edge position, which is defined as the point of maximum slope on the reflectance spectrum of a plant leaf between red and near-infrared wavelengths (Baranoski and Rokne, 2005) was strongly correlated to chlorophyll content (Buschman and Nagel, 1993). Single leaf spectrum measurements can avoid the effects of soil background, neighbor plants, solar angle, and atmospheric conditions (Alvaro et al., 2007). Single leaf spectrum has been reported to generate an accurate estimation of total chlorophyll content of maize (Ciganda et al., 2009) and soybean (Adams et al., 1999). Alvaro et al. (2007) found that NDVI and SR calculated from an individual cereal plant had a significant correlation with the biomass. Single leaf reflectance spectrum also was used to satisfactorily detect wheat nutrient deficiency (Ayala-Silva and Beyl, 2005).

Many indices have been developed and tested. Some of them are first derivatives from spectrum, such as SR, NDVI, stress index (SI), and water index (WI). Others are second derivatives, such as yellow index (YI) (Adams et al., 1999). Different wavelengths have been used in the construction of those indices and in some cases modifications have been made by introducing a third wave length in the algorithm (Sims and Gamon., 2002). A thorough review on different vegetation indices can be found in Volterrani et al. (2005) and in Jiang and Carrow (2007). Little information, however, is available on the effectiveness detecting turfgrass stress based on single leaf spectrum.

The objectives of this study were to compare and contrast between indices of single leaf spectrum and direct physiological parameters in their ability to detect tall fescue responses to salinity stress. The ultimate goal was to develop a practical methodology for screening salt-tolerant turfgrass plants from a large population in the breeding process.

Materials and Methods

The experiment was conducted from August to November in 2009 and repeated from January to April in 2010. Two tall fescue cultivars, Tar Heel II (salt tolerant) and Wolfpack (salt sensitive) (Wipff and Rose-Fricker, 2003), were seeded in plastic tubes (4 cm in diam and 20 cm deep) filled with washed silica sand and thinned to one plant per tube at the 2-leaf stage. The plants were maintained in a greenhouse with temperatures of 25/15 °C (day/night), photoperiod of 14 h, and an average PAR of 400 μmol m^{-2} s^{-1}. The plants were fertilized with half-strength Hoagland solution (Hothem et al., 2003) at a rate of 10 mL per tube twice a week until the 4-leaf stage.

At the 4-leaf stage, full-strength Hoagland solution was applied at a rate of 20 mL per tube every other day, and the experimental treatments were initiated. Salinity treatments were applied by adding NaCl and $CaCl_2$ (1:1 w/w) to the Hoagland solution in 16 000 mg L^{-1} with an electrical conductivity (EC) of approximately 25 dS m^{-1}. A control was included with plants receiving Hoagland solution only. Sixty three plants were included in each treatment unit. The experiment was arranged in a randomized complete block design with three replicates.

On the day prior to the initiation of treatments and weekly thereafter, the following measurements were taken from the 4th leaves of the primary shoot of the seedlings:

Quantum yield (F_v/F_m) of photosystem II (PS II) from three plants in each experimental unit was measured using a portable chlorophyll fluorometer (MINI-PAM, Heinz Walz GmbH, Effeltrich, Germany) with the fiberoptics placed 6 mm from the leaf surface at 60° angle using the leaf-clip.

The same leaves used for quantum yield measurement were clipped on a black background with the adxial side facing up in a leaf chamber that was illuminated by a high intensity halogen light (Warner-lambert Tech. Inc., Buffalo, N.Y.) from the top. The reflectance spectrum from 350 to 1000 nm was collected with an S2000-TR temperature-regulated fiber optic spectrometer (OceanOptics Inc., Dunelin, FL.) which has a read fiber inserted in the leaf chamber at 60° to the surface plane and controlled by a computer. The relative reflectance spectrum was then used in the calculation of different indices used in this study, which are listed in Table 2.1.

After the collection of reflectance spectrum, the leaves were excised and placed in the leaf chamber of a LI-COR 6200 Portable Photosynthesis System (LI-COR, Lincoln, NE.) to measure the Tr and g_s. After the measurements, the 4th leaf from six plants were flash frozen in liquid nitrogen and kept under -80°C for the measurement of chlorophyll (Chl) and malondialdehyde (MDA) content.

The 4th leaf blades of another three plants in each experimental unit also were collected by cutting at the collar to measure RWC. The leaf samples were immediately weighed to obtain the fresh weight (fw) and then submerged in distilled water for 6 h. The soaked leaves were blot-dried with paper towels before weighing again to obtain the turgid weight (sw). The final dry weight (dw) was obtained by weighing the leaves after drying in an oven at 68°C for 48 h. Leaf RWC was calculated using the equation (Lee et al., 2005) as follows,

$$RWC(\%) = (fw - dw)/(sw - dw).$$

Malondialdehyde (MDA) content in the leaves was determined by the thiobarbturic acid (TBA) reaction. The leaf samples were ground and extracted with 1 mL 5% trichloroacetic acid. The sample extraction was centrifuged immediately. Then 0.5 mL of the supernatant was pipetted to a new centrifuge tube, and mixed with equal volume of 0.67% TBA. The mixture was incubated in a water bath at 100 °C for 30 min. The light absorbance of the mixtures at the wavelengths of 450, 532 and 600 nm was read using a Beckman DU 640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA.). The MDA content was calculated using an extinction coefficient 155 mmol L^{-1} cm^{-1} (Heath

Table 2.1. Statistical parameters from the analysis of variance of the indices developed from the single leaf reflectance spectrum using the 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

Index	Algorithm	Source	Statistics in this study						
			Min.	Max.	Mean	R^2	CV	RMSE[†]	$P>F$
SR[‡]$_{900/675}$	R_{900}/R_{675}	Volterrani et al., 2005	1.97	6.23	4.32	0.80	15.48	0.67	0.0046
$SR_{775/675}$	R_{775}/R_{675}	Volterrani et al., 2005	1.89	6.44	4.37	0.90	13.01	0.57	0.0002
$SR_{640/570}$	R_{640}/R_{570}	Foschi et al., 2009	0.72	0.99	0.82	0.89	4.93	0.04	0.0002
mSR[§]$_{750/705}$	$(R_{750}-R_{445})/(R_{705}+R_{445})$	Sims and Gamon, 2002	1.36	3.30	2.55	0.94	7.62	0.19	<0.0001
NDVI[¶]$_{900/675}$	$(R_{900}-R_{675})/(R_{900}+R_{675})$	Volterrani et al., 2005	0.10	0.71	0.58	0.66	19.97	0.12	0.0412
$NDVI_{775/675}$	$(R_{775}-R_{675})/(R_{775}+R_{675})$	Volterrani et al., 2005	0.24	0.72	0.58	0.83	13.55	0.08	0.0016
$NDVI_{950/660}$	$(R_{950}-R_{660})/(R_{950}+R_{660})$	Jiang and Carrow, 2007	0.30	1.94	0.70	0.46	52.84	0.37	0.2749
$NDVI_{780/670}$	$(R_{780}-R_{670})/(R_{780}+R_{670})$	Jones et al., 2007	0.24	0.72	0.59	0.83	13.48	0.08	0.0017
mND[#]$_{750/705}$	$(R_{750}-R_{705})/(R_{750}+R_{705}-2R_{445})$	Sims and Gamon, 2002	0.14	0.53	0.41	0.90	11.54	0.05	0.0001
SI[††]$_{710/760}$	R_{710}/R_{760}	Carter, 1994	0.47	0.85	0.58	0.90	8.32	0.05	0.0002
$SI_{710/810}$	R_{710}/R_{810}	Jiang and Carrow, 2007	0.46	0.84	0.55	0.70	13.48	0.07	0.0363
NPCI[‡‡]	$(R_{675}-R_{430})/(R_{675}+R_{430})$	Penuelas et al., 1994	0.12	0.45	0.27	0.58	24.20	0.07	0.1848
WI[§§]	R_{900}/R_{970}	Penuelas et al., 1997	0.44	10.82	2.58	0.58	82.46	2.13	0.3399
PSR[¶¶]	R_{430}/R_{680}	Penuelas et al., 1994	0.38	0.76	0.57	0.55	14.24	0.08	0.2929
YI[##]	$(R_{600}-2R_{640}+R_{680})/0.016$	Adams et al., 1999	-1.79	10.28	4.27	0.87	46.71	1.99	0.0004

[†] RMSE, root mean square error.

[‡] SR, simple ratio vegetation index.

[§] mSR, modified simple ratio vegetation index.

[¶] NDVI, normalized difference vegetation index.

[#] mND, modified normalized difference vegetation index.

[††] SI, stress index.

[‡‡] NPCI, normalized pigment content index.

[§§] WI, water index.

[¶¶] PSR, simple pigment ratio.

[##] YI, yellow index.

and Packer, 1968).

For Chl measurement, both chlorophyll a (Chl_{a}) and chlorophyll b (Chl_{b}), approximately 0.03 g of each leaf sample was ground and weighed out and transferred to a centrifuge tube. To each sample, 2 mL of 80% acetone were added. The tubes were then capped and kept in darkness at -4°C overnight for extraction. The extract was centrifuged at -4°C and 50 μl of the supernatant were diluted by adding 950 μl of 80% acetone. After thorough mixing, the absorbance of the diluted extraction was measured with a Beckman DU 640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA.) at the wavelengths of 470, 646.8, and 663.2 nm. The Chl was determined following the method and equations by Lichtenthaler (1987).

The data were subjected to analysis of variance (ANOVA) using mixed procedures in SAS 9.2 (SAS Institute, 2008) with replication blocks treated as a random variable. Treatment means were separated using Fisher protected least significant difference (LSD) at 0.05 probability level. Homogeneity test revealed different variance between the results of 2009 and 2010. Therefore, the results are reported separately.

Results and Discussion

Results from both 2009 and 2010 study showed decreasing RWC in the 4th leaf at one week after treatment (WAT) in the salt treated plants (Table 2.2). Although there were slight decreases of RWC in the untreated control as the study progressed, the sensitivity of RWC was good to separate salinity stress from the control. This result was in agreement with other research where water content was directly related to the levels of NaCl treatment (Fricke and Peters, 2002; Leskys et al., 1999). No difference was detected between the two cultivars (Table 2.2) on any date of measurement.

Quantum yield of PS II (F_v/F_m) of the 4th leaf decreased under salinity stress but the difference did not become evident until 3 WAT in 2009 (Table 2.3). Quantum yield showed a general decreasing trend as the leaf aged especially in the 2009 study. However, F_v/F_m was low at the initiation of treatment and increased at 2 WAT in 2010 (Table 2.3), indicating environmental and age effects on the measurements. Likewise, the F_v/F_m ratio did not show difference until very late stage of drought treatment in a study by Huang and Gao (1999) and was considered less indicative. Again, there was no difference of F_v/F_m ratio detected in both the 2009 and 2010 studies between the two cultivars.

In both 2009 and 2010, the total Chl content of the 4th leaf in the salt treated plants decreased significantly as compared to the untreated control at 2 WAT indicating a fair sensitivity. The Chl content also showed a consistent decreasing trend as the leaves aged indicating that the same leaf ages have to be used for comparing or differentiation of salinity stress. It is worth noticing that the difference between treated and untreated plants existed in different cultivars and at different WAT (Table 2.4) rather than in both cultivars during all measurements in both years.

Malondialdehyde (MDA), as an indicator of lipid peroxidation has been investigated in tall fescue under different stresses (Fu and Huang, 2001; Price and Hendry, 1991). In this study, MDA content of the 4th leaf increased in salinity treatment at 2 WAT in both years and was consistent for both cultivars (Table 2.5). The results of 3 WAT and 4 WAT were missing from the 2009 study due to equipment failure. However, MDA content was sensitive to the stress levels as indicated by large differences in contents between salt treated and untreated plants. Although the leaf MDA content increased as the leaves aged, the increase was much less compared to that caused by salinity stress (Table 2.5). Other

Table 2.2. Relative water content (RWC) of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT†	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
					%					
Tar Heel II	95.5	89.3	86.9	84.7	64.9	93.4	86.2	86.4	88.3	78.9
Tar Heel II + Salts		76.3	79.7	70.9	44.4		78.7	68.6	42.7	52.4
Wolfpack	95.7	92.5	88.2	83.2	68.6	93.4	91.3	86.7	76.7	80.4
Wolfpack + Salts		88.3	77.7	71.1	40.9		73.6	63.1	43.1	39.9
$LSD_{0.05}$	NS	7.8	3.2	8.5	16.5	NS	10.9	9.5	20.2	13.3

† WAT, week after treatment.

Table 2.3. Quantum yield (F_v/F_m) of photosystem II of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT†	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
					F_v/F_m					
Tar Heel II	0.73	0.67	0.64	0.54	0.25	0.66	0.64	0.71	0.58	0.48
Tar Heel II + Salts		0.68	0.56	0.06	0.03		0.38	0.22	0.06	0.00
Wolfpack	0.73	0.60	0.64	0.53	0.35	0.58	0.65	0.71	0.53	0.54
Wolfpack + Salts		0.65	0.65	0.17	0.08		0.51	0.13	0.04	0.06
$LSD_{0.05}$	NS	NS	NS	0.18	0.21	NS	0.20	0.16	0.14	0.24

† WAT, week after treatment.

Table 2.4. Total chlorophyll content of 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT[†]	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
	……………mg g^{-1}……………									
Tar Heel II	13.51	14.50	13.52	10.00	6.22	10.84	13.26	11.30	9.36	8.65
Tar Heel II + Salts		14.01	10.39	6.30	4.41		11.47	8.92	4.92	2.60
Wolfpack	12.66	13.47	12.06	10.65	8.53	10.54	12.50	12.58	8.65	8.48
Wolfpack + Salts		13.68	11.32	7.45	3.01		10.98	8.60	4.76	2.70
$LSD_{0.05}$	NS	NS	2.20	2.48	3.22	NS	NS	2.68	4.42	3.43

[†] WAT, week after treatment.

Table 2.5. Malondialdehyde (MDA) content of 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009			2010				
Treatment	0WAT[†]	1WAT	2WAT	0WAT	1WAT	2WAT	3WAT	4WAT
	……………ng g^{-1}……………							
Tar Heel II	6.60	5.75	14.61	3.72	5.13	5.10	7.85	8.34
Tar Heel II + Salts		5.49	44.53		9.44	43.16	76.44	84.64
Wolfpack	5.18	6.28	12.78	3.95	5.15	4.70	6.90	10.72
Wolfpack + Salts		6.62	47.85		13.94	26.87	65.72	64.12
$LSD_{0.05}$	NS	NS	8.15	NS	3.15	18.23	16.39	26.14

[†] WAT, week after treatment.

reports also indicated that the increase of MDA during natural aging was not substantial (Fu and Huang, 2001). 'Tar Heel II' and 'Wolfpack' showed no difference in MDA content in either treated or untreated leaves.

Both Tr and g_s are measurement of the stomatal adjustment of leaves due to environmental and biological conditions and diurnal rhythms. Both are affected by environmental conditions and the time of measurement during the day. The decreased Tr and g_s in salt treated plants were not evident until 3 WAT for both 2009 and 2010 study (Table 2.6, 2.7). At the end of study, Tr and g_s of the 4th leaf of tall fescue under salinity stress showed a sharp decrease and were too low to allow for quantitative separation between materials. A similar trend also was reported by Huang and Gao (1999) in comparing six tall fescue cultivars under drought stress.

The results from this study showed that RWC and MDA are sensitive parameters to measure salinity stress although MDA may also be affected by natural aging of the plants. Chlorophyll retention during aging and under stress is affected by both genetic and environments (Hameed and Ashraf, 2008). The quantum yield, Tr, and g_s can be indicators of instantaneous physiological conditions of the plants and are sensitive to environmental conditions that vary through the day (Katerji et al., 1997; Wilson et al., 2006). Although all those parameters are affected by water status in plants, the primary responses such as RWC are less temporal than other parameters that are down stream of a physiological cascade or highly regulated in response to environmental conditions.

Since the physiological responses to salinity stress in two cultivars of tall fescue became more detectable with the passage of time after treatment, the indices developed from reflectance spectrum at the early stages of stress were analyed with ANOVA to test

Table 2.6. Transpiration rate (Tr) of 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT[†]	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
	....mol m^{-2} s^{-1}..									
Tar Heel II	0.081	0.078	0.046	0.041	0.046	0.037	0.046	0.018	0.025	0.006
Tar Heel II + Salts		0.078	0.039	0.024	0.012		0.031	0.002	0.000	0.000
Wolfpack	0.080	0.080	0.041	0.039	0.042	0.032	0.039	0.019	0.025	0.009
Wolfpack + Salts		0.072	0.036	0.029	0.000		0.033	0.002	0.000	0.000
$LSD_{0.05}$		NS	NS	0.012	0.016	NS	NS	0.004	0.004	0.005

[†] WAT, week after treatment.

Table 2.7. Stomatal conductance (g_s) to CO_2 of 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT[†]	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
	....µmol m^{-2} s^{-1}..									
Tar Heel II	0.121	0.117	0.070	0.062	0.070	0.056	0.070	0.028	0.015	0.009
Tar Heel II + Salts		0.116	0.059	0.037	0.018		0.048	0.003	0.000	0.000
Wolfpack	0.119	0.119	0.062	0.059	0.064	0.049	0.059	0.029	0.014	0.014
Wolfpack + Salts		0.108	0.055	0.044	0.000		0.050	0.003	0.000	0.000
$LSD_{0.05}$	NS	NS	NS	0.018	0.024	NS	NS	0.007	0.008	0.008

[†] WAT, week after treatment.

their sensitivity. The performance of indices from one of the analysis was shown in Table 2.1. Final selection of indices was based on statistical significance, higher values of range and coefficient of determination (R^2), and lower values of CV, root mean square error (RMSE), and P of the F-test (Table 2.1). Six indices were chosen for ANOVA analysis during all dates of measurement in both 2009 and 2010. Those included two SR ($SR_{775/675}$, $SR_{640/570}$), one modified SR ($mSR_{750/705}$), one NDVI ($NDVI_{775/675}$), one modified NDVI ($mND_{750/705}$), and one SI ($SI_{710/760}$).

Simple ratio vegetation index $SR_{775/675}$ showed fair sensitivity because it revealed differences between the control and salt treated plants in both cultivars at 3 WAT in 2009 and 2 WAT in 2010 (Table 2.8). The ratio $SR_{775/675}$ decreased as leaf aged. $SR_{640/570}$, on the other hand, did not show large changes with leaf aging, but had sufficient sensitivity to differentiate the salt treated plants in both cultivars as early as 1 WAT in the 2010 study (Table 2.9). By including the wavelength 445 nm, $mSR_{750/705}$ performed better than $SR_{775/675}$ with certain degree of age effect (Table 2.10). The $NDVI_{775/675}$ showed fair sensitivity in differentiating salt treated plants from the control with little age dependence in both cultivars for 2009 and 2010 study (Table 2.11). Again, by including the wavelength 445 nm, $mND_{750/705}$ performed better than $NDVI_{775/675}$ and showed also sensitivity to the age of leaves (Table 2.12). Finally, $SI_{710/760}$ showed good sensitivity in differentiating salt treated plants from the control at 2 WAT in both 2009 and 2010 study, with certain degree of age dependence, i.e. the index increased with leaf age (Table 2.13). In general, $mSR_{750/705}$, $mND_{750/705}$, and $SI_{710/760}$ showed good potential to be used to detect both salinity stress and leaf senescence.

Simple correlation analysis between the six reflectance indices and six

Table 2.8. Simple ratio vegetation index ($SR_{775/675}$) of the 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT†	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
Tar Heel II	5.11	5.44	4.61	5.14	4.33	4.83	4.50	5.23	5.27	4.21
Tar Heel II + Salts		5.33	4.16	3.14	1.66		4.48	2.42	2.52	1.75
Wolfpack	5.56	5.57	4.25	4.97	4.06	4.78	4.64	5.23	5.90	4.18
Wolfpack + Salts		5.29	4.60	3.10	2.06		4.39	2.68	1.95	1.70
$LSD_{0.05}$	NS	NS	NS	1.49	1.60	NS	NS	0.88	1.26	0.78

† WAT, week after treatment.

Table 2.9. Simple ratio vegetation index ($SR_{640/570}$) of the 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT†	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
Tar Heel II	0.76	0.77	0.83	0.83	0.79	0.75	0.80	0.77	0.79	0.81
Tar Heel II + Salts		0.77	0.86	0.97	1.10		0.82	0.95	0.98	1.13
Wolfpack	0.75	0.74	0.83	0.83	0.75	0.75	0.79	0.77	0.75	0.82
Wolfpack + Salts		0.76	0.82	0.96	1.08		0.85	0.97	1.03	1.14
$LSD_{0.05}$	NS	NS	NS	0.09	0.17	NS	0.04	0.05	0.06	0.11

† WAT, week after treatment.

Table 2.10. Modified simple ratio vegetation index ($mSR_{750/705}$) of the 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT[†]	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
Tar Heel II	2.74	3.50	3.45	3.14	1.61	2.71	3.06	3.06	2.54	2.11
Tar Heel II + Salts		3.30	2.80	2.00	1.18		2.83	1.71	1.37	1.44
Wolfpack	2.92	3.35	3.16	3.23	1.70	2.69	3.01	3.10	2.47	2.28
Wolfpack + Salts		3.32	3.09	2.16	1.13		2.77	1.84	1.24	1.45
$LSD_{0.05}$	NS	NS	0.47	0.60	0.23	NS	NS	0.33	0.40	0.44

[†] WAT, week after treatment.

Table 2.11. Normalized difference vegetation index ($NDVI_{775/675}$) of the 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT[†]	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
Tar Heel II	0.67	0.68	0.63	0.67	0.60	0.65	0.63	0.67	0.67	0.60
Tar Heel II + Salts		0.66	0.58	0.49	0.23		0.63	0.37	0.42	0.27
Wolfpack	0.69	0.69	0.62	0.65	0.67	0.65	0.64	0.67	0.70	0.59
Wolfpack + Salts		0.67	0.64	0.49	0.28		0.62	0.41	0.31	0.26
$LSD_{0.05}$	NS	NS	NS	0.12	0.20	NS	NS	0.12	0.09	0.09

[†] WAT-week after treatment.

Table 2.12. Modified normalized difference vegetation index ($mND_{750/705}$) of the 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT[†]	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
Tar Heel II	0.46	0.55	0.54	0.51	0.23	0.46	0.51	0.50	0.43	0.33
Tar Heel II + Salts		0.53	0.45	0.31	0.08		0.47	0.25	0.15	0.18
Wolfpack	0.49	0.54	0.51	0.52	0.25	0.46	0.50	0.51	0.42	0.36
Wolfpack + Salts		0.53	0.51	0.35	0.06		0.46	0.28	0.11	0.18
$LSD_{0.05}$	NS	NS	0.07	0.10	0.07	NS	NS	0.08	0.08	0.10

[†] WAT, week after treatment.

Table 2.13. Stress index ($SI_{710/760}$) of the 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT[†]	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
Tar Heel II	0.51	0.46	0.47	0.48	0.61	0.54	0.51	0.49	0.52	0.65
Tar Heel II + Salts		0.48	0.54	0.66	0.85		0.53	0.74	0.73	0.78
Wolfpack	0.50	0.48	0.50	0.48	0.56	0.55	0.51	0.49	0.50	0.63
Wolfpack + Salts		0.48	0.49	0.63	0.80		0.55	0.71	0.78	0.79
$LSD_{0.05}$	NS	NS	0.07	0.09	0.19	NS	NS	0.08	0.09	0.09

[†] WAT, week after treatment.

physiological parameters demonstrated that significant strong to fair correlations existed between the three best reflectance indices ($mSR_{750/705}$, $mND_{750/705}$, and $SI_{710/760}$) and three important physiological parameters (RWC, Chl, and MDA) (Table 2.14, 2.15) except that of MDA in the 2009 study. The weak correlation of MDA with other indices in 2009 was probably due to the missing data points. Since these three indices changed in different directions under stress and were calculated from the wavelengths spreading across a wide range of the spectrum, they may be potentially good candidates to be used as indicators for screening salinity tolerance. Additional research may be needed to test those indices with wider variation of genotypes. Macros could also be developed to output those indices directly instead of using post spectrum collection calculation.

Conclusions

This study showed that salinity stress in tall fescue may be reflected by parameters at different levels of plant physiological processes. However, they were not equally sensitive to be used as indicators for salinity tolerance selection. Even the most recommended parameters take a significant amount of time to gain results, such as RWC, and therefore are of value only in physiological studies, rather than in breeding programs. At least three indices calculated based on single leaf spectrum showed promise as sensitive means to differentiate salinity stress from untreated plants. In addition to the sensitivity, those indices also are strongly correlated to many physiological parameters that have been shown to be reliable measures of salinity tolerance in many plant species including tall fescue. With modification of computer algorithms, indices can be generated instantaneously from a scan of single leaf.

Table 2.14. Simple correlation coefficients between reflectance spectral indices and physiological parameters measured on the 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage in the 2009 study. Coefficients are followed by *t*-test probability in parenthesis.

	RWC[†]	Chl[‡]	QY[§]	MDA[¶]	*gs*[#]	Tr[††]
$SR_{640/570}$[‡‡]	-0.29(0.0110)	-0.34(0.0021)	-0.31(0.0064)	0.145(0.3595)	-0.31(0.0064)	-0.30(0.0069)
$SR_{775/675}$	0.56(<0.0001)	0.73(<0.0001)	0.71(<0.0001)	-0.325(0.0359)	0.71(<0.0001)	0.70(<0.0001)
$mSR_{750/705}$[§§]	0.39(0.0004)	0.78(<0.0001)	0.80(<0.0001)	-0.060(0.7072)	0.56(<0.0001)	0.56(<0.0001)
$NDVI_{775/675}$[¶¶]	0.55(<0.0001)	0.76(<0.0001)	0.76(<0.0001)	-0.393(0.0101)	0.68(<0.0001)	0.67(<0.0001)
$mND_{750/705}$[##]	0.45(<0.0001)	0.80(<0.0001)	0.80(<0.0001)	-0.104(0.5102)	0.57(<0.0001)	0.57(<0.0001)
$SI_{710/760}$[†††]	-0.32(0.0039)	-0.60(<0.0001)	-0.63(<0.0001)	-0.05(0.7757)	-0.45(<0.0001)	-0.45(<0.0001)

[†] RWC, relative WATer content.

[‡] Chl, total chlorophyll content.

[§] QY, quantum yield of photosystem II (F_v/F_m).

[¶] MDA, malondialdehyde content.

[#] *gs*, stomatal conductance to CO_2.

[††] Tr, leaf water transpiration rate.

[‡‡] SR, simple ratio vegetation index.

[§§] mSR, modified simple ratio vegetation index.

[¶¶] NDVI, normalized difference vegetation index.

mND, modified normalized difference vegetation index.

[†††] SI, stress index.

Table 2.15. Simple correlation coefficients between reflectance spectral indices and physiological parameters measured on the 4th leaves of two tall fescue varieties irrigated with full strength Hoagland solution containing 16 000 mg L^{-1} NaCl and $CaCl_2$ at the onset of 4th leaf seedling stage in the 2010 study. Coefficients are followed by *t*-test probability in parenthesis.

	RWC[†]	Chl[‡]	QY[§]	MDA[¶]	*gs*[#]	Tr[††]
SR$_{640/570}$[‡‡]	-0.80(<0.0001)	-0.73(<0.0001)	-0.84(<0.0001)	0.87(<0.0001)	-0.51(<0.0001)	-0.50(<0.0001)
SR$_{775/675}$	0.73(<0.0001)	0.69(<0.0001)	0.84(<0.0001)	-0.81(<0.0001)	0.51(0.<0.0001)	0.50(<0.0001)
mSR$_{750/705}$[§§]	0.71(<0.0001)	0.77(<0.0001)	0.82(<0.0001)	-0.78(<0.0001)	0.64(<0.0001)	0.63(<0.0001)
NDVI$_{775/675}$[¶¶]	0.76(<0.0001)	0.72(<0.0001)	0.84(<0.0001)	-0.85(<0.0001)	0.54(<0.0001)	0.53(<0.0001)
mND$_{750/705}$[##]	0.74(<0.0001)	0.78(<0.0001)	0.83(<0.0001)	-0.84(<0.0001)	0.65(<0.0001)	0.64 (<0.0001)
SI$_{710/760}$[†††]	-0.72(<0.0001)	-0.76(<0.0001)	-0.83(<0.0001)	-0.79(<0.0001)	-0.63(<0.0001)	-0.62(<0.0001)

[†] RWC, relative water content.

[‡] Chl, total chlorophyll content.

[§] QY, quantum yield of photosystem II (F_v/F_{max}).

[¶] MDA, malondialdehyde content.

[#] g_s, stomatal conductance to CO_2.

[††] Tr, leaf water transpiration rate.

[‡‡] SR, simple ratio vegetation index.

[§§] mSR, modified simple ratio vegetation index.

[¶¶] NDVI, normalized difference vegetation index.

mND, modified normalized difference vegetation index.

[†††] SI, stress index.

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CHAPTER 3. LEAF SENESCENCE IS DIFFERENT IN TALL FESCUE INDUCED BY SALINITY AND SHADE

Abstract

Salinity is one of the major abiotic factors that adversely affect turfgrass quality. Tall fescue has a moderate to high salinity tolerance and is used as a turfgrass in 42 states through the U.S. Because of the wide adaptation and salinity tolerance, tall fescue has been increasingly used for lawn, golf course fairways and rough areas. Despite the current upward trends in tall fescue use, turf quality may still be affected under saline conditions. The objective of this study was to investigate the morphological and physiological process of leaf senescence in tall fescue under salinity stress as compared to normal growing condition or accelerated aging under shade. Two tall fescue cultivars, Tar Heel II (salt tolerant) and Wolfpack (salt sensitive) were seeded in plastic tubes filled with washed silica sand and were fertilized with half-strength Hoagland solution until the 4-leaf stage. Salinity treatments were applied by adding NaCl and $CaCl_2$ (1:1 w/w) to the Hoagland solution in 16 000 mg L^{-1} with an electrical conductivity (EC) of approximately 25 dS m^{-1} at the 4-leaf stage. A control was included with plants receiving Hoagland solution only. Tall fescue leaf senescence was accelerated by salinity stress which was different from the aging process under shade. Tall fescue had several adaptation strategies in order to conserve water under salinity stress, while the plants under shade had adaptation mechanisms centered on light harvesting. Tall fescue accelerated old leaf senescence, whereas under shade conditions, tall fescue showed slowing down in the new leaf development as well as mature leaf aging. The contrasting mechanisms of tall fescue under shade and salinity warrant further study to investigate the performance of species under

both shade and salinity stress that is likely to happen when used for its shade tolerance and irrigated with recycled water, such as the case in golf course rough, landscapes, or home lawns.

Introduction

Salinity is one of the major abiotic factors that adversely affect turfgrass quality. In addition to the use of recycled waters, saline conditions also occur in salt-affected soils, areas with salt water intrusion (McCarty and Dudeck, 1993; Murdoch, 1987), and roadsides where salts are used for snow and ice melting (Greub et al., 1985). Turfgrasses have a wide range of tolerance and adaptation to salinity stress (Hughes et al., 1975). Alshammary et al. (2004) ranked the salinity tolerance of four cool-season grasses in the order of saltgrass (*Distichlis spicata* (L.) Greene), alkaligrass (*Puccinellia distans* (L.) Parl.), tall fescue (*Festuca arundinacea* Schreb.), and kentucky bluegrass (*Poa pratensis* L.). Tall fescue has a moderate to high salinity tolerance (Beard, 1973; Marcum, 2006; Wu et al., 1988) and is used as a turfgrass in 42 states through the U.S. (Murray and Powell, 1979). It was reported that tall fescue seed germination was not affected by moderate salinity (Johnson, et al., 2007), but that seedling penetration was inhibited by salt crust (Frelich et al., 1973). However, Horst and Beadle (1984) found large variations of germination rates among 16 tall fescue cultivars evaluated at 7 500 to 15 000 mg L^{-1} of NaCl and $CaCl_2$. Wipff and Rose-Fricker (2003) screened 59 cultivars and experimental lines at mature stage using 17 000 and 24 000 mg L^{-1} of NaCl, and found that 'Tar Heel II' and 'Pure Gold' had the highest survival rate, while 'Wolfpack' had the lowest survival rate.

Because of the wide adaptation and salinity tolerance, tall fescue has been increasingly used for lawn, golf course fairways and rough areas (Barnes, 1990). Despite the current upward trends in tall fescue use, turf quality may still be affected under saline conditions. Turfgrass breeders have identified cold tolerance, drought tolerance, disease resistance, and leaf texture as important traits for further improvement (Meyer and Watkins, 2003). Leaf firing, which is a form of chlorosis, is common to tall fescue managed under severe salinity stress (Alshammary et al., 2004). Leaf firing initiates from the tip and margins of a leaf and progresses down to the entire blade. Meanwhile, the leaf color turns from green to yellow, then to tan/brown in the dead area (Carrow and Duncan, 2003). A visual rating system of leaf firing based on the percentage leaf area discoloration has been extensively used in salinity and drought tolerance evaluation (Alshammary et al., 2004; Bowman et al., 2006; Chen et al., 2009; Dean et al., 1996; Lee et al., 2005).

Salinity stress often leads to accelerated leaf senescence which is normally considered age-dependent. Leaf senescence is defined as a series of degenerative changes that lead to death (Nooden, 1980). Aging, on the other hand, refers to processes of accruing maturity with passage of time (Leopold, 1980). Both biotic (pathogens) and abiotic (such as shade, water, and temperature) stress can induce senescence. The process of leaf senescence is a succession of physiological and molecular events in three stages: initiation, degeneration, and terminal phase (Yoshida, 2003). Previous research on salt tolerance focused only on survival rate and yield reduction (Johnson et al., 2007; McKenzie et al., 1994; Wipff and Rose-Fricker, 2003). Limited research has been conducted to understand the physiological responses of tall fescue to salinity.

At the cellular level, leaf senescence includes disassembling of chloroplasts and other organelles (Benjamin et al., 1999). The biochemical process consists of hydroxylation of chlorophyll, proteins, lipids, nucleic acids, and carbohydrates (Aliferis et al., 2006; Nooden et al., 1997; Quirino et al., 2000; Rajcan et al., 1999; Roca et al., 2004; Stirpe et al., 1996). All known plant hormones have been reported in the senescence process (Abreu and Munne-Bosch, 2008; Carimi et al., 2004; Dugardeyn and Straeten, 2008; Ella et al., 2003; Hogan et al., 2006; Hung and Kao, 2003; Rakwal and Komatsu, 2001; Yu et al., 2008). The levels of many reactive oxygen species increase at certain stages of senescence (Niewiadomska et al., 2009; Orendi et al., 2001). Ion detoxification (Murgia et al., 2007), pH adjustment (Bao et al., 2009; Berkowitz and Gibbs, 1983), and water conductivity (Azaizeh and Steudle, 1991) have been studied in the process of salt-induced senescence.

The nutrients released by hydrolysis in senescing tissues are often transported to growing and storage tissues (Nooden et al., 1997; Quirino et al., 2000). This recycling process is selective; elements such as Cl and Na are retained in the dead leaves, while P and K are mobilized (Lin and Wang, 2001). Nutrient mobilization under salinity stress therefore is considered as an adaptation mechanism (Hasegawa et al., 2000). Essentially, the whole process of senescence and death of leaves caused by salinity stress may be an avoidance mechanism that minimizes water loss (Herralde et al., 1998). Nutrient mobilization during senescence was reported in *Arabidopsis* (Himelblau and Amasino, 2001), barley (*Hordeum vulgare* L.) (Wiedemuth et al., 2005), and French beans (*Phaseolus vulgaris* L.) (Yordanov et al., 2008).

Leaf senescence also can be accelerated by shading or jasmonic acid treatment (Ananieva et al., 2006). This provides tools to study mechanisms of senescence and different adaptive strategies of plants under different stress, especially of contrasting environmental conditions, such as shade vs drought, heat vs cold. Different types of senescence were studied by Becker and Apel (1993) at the gene level of barley leaves. Leaves are the primary photosynthetic organ where plants obtain most energy and photo-assimilates. During the senescence process, the leaves are subjected to the most physiological and biochemical changes. The loss of leaf turgor, decoloration, thickening of cuticle, accumulation of wax, and chlorosis are some of the primary results from senescence (Richardson et al., 2005).

The mechanism of leaf senescence in tall fescue under saline stress is not fully understood. The knowledge will be useful for breeders to develop more effective selection criterion, selective pressure, and screening methods. Turgrass managers can also apply the new findings to adjust cultural practices, such as mowing, fertilization, irrigation, and application of plant growth regulators.

The objective of this study was to investigate the morphological and physiological process of leaf senescence in tall fescue under salinity stress as compared to normal growing condition or accelerated aging under shade.

Material and Methods

The experiment was conducted in a greenhouse at North Dakota State University (Fargo, ND) from September, 2009 to July, 2010. Two tall fescue cultivars, Tar Heel II (salt tolerant) and Wolfpack (salt sensitive) (Wipff and Rose-Fricker, 2003), were seeded in plastic tubes measuring 4 cm in diameter and 20 cm in depth with silicon sand as the growth medium. The plants were maintained at 25/15 °C (day/night), 14-h photoperiod, and an average PAR of 400 μmol m^{-2} s^{-1}. The growth medium was maintained at field capacity by watering twice daily until the seeds germinated, when the watering was reduced to once daily. The seedlings were fertilized by watering with half strength

Hoagland solution (Hothem et al., 2003) at 10 ml per tube twice a week. At the 2-leaf stage, the plants were thinned to keep one in each tube.

At the 4th leaf stage, full strength Hoagland solution was applied to the sand medium at 20 ml per tube every other day and the experimental treatment was initiated. The salt treatment was applied by adding 16 000 mg L^{-1} of NaCl and $CaCl_2$ to the full strength Hoagland solution to reach an EC of about 25 dS m^{-1}. The shade treatment was applied by covering the plants with black knitted polyethylene cloth that allows penetration of 30% full light. Plants that received Hoagland solution only were included as control. The experiment was arranged in a randomized complete block design with three replicates and was conducted twice.

On the day prior to the initiation of treatments and weekly thereafter, the following measurements were taken from the 4th to the youngest mature leaves on the primary shoot of the seedlings:

Quantum yield of photosystem II (PS II) from three plants in each experiment unit was measured using a portable chlorophyll fluorometer MINI-PAM (Heinz Walz GmbH, Effeltrich, Germany.) with the fiberoptics placed 6 mm to the leaf surface at 60° angle using the leaf-clip. The same leaves used for quantum yield measurement were clipped on a black background with the adxial side facing up in a leaf chamber that was illuminated by a high intensity halogen light (Warner-lambert Tech. Inc., Buffalo, N.Y.) from the top. The reflectance spectrum from 350 to 1000 nm was collected with an S2000-TR temperature-regulated fiber optic spectrometer (OceanOptics Inc., Dunelin, FL.) which has a miniature fiberoptics inserted in the leaf chamber at 60° to the surface plane and controlled by a computer. Three reflectance indices, $mSR_{750/705}$ (Sims and Gamon, 2002), $mND_{750/705}$

(Sims and Gamon, 2002), and $SI_{710/760}$ (Carter, 1994), were calculated from the relative reflective spectra using the following equation:

$$mSR_{750/705} = (R_{750} - R_{445})/(R_{705} + R_{445})$$

$$mND_{750/705} = (R_{750} - R_{705})/(R_{750} + R_{705} - 2R_{445})$$

$$SI_{710/760} = R_{710}/R_{760}$$

where R is the relative reflectance at a given wave length.

After the collection of reflectance spectrum, the leaves were excised and placed in the leaf chamber of a LI-COR 6200 Portable Photosynthesis System (LI-COR, Lincoln, NE) to measure the transpiration (Tr), stomatal conductance (g_s). After the measurements, the 4th leaves from six plants were flash frozen in liquid nitrogen and kept under -80°C for the measurements of chlorophyll (Chl) and malondialdehyde (MDA) content.

The 4th leaf blades of another three plants from each experimental unit also were collected by cutting them at the collar to measure the relative water content (RWC). The leaf samples were immediately weighed to obtain the fresh weight (fw) and then submerged in distilled water for 6 h. The soaked leaves were blot-dried with paper towels before weighing again to obtain the turgid weight (sw). The final dry weight (dw) was obtained by weighing the leaves after drying in an oven at 68°C for 48 h. Leaf RWC was calculated from

$RWC(\%) = (fw - dw)/(sw - dw)$ (Lee et al., 2005).

Malondialdehyde (MDA) content of the 4th leaves was determined by the thiobarbturic acid (TBA) reaction. The leaf samples were ground and extracted with 1 ml 5% trichloroacetic acid. The extract was centrifuged immediately. Then 0.5 ml of the supernatant was pipetted to a new centrifuge tube, and mixed with equal volume of 0.67%

TBA. The mixture was incubated in a water bath at 100°C for 30 min. The light absorbance of the mixtures at the wavelengths of 450, 532 and 600 nm was read using a Beckman DU 640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA.). The MDA content was calculated using an extinction coefficient of 155 mmol L^{-1} cm^{-1} (Heath and Packer, 1968).

For Chl measurement, approximately 0.03 g of each leaf sample was ground, weighed, and placed in a centrifuge tube. A volume of 2 mL of 80% acetone was added to each sample. The tubes then were capped and kept in darkness at -4°C overnight. The extraction was centrifuged at -4°C and 50 μL of the extraction were diluted by adding 950 μL of 80% acetone. After thorough mixing, the absorbance of the diluted extraction was measured with a Beckman DU 640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA.) at the wavelengths of 470, 646.8, and 663.2 nm. The Chl content was determined following the method and equations by Lichtenthaler (1987).

Leaf epicuticular wax accumulation was estimated by measuring the leaf surface water wetting angle of the 5th leaf. The wetting angles were measured at room temperature (20°C) using a pipette to deliver a droplet of 5 μL distilled water onto the center of the abaxial and adaxial surface (Cape, 1983), and a picture was taken with a digital camera and analyzed using ImageJ software (National Institutes of Health, USA.). The status of leaf surface wax also was confirmed by scanning electron microscopy (SEM). Fresh samples were cut into small squares, fixed with 2.5% glutaraldehyde in 0.1 *M* sodium phosphate buffer, pH 7.35 (Tousimis Research Corporation, Rockville, MD.), dehydrated using a graded alcohol series. The fractured pieces were then critical point dried using an autosamdri-810 critical point drier (Tousimis Research Corporation, Rockville, MD.) with liquid carbon dioxide as the transitional fluid. The small fractured pieces were attached to aluminum mounts by silver paint and coated with gold using a Balzers SCD 030 sputter coater (BAL-TEC RMC, Tucson, AZ.). Images were obtained using a JEOL JSM-6490LV Scanning Electron Microscope (JEOL Inc., Peabody, MA.).

Specimens for transmission electron microscopy (TEM) were fixed in 2.5% glutaraldehyde in 0.1 *M* sodium phosphate buffer, pH 7.35 (Tousimis Research Corporation, Rockville, MD.) for at least two hours in a refrigerator. They were rinsed with the sodium phosphate buffer and then placed in 2% osmium tetroxide in buffer for two hours at room temperature. Following dehydration in a graded acetone series, samples were embedded in Epon-Araldite-DDSA with a DMP-30 accelerator and sectioned at 60 nm thickness on a RMC MTXL ultramicrotome (Boeckeler Instruments, Tucson, AZ.). Sections on grids were stained with lead citrate for 2.5 minutes and dried before observation on a JEOL JEM-100CX II electron microscope (JEOL Inc., Peabody, MA.)

The data were subjected to analysis of variance (ANOVA) using mixed procedures in SAS 9.2 (SAS Institute, 2008) with replication blocks treated as a random variable. Treatment means were separated using Fisher protected least significant difference (LSD) at 0.05 probability level. The results of 2009 and 2010 are reported separately because of the heterogeneous variance between the two years.

Results and Discussion

At the conclusion of the 2009 study, the pH of the sand medium for the control, salt, and shade treatments were 7.35, 6.81, and 7.33, respectively; the EC of sand medium for the control, salt, and shade treatments were 0.15, 0.97, and 0.16 dS m^{-1}, respectively. At the conclusion of 2010 study, the pH of sand medium for the control, salt, and shade treatments were 7.42, 6.85, and 7.25, respectively; the EC of sand medium for the control, salt, and shade treatments were 0.10, 0.71, and 0.12 dS m^{-1}, respectively. Salinity treatments resulted in higher soil EC and lower pH compared to the treatments receiving only Hoagland solution.

Relative water content of the leaves reflects the water potential of the cells and decreases as a result of stress or senescence because of the degeneration of cell membranes (Bolger et al., 2005). In general, RWC of the 4th leaves decreased with time (Table 3.1). The RWC decreased in the shade treatments was the least because the plants were well watered and subjected to lower evapotranspiration than the other treatments. The RWC decrease was highest in salt treatments. There was no difference between the cultivars in either 2009 or 2010.

While total Chl content decreased with time in the 4th leaf of the primary shoot, salt treated plants showed lower Chl content than the untreated control as early as one week after treatment (WAT) in both 2009 and 2010 study. 'Wolfpack' in the shade treatment showed higher total Chl content occasionally, but 'Tar Heel II' did not (Table 3.2). The differences in Chl were similarly influenced by Chl a and Chl b (Table, 3.2, 3.3, and 3.4). No consistent differences between the two cultivars were observed in the decrease of Chl content caused by salt treatment.

Lipid peroxidation, as indicated by excessive production of MDA, increased as the plants senesced. The salinity treatments showed higher MDA levels than the shade treatments and control throughout the experiment in the 2010 study (Table 3.5), and likewise at 2 WAT in 2009. Data were missing at 3 WAT and 4 WAT in 2009 due to equipment failure during the measurement. Although the intention of applying a shade

Table 3.1. Relative water content (RWC) of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT[†]	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
					……………%……………					
Tar Heel II	95.5	89.3	86.9	84.7	64.9	93.4	86.2	86.4	88.3	78.9
Tar Heel II + Salts		76.3	79.7	70.9	44.4		78.7	68.6	42.7	52.4
Tar Heel II + Shade		93.7	93.5	89.7	78.8		89.7	86.2	84.9	86.2
Wolfpack	95.7	92.5	88.2	83.2	68.6	93.4	91.3	86.7	76.7	80.4
Wolfpack + Salts		88.3	77.7	71.1	40.9		73.6	63.1	43.1	39.9
Wolfpack + Shade		93.0	91.4	90.8	85.3		85.8	88.0	84.6	85.4
$LSD_{0.05}$	NS	7.8	3.2	8.5	16.5	NS	10.9	9.5	20.2	13.3

[†] WAT, week after treatment.

Table 3.2. Total chlorophyll content of the 4th leaves of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT†	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
	....mg g^{-1}..									
Tar Heel II	13.5	14.5	13.5	10.0	6.2	10.8	13.3	11.3	9.4	8.7
Tar Heel II + Salts		14.0	10.4	6.3	4.4		11.5	8.9	4.9	2.6
Tar Heel II + Shade		14.0	13.4	11.7	8.0		14.6	12.3	9.7	8.6
Wolfpack	12.7	13.5	12.1	10.6	8.5	10.5	12.5	12.6	8.6	8.5
Wolfpack + Salts		13.7	11.3	7.4	3.0		11.0	8.6	4.8	2.7
Wolfpack + Shade		15.8	14.3	11.2	10.8		15.2	13.4	12.9	12.1
$LSD_{0.05}$	NS	2.1	2.2	2.5	3.2		1.9	2.7	4.4	3.4

† WAT, week after treatment.

Table 3.3. Chlorophyll a content of the 4th leaves of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT†	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
	....mg g^{-1}..									
Tar Heel II	10.0	10.6	9.9	7.0	4.2	8.1	9.8	8.4	6.8	6.1
Tar Heel II + Salts		10.3	7.5	4.3	2.7		8.5	6.4	3.3	1.6
Tar Heel II + Shade		10.2	9.7	8.2	5.4		10.7	9.0	7.0	6.0
Wolfpack	9.3	9.9	8.8	7.5	5.9	7.9	9.3	9.2	6.3	5.9
Wolfpack + Salts		10.1	8.3	5.1	1.7		8.1	5.9	2.9	1.6
Wolfpack + Shade		11.5	10.3	7.9	7.4		11.2	9.7	9.2	8.5
$LSD_{0.05}$	NS	1.5	1.7	1.8	2.2	NS	1.3	2.0	3.1	2.4

† WAT, week after treatment.

Table 3.4. Chlorophyll b content of the 4th leaves of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT†	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
					………………mg g^{-1}………………					
Tar Heel II	3.6	3.9	3.6	3.0	2.0	2.8	3.5	2.9	2.6	2.5
Tar Heel II + Salts		3.8	2.9	2.0	1.7		2.9	2.5	1.6	1.0
Tar Heel II + Shade		3.7	3.7	3.5	2.6		3.9	3.3	2.7	2.6
Wolfpack	3.4	3.6	3.3	3.1	2.7	2.7	3.2	3.3	2.4	2.6
Wolfpack + Salts		3.6	3.0	2.4	1.3		2.9	2.7	1.8	1.1
Wolfpack + Shade		4.3	4.0	3.4	3.4		4.0	3.7	3.6	3.6
$LSD_{0.05}$	NS	0.6	0.5	0.7	1.0	NS	0.5	0.8	1.3	1.1

† WAT, week after treatment.

Table 3.5. Malondialdehyde (MDA) content of the 4th leaves of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and CaCl$_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009			2010				
Treatment	0WAT†	1WAT	2WAT	0WAT	1WAT	2WAT	3WAT	4WAT
	ng g^{-1}							
Tar Heel II	6.60	5.75	14.61	3.72	5.13	5.10	7.85	8.34
Tar Heel II + Salts		5.49	44.53		9.44	43.16	76.44	84.64
Tar Heel II + Shade		5.31	5.38		4.62	4.67	5.98	5.85
Wolfpack	5.18	6.28	12.78	3.95	5.15	4.70	6.90	10.72
Wolfpack + Salts		6.62	47.85		13.94	26.87	65.72	64.12
Wolfpack + Shade		4.34	4.93		3.92	3.55	5.40	5.22
LSD$_{0.05}$	NS	2.14	8.15	NS	3.15	18.23	16.39	26.14

† WAT, week after treatment.

the MDA of the 4th leaves of tall fescue under shade stress was lower than that of the control indicating that senescence was not necessarily accelerated in the 4th leaf using MDA as an indicator (Table 3.5).

The Tr rate of the 4th leaves showed different trends for shade and salinity treatments compared to the control (Table 3.6). The Tr rates decreased under salinity stress and the decrease depended not only on the time after salt treatment but also influenced by the environmental conditions as indicated by the variations over different dates of measurements. The decrease caused by the salt treatment was more dramatic in the 2010 study but rather gradual in the 2009 study (Table 3.6). The Tr rates of plants were higher in the shade treatment than the control in 2009 but lower than the control starting at 2 WAT in the 2010 study. The reason for the difference between the two years was not clear except that the relative humidity during the measurements taken in 2010 ranged from 40 to 60% while that in the 2009 ranged from 50 to 70%. Clearly, the plants had different adaptation strategies under the shade and salinity treatments regarding conservation of water. The g_s of the 4th leaf (Table 3.7) of tall fescue followed a similar trend as Tr as both regulated by stomatal resistance.

Leaf surface water wetting angle is often used as an indirect estimation of epicuticular wax accumulation on the leaf surface (Bolger et al., 2005). Salt treatments resulted in larger wetting angle on the adaxial side of the 5th leaves compared to the control and shade treatments in both 2009 and 2010 studies (Fig. 3.1). The results suggested that the salt treated plants accumulated more wax on the abaxial leaf surface than those in the shade and untreated control. There was no difference in wetting angles on the abaxial side of the leaves. No difference was detected between shade and untreated control (Table 3.8).

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Table 3.6. Transpiration rate (Tr) of the 4th leaves of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT†	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
	……………………………mol m^{-2} s^{-1}……………………………									
Tar Heel II	0.081	0.078	0.046	0.041	0.046	0.037	0.046	0.018	0.025	0.006
Tar Heel II + Salts		0.078	0.039	0.024	0.012		0.031	0.002	0.000	0.000
Tar Heel II + Shade		0.080	0.059	0.045	0.048		0.046	0.010	0.014	0.012
Wolfpack	0.080	0.080	0.041	0.039	0.042	0.032	0.039	0.019	0.025	0.009
Wolfpack + Salts		0.072	0.036	0.029	0.000		0.033	0.002	0.000	0.000
Wolfpack + Shade		0.081	0.046	0.042	0.058		0.061	0.012	0.013	0.016
$LSD_{0.05}$	NS	NS	NS	0.012	0.016	NS	0.019	0.004	0.004	0.005

†WAT, week after treatment.

Table 3.7. Stomatal conductance (g_s) of the 4th leaves of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and CaCl$_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	0WAT†	1WAT	2WAT	3WAT	4WAT	0WAT	1WAT	2WAT	3WAT	4WAT
					...............µmol m^{-2} s^{-1}...............					
Tar Heel II	0.121	0.117	0.070	0.062	0.070	0.056	0.070	0.028	0.015	0.009
Tar Heel II + Salts		0.116	0.059	0.037	0.018		0.048	0.003	0.000	0.000
Tar Heel II + Shade		0.118	0.089	0.068	0.073		0.070	0.016	0.019	0.019
Wolfpack	0.119	0.119	0.062	0.059	0.064	0.049	0.059	0.029	0.014	0.014
Wolfpack + Salts		0.108	0.055	0.044	0.000		0.050	0.003	0.000	0.000
Wolfpack + Shade		0.121	0.069	0.064	0.087		0.091	0.019	0.024	0.024
LSD$_{0.05}$	NS	NS	0.022	0.018	0.024	NS	0.028	0.007	0.008	0.008

† WAT, week after treatment.

Table 3.8. Surface water wetting angles on the 5th leaves of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and CaCl$_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009		2010	
Treatment	Adaxial	Abaxial	Adaxial	Abaxial
	………………………	…………………°	………………………	………………………
Tar Heel II	122.2	69.6	107.4	61.7
Tar Heel II + Salts	146.3	55.3	115.4	56.9
Tar Heel II + Shade	130.9	64.7	110.9	62.4
Wolfpack	125.9	68.2	112.1	56.0
Wolfpack + Salts	151.9	60.2	130.2	59.4
Wolfpack + Shade	131.1	67.0	111.4	67.3
LSD$_{0.05}$	11.4	NS	3.8	NS

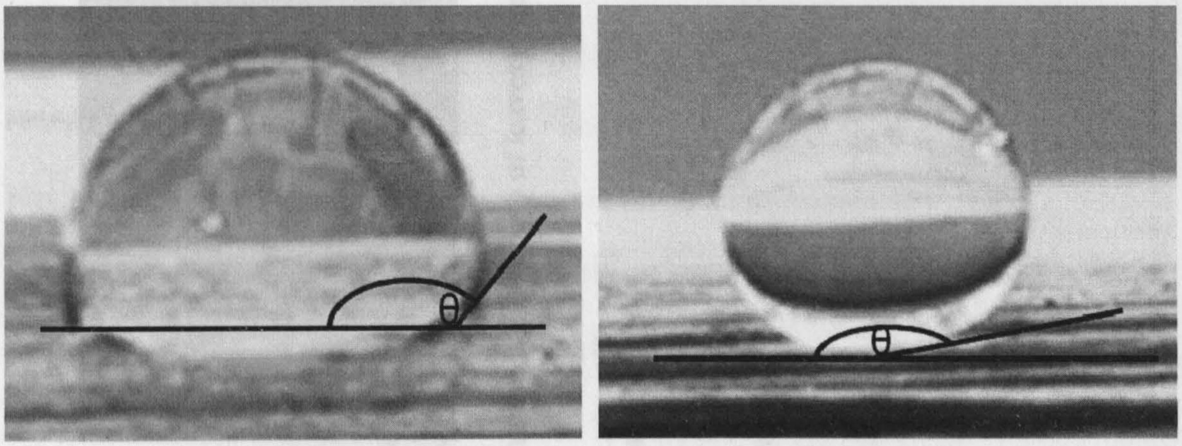

Figure 3.1. Measurement of leaf surface water wetting angle.

The hypothesized epicuticular wax accumulation on the leaf surface under salinity stress was confirmed by SEM images of the leaf surface (Fig. 3.2). The accumulation of epicuticular wax as a means to conserve water by leaves under drought or other stress was reported in other grasses (Bolger et al., 2005; Garcia et al., 2002; Honour et al., 2009;

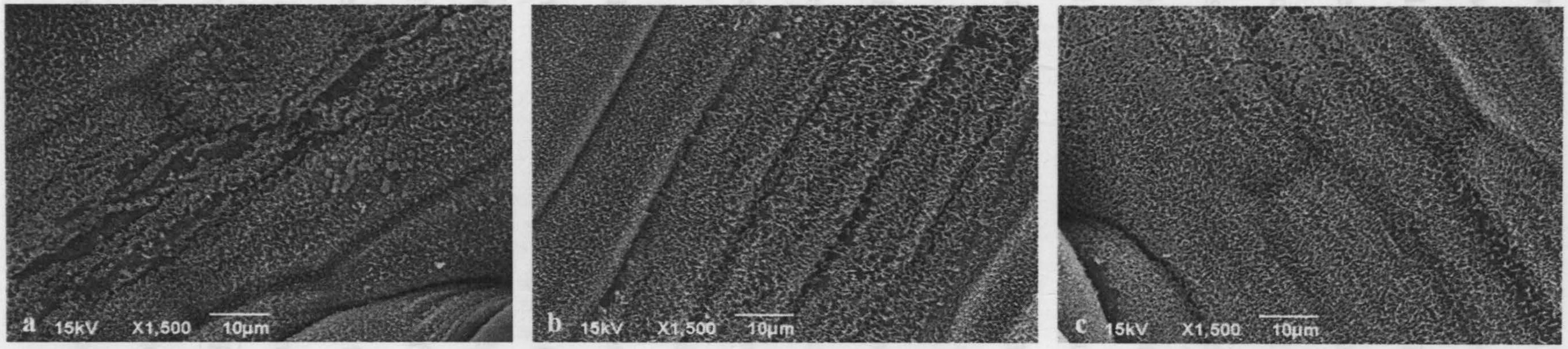

Figure 3.2. Epicuticular wax accumulation on the adaxial surface of leaves under (a) natural aging, (b) shade, and (c) salinity.

Jefferson, 2008; Richardson, et al., 2005).

The vegetation indices calculated from reflectance spectrum for the 4th, 5th, and 6th fully developed leaves showed that salinity stress was more obvious on the older leaves (4th), while shade stress was more obvious on the younger leaves (6th) compared to the control, as indicated by high $mSR_{750/705}$ and $SI_{710/760}$, or low $mND_{750/705}$ (Fig. 3.3, 3.4). The stress levels of the 5th leaves for both shade and salinity fell between the levels of 4th and 6th leaves. This showed different adaptation strategies between shade and salinity stress. The trend also was confirmed by the quantum yield (F_v/F_m) data, where low F_v/F_m ratio indicated higher stress levels (Fig. 3.3, 3.4).

The cellular ultra structure (Fig. 3.5) from the TEM showed better integrity of grana and thylakoids in chloroplasts that were in a spherical shape and distributed close to the center of the cell in untreated control. The grana and thylakoids in the cells of salt treated plants showed signs of deterioration and less clear thylakoid membranes. The chloroplasts were more oblong and distributed along the cell wall in shaded plants with thinner thylakoids, indicating adaptation for more light harvesting.

In general, tall fescue had different adaptions to salinity stress and shade stress with respect to water. Decreasing RWC was only found in salt treated plants. Stomatal conductance and Tr also decreased as a means of water conservation in tall fescue under salinity stress while the opposite was true for tall fescue in the shade except under very dry conditions. Leaf epicuticular wax accumulation, as another means of water conservation, also was shown substantially increased in salt treated plants.

As plant aged and senesced, Chl decreased, while MDA increased. However, the content of Chl diminished much slower in shade treated plants than salt treated plants. In certain

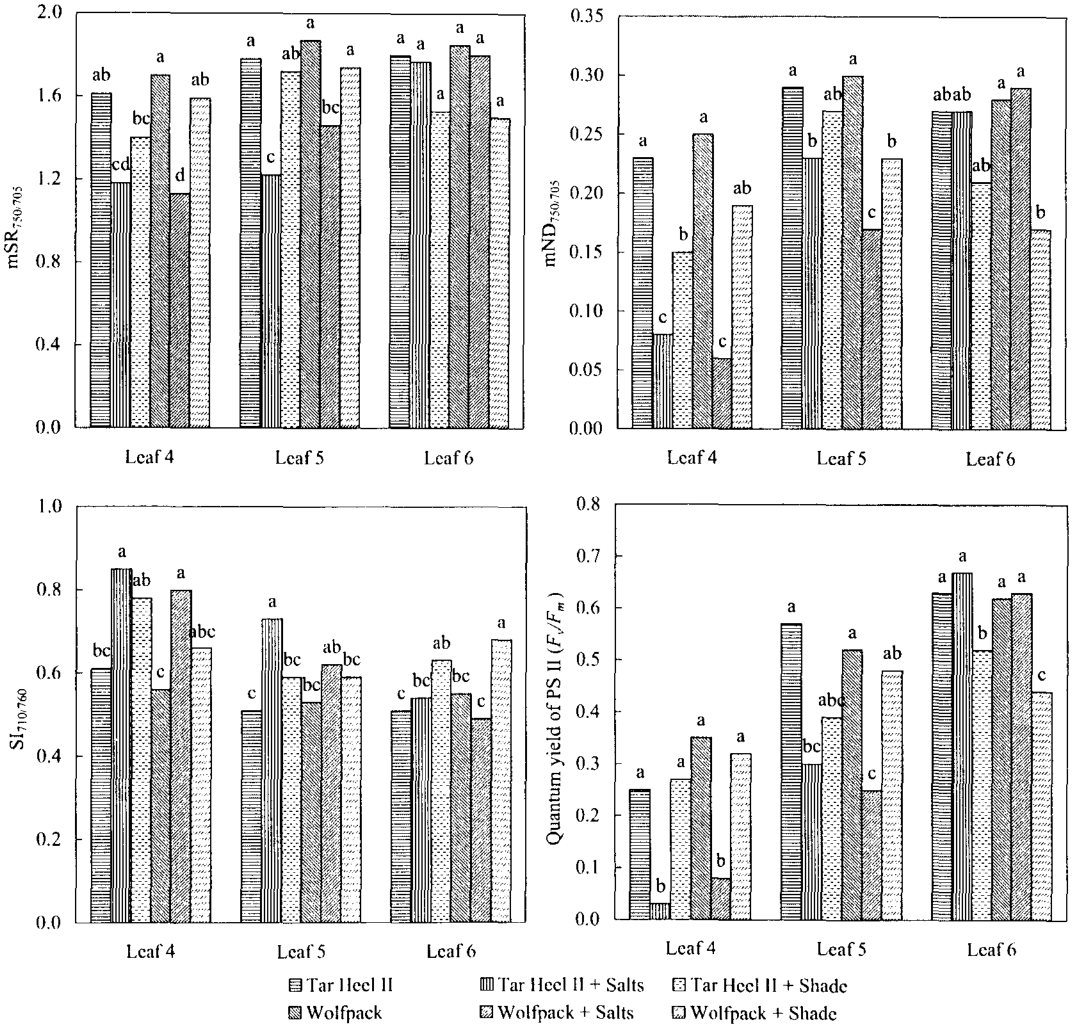

Figure 3.3. Three reflectance indices, $mSR_{750/705}$, $mND_{750/705}$, and $SI_{710/760}$, calculated from the relative reflective spectra, and the quantum yield (F_v/F_m) of the 4th to 6th leaves of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage conducted in 2009.

cases, Chl content of plants under shade might increase compared to the control as a means of adaptation. The Chl level in shaded plants may vary with genotypes. Although MDA increased as tall fescue aged or because of the salinity stress, shade treatment did not result in MDA increase at the level of shade used in this study.

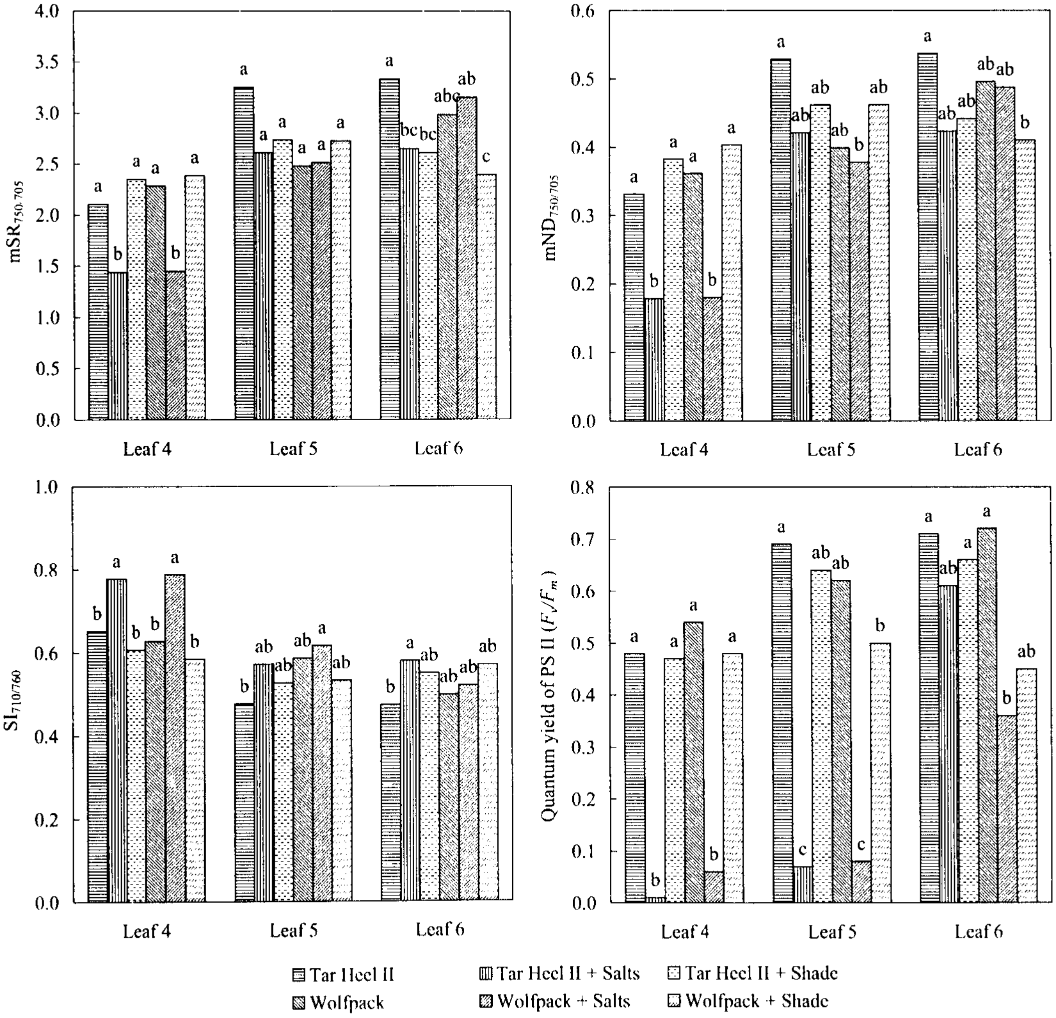

Figure 3.4. Three reflectance indices, $mSR_{750/705}$, $mND_{750/705}$, and $SI_{710/760}$, calculated from the relative reflective spectra, and the quantum yield (F_v/F_m) of the 4th to 6th leaves of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage conducted in 2010.

Salinity and shade stress appeared differently in older leaves than younger leaves. Judging by the vegetation indices, shade was more stressful than salinity in younger leaves while salinity was more stressful than shade for older leaves. Younger leaves are sinks for photosynthate and Chl synthesis and chloroplast development requires light (Salisbury and

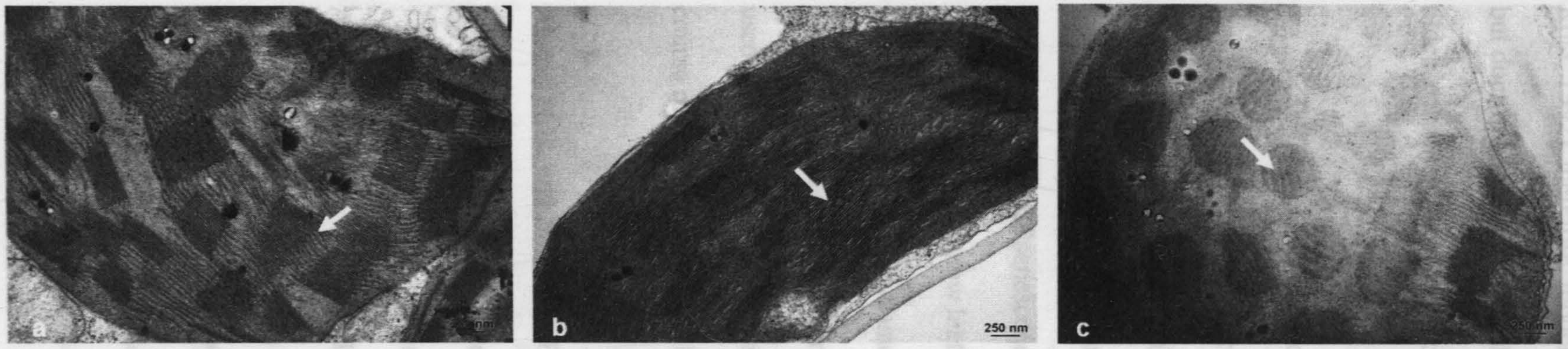

Figure 3.5. Thylakoids (indicated by arrows) in the leave mesophyll cells under (a) natural aging, (b) shade, and (c) salinity.

Ross, 1992), therefore, moderate shade inhibits the development and growth of new leaves, but does not necessarily accelerate the senescence of mature leaves. On the other hand, older leaf senescence was accelerated by salinity stress.

Conclusions

Tall fescue leaf senescence was accelerated by salinity stress which was different from the aging process under shade. Tall fescue had several adaptation strategies in order to conserve water under salinity stress, while the plants under shade had adaptation mechanisms centered on light harvesting. It seems that under salinity stress, tall fescue accelerated old leaf senescence, whereas under shade conditions, tall fescue showed slowing down in the new leaf development as well as mature leaf aging. The contrasting mechanisms of tall fescue under shade and salinity warrant further study to investigate the performance of species under both shade and salinity stress that is likely to happen when used for its shade tolerance and irrigated with recycled water, such as the case in golf course rough, landscapes, or home lawns.

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CHAPTER 4. COMPARATIVE GROWTH RESPONSES OF TALL FESCUE CULTIVARS TO SALINITY AND SHADE STRESS

Abstract

Tall fescue has good tolerance to shade and soil salinity. Those stresses often interact to make the turfgrass management more challenging. Salinity or shade reduces the turfgrass quality, such as density and texture, which naturally requires improvement as proposed by turfgrass breeders. With increased use of recycled water for irrigation, tall fescue may experience extra stress under existing adverse environmental conditions, such as shade and high temperature. The objective of this study was to investigate the growth habit and nutrient uptake of tall fescue under salinity stress as compared to those under reduced light conditions. Two tall fescue cultivars, Tar Heel II (salt tolerant) and Wolfpack (salt sensitive) were seeded in plastic tubes filled with washed silica sand. Salinity treatments were applied by adding NaCl and $CaCl_2$ (1:1 w/w) to the Hoagland solution in 16 000 mg L^{-1} with an electrical conductivity (EC) of approximately 25 dS m^{-1} at the 4-leaf stage. A control was included with plants receiving Hoagland solution only. Tall fescue leaf senescence was accelerated by salinity stress which was different from the aging process under shade. Tall fescue growth responded to salinity stress differently from shade stress, such as leaf length, root to shoot ratio. Leaf appearance rate may be different with stress tolerance levels and mediate the tiller number and shoot density. By comparing and contrasting the effects on nutrients uptake, it was shown that K, Ca, Mg may be very important in the ion balance and salinity stress tolerance.

Introduction

Tall fescue has been increasingly used as turfgrass since the middle of last century due to intensive breeding efforts. Its wide adaptation and improved genetic traits made the species the grass of choice for medium or low maintenance uses (Watkin et al., 2010) and for the transition zone where extreme temperatures and drought predominate (Funk and Clarke, 1989). In addition to the breeding contributions, physiological studies since the 1980s revealed many aspects of stress tolerance in tall fescue including heat, drought, shade, salinity, and wear (Beard, 1981). Tall fescue has good tolerance to shade (Beard, 1969; Gardner and Taylor, 2002) and soil salinity (Lunt et al., 1961). Those stresses often interact to make the turfgrass management more challenging. Salinity or shade reduces the turfgrass quality, such as density and texture (Dean et al., 1996), which naturally requires improvement as proposed by turfgrass breeders (Hopkins et al., 2009). Tall fescue density is affected by tiller and leaf number. Tiller development depends largely on environmental conditions and cultural practices. Tall fescue tillering rate is limited by and negatively correlated to leaf appearance (Templeton et al., 1961). Tillering rate was not affected by water soluble carbohydrates in the leaves, roots, or stem base tissues (Zarrough et al., 1984). However, shoot biomass is closely related to the tiller carbohydrate reserve and photosynthesis capacity of leaves (Smith, 1973). Usually three leaves per tiller are active at any time (Alburquerque, 1967) and individual leaves are photosynthetically active for about 6 weeks during spring and summer (Jewiss and Woledge, 1967).

Nutrient uptake also affects biomass, tiller density and leaf number (Beard, 1985; Pitman, 1999). Increasing N rates commonly result in increased tall fescue biomass. Nitrate-N accumulates when total protein maximizes. Maximum growth of tall fescue was obtained at 0.09 to 0.11% NO_3-N (Duncan et al., 1969). Lechtenberg et al. (1972) found that N deficiency resulted in accumulation of nonstructural carbohydrates and restricted growth but did not affect the diurnal trend of sucrose and fructosan contents. When available soil K levels were at 160 kg ha^{-1} or less, Mg concentration increased from around 0.2 to 0.3%; while when available K increased above 160 kg ha^{-1} tissue Mg maintained at about 2.0% (Wilkinson and Mays, 1979). Gross (1973) ranked tall fescue as a high Mg user among the cool-season grasses. Although DeWit et al. (1963) reported critical levels for Ca and Mg in grass to be about 0.1 and 0.06%, respectively, no exact levels are known except twice the amount of Ca as Mg is usually recycled through harvesting (Wilkinson and Lowrey, 1973).

Under salinity stress, tall fescue nutrient uptake and growth rate are affected (Bowman et al., 2006; Lunt et al., 1961). The detrimental effects of salinity can be attributed to toxicity of excessive Na^+, Cl^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-, and BO_3^-, as well as nutrient imbalance and deficiency (Bowman et al., 2006). High levels of salinity disrupt ion homeostasis in plants by inhibiting the uptake of essential nutrients like K^+, Ca^{2+}, NO_3^-, and excessive accumulation of Na^+ and Cl^- (Lacerda et al., 2003; Marschner, 1995; Zhu, 2001). As a consequence, cell division and elongation decline or stop completely (Hasegawa et al., 2000). Salinity reduced the uptake and partitioning of N to leaves and increased the retention of N in the roots of tall fescue (Bowman et al., 2006). Fast root growth and low Na^+/K^+ ratio are attributed to salt tolerance in Kentucky bluegrass cultivars compared to sensitive ones (Qian, et al., 2001).

With increased use of recycled water for irrigation, tall fescue may experience extra stress under existing adverse environmental conditions, such as shade and high temperature. Recycled waters, such as reclaimed municipal effluent and brackish drainage water typically have elevated salt levels (Miyamoto and Chacon, 2006). There is a renewed interest in understanding how tall fescue growth and tissue mineral nutrients uptake are affected by salinity stress and their influence on turf quality. The objective of this study was to investigate the growth habit and nutrient uptake of tall fescue under salinity stress as compared to those under reduced light conditions.

Materials and Methods

The experiment was conducted in a greenhouse at North Dakota State University from September, 2009 to July, 2010. Two tall fescue cultivars, Tar Heel II (salt tolerant) and Wolfpack (salt sensitive) (Wipff and Rose-Fricker, 2003), were seeded in plastic tubes measuring 4 cm in diameter and 20 cm in depth with silica sand as growth medium. The plants were maintained in a greenhouse with 25/15 °C (day/night), 14-h photoperiod, and an average PAR of 400 μmol m^{-2} s^{-1}. The sand growth medium was maintained at field capacity by watering twice a day until the seeds germinated when the seedlings were watered once a day. The seedlings were fertilized by watering with half strength Hoagland solution (Hothem et al., 2003) in 10 ml per tube twice a week. At the 2-leaf stage, the plants were thinned to keep one plant in each tube.

At the 4th leaf stage, the experimental treatments were initiated. Full strength Hoagland solution was applied to the sand medium in 20 ml per tube every other day. The salt treatment was applied by adding 16 000 mg L^{-1} of of NaCl and $CaCl_2$ to the full strength Hoagland solution to reach an EC about 25 dS m^{-1}. The shade treatment was applied by covering with a black knitted polyethylene cloth that allowed 30% passing of light. Plants that received Hoagland solution only were included as control. The experimental design was a randomized complete block with three replicates.

On the day prior to the initiation of treatments and weekly thereafter, tiller number and leaf number on the primary shoot of the seedlings were recorded. A tiller was defined as one shoot with at least one fully developed leaf. A leaf with ligule and auricles exposed was considered as fully developed. Leaf appearance rate (LAR) (number of leaves plant^{-1} d^{-1}) and tiller appearance rate (TAR) (number of new tiller plant^{-1} d^{-1}) were calculated based on the leaf and tiller number data.

At the end of four weeks after treatment (WAT), the plant height was measured from 10 plants in each treatment unit by measuring the distance from the soil surface to the tip of the youngest fully developed leaf on the primary shoot.

At the end of each experiment, the shoot biomass was harvested by clipping at the soil surface. The roots were harvested by washing the sand off on a 2-mm sieve with tap water. The root and shoot dry mass were recorded after drying at 68°C for 48 h. The dry tissues were ground to pass 0.178 mm for tissue nutrient analysis. The K, Ca, Mg, Na, and Fe of the tissues were analyzed using an AA7000w flame atomic absorption spectrophotometer (Beijing East & West Electronic Company, Beijing China) following dry ashing in a muffle furnace at 490°C for 8 hours and digestion with 5 *M* aqua regia (Greweling, 1976; Jones et al., 1991).

The data were subjected to analysis of variance (ANOVA) using mixed procedures in SAS 9.2 (SAS Institute, 2008) with replication blocks treated as a random variable. Treatment means were separated using Fisher protected least significant difference (LSD) at 0.05 probability level. Homogeneity test revealed different variance between the results of 2009 and 2010. Therefore, the results are reported separately.

Results and Discussion

Both salt and shade treatments reduced tiller number and leaf number compared to the untreated control in 2009 and 2010 study (Table 4.1). Plants treated with salts were shorter than the control in 2010 and were not different from the control in 2009 (Table 4.1). 'Wolfpack' in shade treatment were taller than those in the control and salt treatment in both 2009 and 2010. 'Tar Heel II', on the other hand, showed no difference in plant heights among treatments (Table 4.1). Similar results were reported in tall fescue and Kentucky bluegrass under salinity stress (Alshammary et al., 2004). Leaf length of warm-season grasses also decreased as a result of salinity stress (Chen et al., 2009; Muscolo et al., 2003). The increase of plant height under shade was mainly due to the elongation of young leaves as commonly happens in many plants (Beard, 1997). The result corroborates

Table 4.1. Growth features of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

Treatment	2009 Tiller number†	2009 Leaf number‡	2009 Plant height§ ….cm….	2010 Tiller number	2010 Leaf number	2010 Plant height ….cm….
Tar Heel II	8.8	7.0	14.47	11.6	7.1	13.07
Tar Heel II + Salts	5.8	6.5	14.13	3.8	5.7	11.71
Tar Heel II + Shade	4.7	6.4	14.68	5.9	6.3	13.31
Wolfpack	10.5	7.2	13.70	13.0	7.2	15.39
Wolfpack + Salts	6.4	6.5	13.25	3.5	5.3	12.13
Wolfpack + Shade	5.7	6.4	15.87	4.9	6.1	18.50
$LSD_{0.05}$	3.1	0.5	1.51	2.0	0.5	2.24

† Tiller number, total tiller number per seedling.

‡ Leaf number, number of leaves on the main shoot of the seedling.

§ Plant height, measured to the tip of the youngest mutual leaf on the main shoot of the seedling.

previous results for tall fescue (Allard et al., 1991; Woledge, 1971). The different response to shade between the two cultivars suggests possible genetic variation in shade tolerance. The salinity response was not different between the two cultivars, and that disagreed with Wipff and Rose-Fricker (2003) who used mature plants for the test while this study involved the seedling stage. The other reason may be from the different salts that were used: the ocean salt mix was used in previous study instead of NaCl and $CaCl_2$ as in this study.

Both shoot and root biomasses decreased in the salt treatment and shade treatment as compared to the control (Table 4.2). Decreased biomass of tall fescue in shade as associated with reduction of tiller number and leaf number and their weight also were reported by Allard et al. (1991). The root/shoot ratio decreased in the shade treatment compared to the control (Table 4.2), indicating relatively more allocation of photosynthate to shoot under reduced irradiance. This finding was in agreement with other studies (Allard et al., 1991; Beard, 1969). In the salinity treatment, root/shoot ratio showed no change, which is in agreement with the results of Alshammary et al. (2004), who reported a positive quadratic regression relationship between root/shoot ratio and EC. In contrast, the root/shoot ratio of the euhalophyte species *Sporobolus virginicus* showed negative quadratic regression with EC (Bell and O'Leary, 2003; Chen, et al., 2009; Muscolo et al., 2003).

The TAR and LAR of the plants in salt and shade treatments were lower than the control in both 2009 and 2010 as indicated by the slope of tiller number and leaf number changes over time (Fig. 4.1), where larger slopes meant higher rate. In both years, TAR was more affected by stresses than LAR. Similar results were reported in a warm-season

Table 4.2. Biomass of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009			2010		
Treatment	Shoot	Root	Root/shoot	Shoot	Root	Root/shoot
	.....g....			....g.....		
Tar Heel II	27.5	9.73	0.35	37.7	16.31	0.44
Tar Heel II + Salts	19.4	6.23	0.32	8.7	3.19	0.38
Tar Heel II + Shade	16.2	4.30	0.27	17.9	3.67	0.21
Wolfpack	28.5	9.76	0.34	40.1	15.45	0.39
Wolfpack + Salts	19.7	6.69	0.34	8.8	3.10	0.35
Wolfpack + Shade	17.4	3.80	0.22	19.1	5.35	0.28
$LSD_{0.05}$	2.3	1.28	0.05	3.4	1.18	0.08

slope, a break point, between stage one and stage two after certain days of exposure to salinity (Harris et al., 2010). The present study showed tall fescue had a similar trend of changing slope for LAR in salinity treatment in 2010 study although a break point could not be clearly established to associate with plant development stage as in barley or wheat. The leaf and tiller appearance rate of the plants in the salt treatment were also lower than those in the shade treatment in 2010 study (Fig. 4.1), indicating different allocation of photosynthate under salt and shade stress in tall fescue. No difference between the two cultivars was detected.

The adaptation and impact on turf quality of this finding warrant further study. For example, selection of long- or short leaved-perennial ryegrass (*Lolium perenne* L.) under shade conditions based on leaf elongation rate and leaf length did not result in reduced tiller number per plant because of the traits were more mediated by LAR (Bahmani, et al., 2000). Similar strategies may be used in tall fescue breeding if it followed a same pattern. Salt treatment reduced the uptake of K and Mg, but increased the uptake of Na and

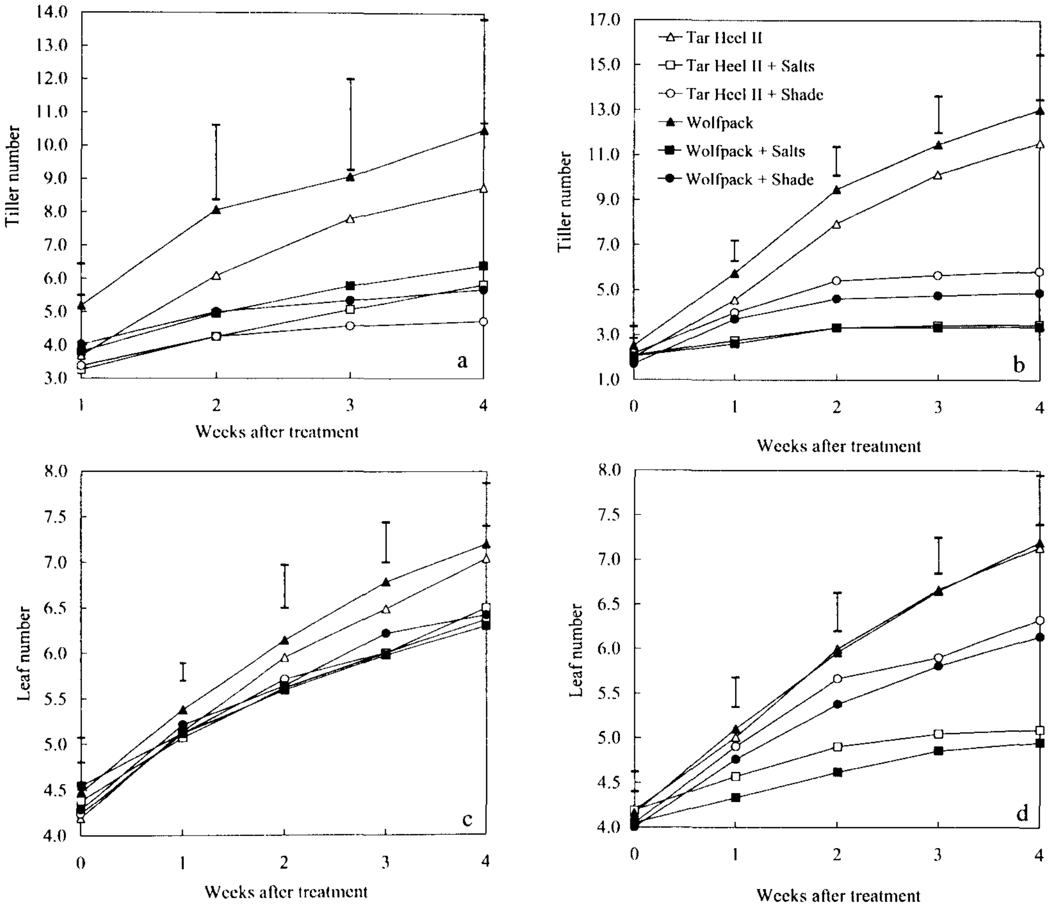

Figure 4.1. Tiller and leaf appearance after initiation of salinity or shade treatment in two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage. a) and c), study in 2009. b) and d), study in 2010. Vertical bars indicate least significant difference at 0.05 probability level between treatments within a given week after treatment.

Ca in the shoot in both 2009 and 2010 (Table 4.3). Shoot Na increased by approximately 1.5 times, while Ca increased by 2 times in 2010 and 7 times in 2009 (Table 4.3). Since Na and Ca was applied in 1:1 ratio (W/W), it appeared that tall fescue shoot selectively uptook Ca under salinity stress. These results agree with those of Lunt et al. (1961) found in ‘Alta’ tall fescue, and those of Torello and Rice (1986) found in red fescue (*F. rubra* L.) and Kentucky bluegrass.

Table 4.3. Shoot tissue nutrients analysis of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage..

	2009					2010				
Treatment	K	Na	Ca	Mg	Fe	K	Na	Ca	Mg	Fe
	……………………………….mg kg^{-1}………………………………									
Tar Heel II	615.0	275.3	794.6	218.8	524.5	554.1	359.1	1749.7	75.0	370.3
Tar Heel II + Salts	578.3	416.0	5624.6	180.7	444.6	519.5	544.8	3409.2	35.5	396.7
Tar Heel II + Shade	607.2	312.3	941.5	221.3	558.8	550.4	332.0	1679.2	64.4	365.6
Wolfpack	604.1	294.4	1010.6	221.8	447.4	553.3	329.5	1829.2	70.6	356.0
Wolfpack + Salts	579.9	420.5	6707.3	181.1	393.1	521.0	544.7	3441.4	27.1	376.8
Wolfpack + Shade	608.3	281.0	795.7	215.5	448.3	547.2	322.4	1647.1	65.4	312.1
$LSD_{0.05}$	21.1	26.3	1337.6	7.1	NS	4.7	41.2	441.5	25.6	NS

The effects of salinity on mineral nutrients in leaves vary with species. For example, high exogenous Na^+ causes decreases in K^+, Ca^{2+}, and Mg^{2+} in the leaves of Kentucky bluegrass (Qian et al., 2001), K^+ and Ca^{2+} concentrations in leaf tissues of barley (Greenway and Munns 1980; Halperin et al., 1997) and maize (Neves-Piestun and Bernstein, 2005), and K^+ concentration in the leaves of chewing fescue (*F. rubra* L.) (Khan and Marshall, 1981). On the other hand; Hu and Schmidhalter (1998) reported that K^+, Ca^{2+} and Mg^{2+} concentrations were increased by Na^+ in wheat. The Na^+:K^+ ratio in some warm-season grasses increased with increasing NaCl content in soil (Chen et al., 2009). In the shoot of euhalophyte species *S. virginicus*, Na^+ and K^+ increased, Ca^{2+} decreased, Mg remained at same levels, and Na^+:K^+ ratio increased, after grown in 450 m*M* of NaCl (Bell and O'Leary, 2003).

Iron uptake in the shoot was not affected by salt treatment in either 2009 or 2010 (Table 4.3). Shade treatment did not affect shoot uptake of K, Ca, Mg, Na, and Fe in either year (Table 4.3). Under shade conditions, K, Ca, and Mg content in the shoot of cocksfoot (*Dactylis glomerata* L.) increased in different seasons (Peri et al., 2007). Increased K content also was reported in *D. glomerata* and *F. ovina* under 50% and 90% shade (Koukoura et al., 2009).

Salinity treatment increased the Na uptake in roots by approximately 2.5 times in both 2009 and 2010 (Table 4.4). However, unlike the case in shoots, Ca content in roots decreased under salinity stress. This may be caused by more transport of Ca to shoot which might be involved in the mechanism of countering the toxic effect of Na. The Mg content in roots showed a decreasing trend and was only significant in the 2010 study. The Fe content in roots also showed a decreasing trend but was significant only for 'Wolfpack'

Table 4.4. Root tissue nutrient analysis of two tall fescue cultivars irrigated with full strength Hoagland solution either containing 16 000 mg L^{-1} NaCl and $CaCl_2$, or shaded to allow 30% passing of full light at the onset of 4th leaf seedling stage.

	2009					2010				
Treatment	K	Na	Ca	Mg	Fe	K	Na	Ca	Mg	Fe
			……………………… mg kg^{-1} ………………………							
Tar Heel II	565.5	933.4	4175.9	165.9	913.5	393.5	1059.2	8034.1	183.8	915.0
Tar Heel II + Salts	581.0	2247.2	3357.1	153.7	795.1	378.3	2580.1	7965.0	160.1	803.5
Tar Heel II + Shade	561.7	1008.4	2209.4	149.9	900.2	402.9	1069.8	3665.1	171.5	859.1
Wolfpack	570.0	952.5	3910.1	160.1	899.8	405.6	1143.0	8957.7	197.7	854.7
Wolfpack + Salts	582.1	2330.1	3732.5	154.5	760.1	378.2	2592.9	8413.4	157.8	808.1
Wolfpack + Shade	583.9	940.1	3064.5	168.4	915.2	394.8	1041.0	5161.7	183.6	914.6
$LSD_{0.05}$	13.9	121.8	709.5	NS	129.1	15.9	107.9	742.7	22.3	93.4

in 2009. The K content in roots showed no consistent trend, it increased in 'Tar Heel II' in 2009 but decreased in 'Wolfpack' in 2010 (Table 4.4). Increased Na^+:K^+ ratio was reported in the root cell vacuoles of salt-tolerant tall fescue (Zhao et al., 2007). The Na^+:K^+ ratio in the roots of warm-season grasses also increased with increasing soil NaCl content (Chen et al., 2009). In the roots of *S. virginicus*, Na^+ increased, Ca^{2+}, Mg^{2+}, and K^+ decreased, and Na^+:K^+ ratio increased, after grown in 450 m*M* of NaCl (Bell and O'Leary, 2003). Under shade treatment, Ca content in roots decreased compared to the control, while other elements were not affected (Table 4.4). The function of Ca in mediating stress tolerance needs further study.

The growth pattern of tall fescue was affected by salinity stress and some of the characteristics may be used in breeding selection, such as the biomass ratio of root/shoot, TAR, and LAR. The nutrient uptake of K, Na, Ca, and Mg in the shoot under salinity stress did not show clear pattern and may be controlled by the antagonism between the nutrients at the sites of uptake in the roots, or the inhibitive effect of Na on transport of K and Mg in xylem (Lych and Lauchli, 1984; 1985), or other active transportation affected by H^+-ATPase activity and hormones (Gronwald et al., 1990; Van Steveninck, 1972). The understanding of nutrient uptake affected by salinity is further complicated by the different growth and development patterns of leaf and tiller, and the effect of nutrient movement between tissues and organs. The complexity also is demonstrated by differences among species, cool-season vs warm-season, and glycophytes vs halophytes. Such diversity is not only shown in saline conditions but also in shade conditions.

Conclusions

Tall fescue growth responded to salinity stress differently from shade stress, a fact that has to be taken into consideration when selecting for stress tolerant traits, such as leaf length, root to shoot ratio. The root/shoot ratio may even be different on species level. Leaf appearance rate may be different with stress tolerance levels and mediate the tiller number and shoot density. Evaluation of LAR at different salt levels may provide more information about the role it plays in regulating tiller density.

Nutrient uptake under salinity stress varies with species. Since the nutrient uptake involves many steps and is very delicately regulated, it is important to study those essential elements and the function in salinity stress tolerance. By comparing and contrasting the effects on nutrients uptake, it was shown that K, Ca, Mg may be very important in the ion balance and salinity stress tolerance.

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CHAPTER 5. DIFFERENTIATION OF CARBONATE, CHLORIDE, AND SULPHATE SALINITY RESPONSES IN TALL FESCUE

Abstract

Managing turfgrass under salinity stress is becoming more and more important with the increasing use of recycled water for irrigation. Tall fescue is a cool-season turfgrass with moderate to high tolerance to salinity. Very little research investigated different anions and cations simultaneously to determine the underlying mechanism of salinity stress. The objective of this study was to compare physiological responses of tall fescue leaves to carbonate, chloride, and sulfate under iso-osmotic, iso-Na^+ strength conditions. The study was conducted in April to July, 2010. Two tall fescue cultivars, Tar Heel II (salt tolerant) and Wolfpack (salt sensitive) were seeded in plastic tubes using washed sand as growth medium with pH of 7.7 and EC of 0.04 dS m^{-1}. Four salts, NaCl, Na_2CO_3, Na_2SO_4, and $CaCl_2$ were used in the study at 0 to 225 m*M*. Tall fescue leaf firing was mainly caused by an alkaline condition with pH higher than 9 as in Na_2CO_3, or moderate pH combined with high salinity (high EC) as in $CaCl_2$. Plant growth was more affected by sodicity which was high in Na_2CO_3 and Na_2SO_4. The combination of different salts along with the variation of their physical and chemical properties, such as EC, pH, and osmotic potential, made the differentiation of their influence on tall fescue stress rather difficult. Multiple properties, growth, morphological, and physiological, should be measured to better understanding the effects of different salts. Current study showed that evaluating tall fescue for alkali, sodic, and saline soil adaptation was often complicated by interaction of ions and pH.

Introduction

Managing turfgrass under salinity stress is becoming more and more important with the increasing use of recycled water for irrigation (Leskys et al., 1999). The acute water shortage has forced turfgrass managers to look into alternative water sources. Lower prices and a consistent supply make recycled waters very practical alternative for irrigation. Recycled waters, such as reclaimed municipal effluent and brackish drainage water, usually have high levels of salts, which can cause physiological stress to turfgrass and deteriorate soil structure. Saline conditions also occur in salt-affected soils, areas with salt water intrusion (McCarty and Dudeck, 1993; Murdoch, 1987), and roadsides where salts are used for snow and ice removal (Greub et al., 1985). One of the solutions to this problem is to use salt-tolerant species or cultivars. Tall fescue (*Festuca arundinacea* Schreb.) is a cool-season turfgrass with moderate to high tolerance to salinity (Beard, 1973; Marcum, 2006; Wu et al., 1988). It also has a wide range of adaptation to heat, drought, and wide soil pH range (4.7 to 9.0) (USDA-NRCS, 2006), and therefore a great potential to be used in salt affected soils (Barnes, 1990).

The detrimental effects of salinity can be attributed to toxicity of excessive Na^+, Cl^-, SO_4^{2-}, CO_3^-, HCO_3^-, and BO_3^-, as well as nutrient imbalance and deficiency (Bowman et al., 2006). High levels of salinity disrupt ion homeostasis in plants by inhibiting the uptake of essential nutrients like K^+, Ca^{2+}, and NO_3^- and excessive accumulation of Na^+ and Cl^- (Lacerda et al., 2003; Marschner, 1995; Zhu, 2001). One of the symptoms of tall fescue under severe salinity stress is leaf firing (Alshammary et al., 2004), which is a form of chlorosis. Leaf firing can result in direct reduction of turfgrass visual quality. Leaf firing initiates from the tip and margins of a leaf and progresses down to the whole blade.

Meanwhile, the leaf color turns from green to yellow, and to tan/brown in the dead area (Carrow and Duncan, 2003). A visual rating system of leaf firing based on the percentage of leaf area discoloration has been extensively used in salinity and drought tolerance evaluation (Alshammary et al., 2004; Bowman et al., 2006; Chen et al., 2009; Dean et al., 1996; Lee et al., 2005).

It has long been reported salinity problems caused by sulfate and chloride are different (Hanson-Porath and Poljakoff-Mayber, 1970; Meiri et al., 1971). Chloride salt suppressed P uptake and the growth of shoot and root more than sulfate salt in a study with wheat (*Triticum aestivum* L.) (Zahoor et al., 2007). With salinity level at the same electrolyte concentration, sulfate had less reduction impact than chloride on the yield of wheat and rice (*Oryza sativa* L.) (Minhas et al., 2007). Research on French beans (Bhivare and Nimbalkar, 1984) and corn (*Zea mays* L.) (Kostandi and Soliman, 1998) also showed that sulfate is less detrimental to yield. Colmer et al. (1996) reported that external sulfate concentration ranging from 0.1 to 100 mol m^{-3} had no effect on leaf glycine betaine, proline, or asparagine in *Spartina alterniflora* Loisel. In a trial with tomato (*Lycopersicon esculentum* Mill.), K_2SO_4 corrected salinity problems and K deficiency caused by treatment with 60 mol m^{-3} NaCl (Kaya et al., 2002).

In an iso-osmotic study with pepper (*Capsicum annuum* L.), Navarro et al. (2003) found that sulfate salt resulted in more growth reduction than chloride salt due to the higher concentration of sodium and less osmotic adjustment in sulfate. However, the same author reported more yield and quality reduction from sulfate than chloride (Navarro et al., 2002). Sulfate was reported more detrimental to barley yield than chloride (Datta et al., 1994, 1995). Germination of sunflower (*Helianthus annuus* L.) seeds was inhibited by carbonate, followed by sulfate and chloride when the seeds were treated with 10, 20, and 50 mol m^{-3} NaCl and Na_2SO_4, and 5, 10, and 15 mol m^{-3} Na_2CO_3 (Manivannan et al., 2008). In a study with pea (*Pisum sativum* L.), Na_2CO_3 caused the most reduction in osmotic pressure of leaves, followed by Na_2SO_4 and NaCl while the shoot and root dry weight reduction was greater with NaCl, followed by Na_2CO_3 and Na_2SO_4 (Hamdia and Shaddad, 1996).

Clearly, there is no agreement on both the research results and the hypotheses accounting for the responses of plants to different salts. Elucidating the underlying mechanisms of physiological responses to different salts in tall fescue is important for turfgrass management and also may cast light on the understanding of mechanisms of salinity stress tolerance in other grasses. One of the early salinity tests in tall fescue used equal amount of NaCl and $CaCl_2$ up to 21 000 mg L^{-1}, or in different ratios of NaCl and $CaCl_2$ up to 42 000 mg L^{-1}, revealed that Na^+ was more detrimental to the turf quality (Lunt et al., 1961). However, most research on salinity used either NaCl alone, or in combination with $CaCl_2$ (Qian et al., 2001; Robins et al., 2009), a few tests also used simulation of ocean salts (Peacock and Dudeck, 1985; Qian, et al., 2004). Very little research investigated different anions and cations simultaneously to determine the underlying mechanism of salinity stress.

We hypothesized that if osmotic stress is more important, then sodium carbonate, chloride, and sulfate salts should have similar responses at iso-osmotic conditions. If cations are more important, then sulfate should result in more injuries at the same ion concentration strength. The objective of this study was to compare physiological responses of tall fescue leaves to carbonate, chloride, and sulfate under iso-osmotic, iso-Na^+ strength conditions.

Materials and Methods

The study was conducted in April to July, 2010. The growth medium was washed sand with pH of 7.7 and EC of 0.04 dS m^{-1}. Two tall fescue cultivars, Tar Heel II (salt tolerant) and Wolfpack (salt sensitive) (Wipff and Rose-Fricker, 2003), were seeded in plastic tubes (4 cm in diam and 20 cm deep) and watered with distilled water twice a day. Upon germination, the seedlings were watered with half strength Hoagland solution (Hothem et al., 2003) at 10 mL per tube twice a week until the 3-leaf stage. The plants were maintained in a greenhouse with 25/15 °C (day/night), 14-h photoperiod, and an average PAR of 400 μmol m^{-2} s^{-1}. The seedlings were thinned to three plants of uniform size/stage in each tube at the 2-leaf stage.

The experimental treatments were initiated at the 4-leaf stage. Four salts, NaCl, Na_2CO_3, Na_2SO_4, and $CaCl_2$ were used in the study with NaCl at 0, 25, 75, 125, 175, 225 m*M*, while other salts at 0, 25, 50, 75, 100, 125 m*M*. The different concentration range for NaCl was used in order to achieve either a similar range of electric conductivity (EC) or osmotic potential among the four salts (Fig. 5.1). The osmotic potential was measured with a WP4 dewpoint potential meter (Decagon Devices, Inc. Pullman, WA). The treatments were applied with full strength Hoagland solution once a week at 20 mL per tube, which had pH 6.07, EC 1.3 dS m^{-1}, and osmotic potential -0.12 MPa. The plants were watered every two days to maintain the soil moisture at field capacity. The experiment was a split-split plot design with cultivar as main plot, salt type as sub-plot, and salt concentration as sub-sub plot. The main plots were arranged in a randomized complete block design with three replicates.

On the day prior to the initiation of treatments and weekly thereafter, the following.

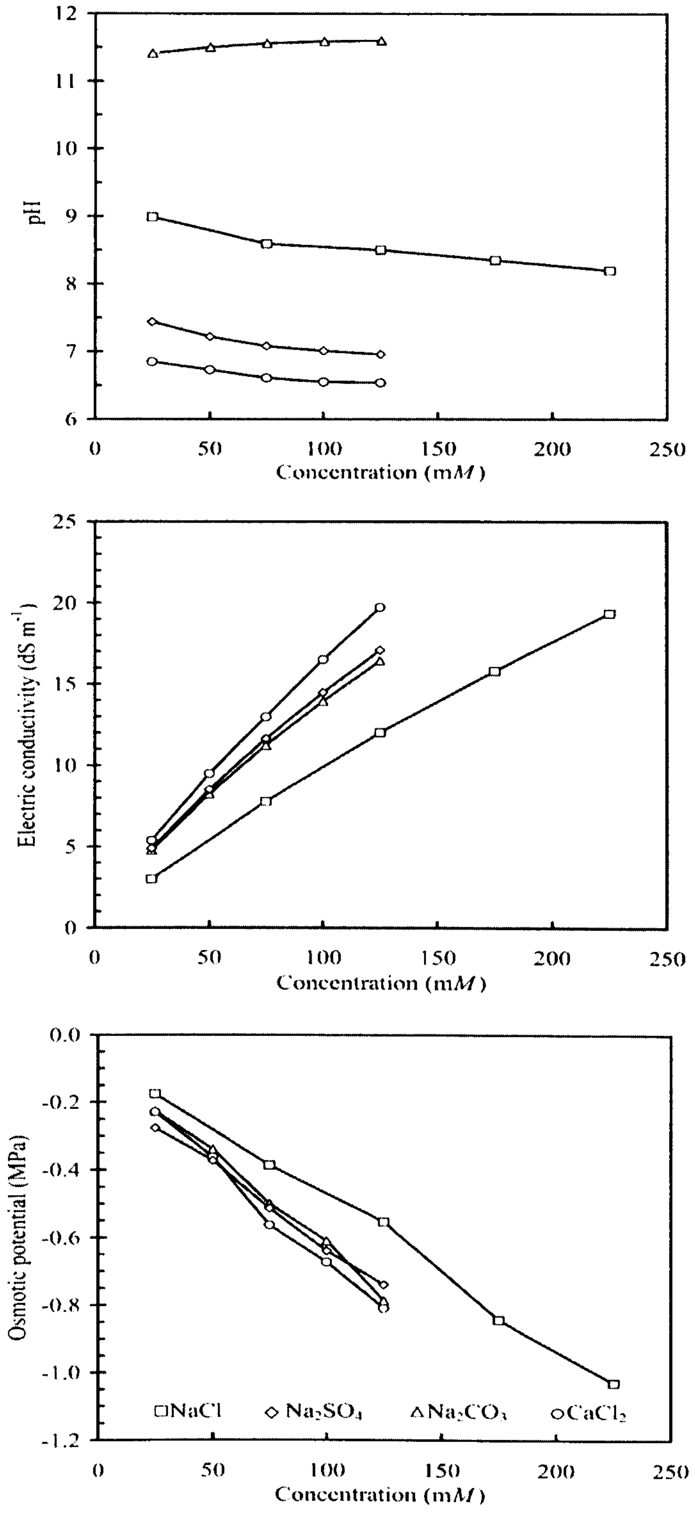

Figure 5.1. Salt properties used for two tall fescue cultivars, Tar Heel II and Wolfpack, at 4th leaf stage, applied with Hoagland solution.

measurements were taken from the 4th leaf of the primary shoot of the seedlings:

Quantum yield (F_v/F_m) of photosystem II (PS II) from three plants in each experimental unit was measured using a portable chlorophyll fluorometer (MINI-PAM, Heinz Walz GmbH, Effeltrich, Germany) with the fiberoptics placed 6 mm from the leaf surface at 60° angle using the leaf-clip.

The same leaves used for quantum yield measurement were clipped on a black background with the adxial side facing up in a leaf chamber that was illuminated by a high intensity halogen light (Warner-lambert Tech. Inc., Buffalo, N.Y.) from the top. The reflectance spectrum from 350 to 1000 nm was collected with an S2000-TR temperature-regulated fiber optic spectrometer (OceanOptics Inc., Dunelin, FL) which has a read fiber inserted in the leaf chamber at 60° to the surface plane and controlled by a computer. Two reflectance indices, $NDVI_{775/675}$ (Volterrani et al., 2005) and $SI_{710/760}$ (Carter, 1994), were calculated from the spectra using the following equation:

$$NDVI_{775/675} = (R_{775} - R_{675})/(R_{775} + R_{675})$$

$$SI_{710/760} = R_{710} / R_{760}$$

where R is relative reflectance at different wave length.

After the measurement, the 4th leaf from six plants were flash frozen in liquid nitrogen and kept under -80°C for the measurement of chlorophyll (Chl) content.

The 4th leaf blades of three different plants in each experimental unit also were collected by cutting at the collar to measure RWC. The leaf samples were immediately weighed to obtain the fresh weight (fw) and then submerged in distilled water to soak for 6 h. The soaked leaves were blot-dried with paper towels before weighing again to obtain the turgid weight (sw). The final dry weight (dw) was obtained by weighing the leaves after drying in an oven at 68°C for 48 h. Leaf RWC was calculated using the equation (Lee et al., 2005) as follows.

$$RWC\ (\%) = (fw - dw)/(sw - dw).$$

For Chl measurement, approximately 0.03 g of each leaf sample was ground and weighed out to a centrifuge tube. To each sample, 2 mL of 80% acetone were added before the tubes were capped and kept in dark at -4°C overnight. The extraction was centrifuged at -4°C and 50 μL of the supernatant were diluted by adding 950 μL of 80% acetone. After thorough mixing, the absorbance of the diluted extraction was measured with a Beckman DU 640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA.) at the wavelengths of 470, 646.8, and 663.2 nm. The Chl content was determined following the method and equations by Lichtenthaler (1987).

At the end of four weeks after treatment (WAT), the plant height was measured from 10 plants in each treatment unit by measuring the distance from the soil surface to the tip of the youngest fully developed leaf on the primary shoot. Tiller number and leaf number on the primary shoot of the seedlings were recorded. A tiller was defined as one shoot with at least one fully developed leaf. A leaf with ligule and auricles exposed was considered as a fully developed leaf. At the end of the experiment, the shoot biomass was harvested by clipping at the soil surface. The roots were harvested by washing the sand off on a 2-mm sieve with tap water. The root and shoot dry mass were recorded after drying at 68°C for 48 h.

The data were subjected to analysis of variance (ANOVA) using general linear model procedures in SAS 9.2 (SAS Institute, 2008). Treatment means were separated using Fisher protected least significant difference (LSD) at 0.05 probability level. The

PROC REG procedure with a quadratic polynomial model was used between salt concentration and growth, and between salt concentration and physiological indices. Model fitting were tested from studentized residual, residual distribution, and Cook's distance.

Results and Discussion

The selection of molar concentrations of the four salts used in this study created a similar range of EC about 5 to 20 dS m^{-1} (Fig. 5.1). However, the osmotic potential for NaCl was in a range of -0.2 to -1.0 MPa, differing from that of Na_2CO_3, Na_2SO_4, and $CaCl_2$, which ranged from -0.2 to -0.8 MPa. Furthermore, the pH of these salt solutions were different, which were 11.5, 7.1, 6.7, and 8.5 for Na_2CO_3, Na_2SO_4, $CaCl_2$, and NaCl, respectively. Therefore, those must be borne in mind when interpreting the effects of different salts.

The ANOVA for salinity induced changes in biomass, plant height, tiller number, and leaf number is shown in Table 5.1. There were no differences in shoot and root biomass between 'Tar Heel II' and 'Wolfpack' before salinity stress (Table 5.2). When expressed as relative value (percentage of the control), shoot showed no difference (87% vs 89%), but root decreased less for 'Tar Heel II' (95%) compared to 'Wolfpack' (85%). The root/shoot ratio showed no difference as reported in last chapter. The two cultivars had different initial plant height, but the reductions due to salinity stress were 11% for both. 'Tar Heel II' had more initial tiller and leaf number and showed more reduction in tiller and less reduction in leaf number than 'Wolfpack' after salt treatment (Table 5.2). Judging by the tiller and leaf number change, it is not certain which cultivar was more tolerant to salts.

Table 5.1. Analysis of variance of the growth two tall fescue cultivars effected by sodium chloride, sodium carbonate, sodium sulfate, and calcium chloride in six concentration levels from 0 to 250 m*M*.

Source of variance	df	Shoot mass	Root mass	root/shoot	Plant height[†]	Tiller number[‡]	Leaf number[§]
Replication	2	21.50**	11.26**	16.53**	5.89**	15.72**	16.08**
Cultivar	1	0.39	10.29**	0.72	0.06	35.87**	23.38**
Salt	3	93.84**	56.26**	12.37**	66.96**	6.89**	0.65
Level	4	7.73**	14.00**	12.88**	10.32**	3.37*	1.10
Cultivar*Salt	3	1.14	1.33	0.78	0.11	0.63	0.46
Cultivar*Level	4	1.16	0.57	0.85	0.29	1.41	0.45
Salt*Level	12	4.05**	1.15	0.69	3.75**	1.09	0.44
CV(%)		13.06	19.43	11.20	4.74	7.62	8.27

[†] Plant height, measured to the tip of the youngest mutual leaf on the main shoot of the seedling.
[‡] Tiller number, total tiller number per seedling.
[§] Leaf number, number of leaves on the main shoot of the seedling.

However, it is speculated that 'Tar Heel' had more leaves to compensate the reduction of tillers with the final root/shoot ratio similar between the two cultivars.

Sodium carbonate resulted largest shoot mass reduction, followed by NaCl and Na_2SO_4, while $CaCl_2$ did not change the initial shoot biomass (Table 5.3). Sodium carbonate also had the most reduction in root biomass, and as a result, had the lowest root/shoot ratio. Since plant height and tiller number were reduced by Na_2CO_3 more than by other salts, this may contribute to the reduction in shoot biomass. The four salts resulted in no difference in leaf numbers (Table 5.3). El-Samad and Shaddad (1996) reported that NaCl induced more obvious decrease in shoot and root dry weight in pea plant than Na_2SO_4 and Na_2CO_3, and Na_2CO_3 reduced more than Na_2SO_4 did. However, the fresh weight of shoot was reduced by Na_2CO_3 more than other two salts (El-Samad and Shaddad, 1996), indicating water content and osmotic adjustment contributed to the differences.

Table 5.2. Growth of two tall fescue cultivars affected by sodium chloride, sodium carbonate, sodium sulfate, and calcium chloride in six concentration levels from 0 to 250 m*M*. Data were pooled across salt types and concentrations.

	Shoot mass		Root mass		Root/shoot	Plant height[†]		Tiller number[‡]		Leaf number[§]	
Cultivar	Control	Relative[¶]	Control	Relative		Control	Relative	Control	Relative	Control	Relative
	...g...	...%...	...g...	...%...		...cm...	...%...		...%...	...cm...	...%...
Tar Heel II	0.19a	87a	0.05a	95a	0.29a	22.0a	89a	6.6a	90a	16.4a	105a
Wolfpack	0.20a	89a	0.06a	85b	0.28a	24.7b	89a	5.2b	98b	16.0b	98b

[†] Plant height, measured to the tip of the youngest mutual leaf on the main shoot of the seedling.

[‡] Tiller number, total tiller number per seedling.

[§] Leaf number, number of leaves on the main shoot of the seedling.

[¶] Relative value after salt treatment based on control as 100%.

Table 5.3. Relative growth (percentage of untreated control) of two tall fescue cultivars affected by sodium chloride, sodium carbonate, sodium sulfate, and calcium chloride in six concentration levels from 0 to 250 m*M*. Data were pooled across cultivars and salt concentrations.

Salt treatment	Shoot mass	Root mass	Root/shoot ratio	Plant height[†]	Tiller number[‡]	Leaf number[§]
	........%.........				........................%.............................	
NaCl	97b	103a	0.30c	92ab	97a	100a
Na_2SO_4	95b	100a	0.30c	90b	93ab	103a
Na_2CO_3	58c	54b	0.26a	79c	89b	102a
$CaCl_2$	103a	102a	0.28b	93a	97a	101a

[†] Plant height, measured to the tip of the youngest mutual leaf on the main shoot of the seedling.

[‡] Tiller number, total tiller number per seedling.

[§] Leaf number, number of leaves on the main shoot of the seedling.

There was no difference between cultivars in RWC (Table 5.4, 5.5). 'Tar Heel II' had more leaf firing and lower Chl content compared to 'Wolfpack'. The differences between the two cultivars also were reflected in the vegetation indices, NDVI$_{775/675}$ and SI$_{710/760}$, and the quantum yield (F_v/F_m), all indicating higher level of stress in 'Tar Heel II' than in 'Wolfpack'. This is different from the tiller and leaf number results. Since the RWC is related to water potential, it implied that osmotic adjustment in plants may be involved in the adaptation to different salts as reported by El-Samad and Shaddad (1996).

Table 5.4. Analysis of variance of physiological properties of two tall fescue cultivars affected by sodium chloride, sodium carbonate, sodium sulfate, and calcium chloride in six concentration levels from 0 to 250 m*M*. *F* values are followed by significant indicators.

Source of variance	df	RWC[†]	Leaf firing	SI[‡]	NDVI$_{775/675}$[§]	QY[¶]	Chl[#]
Replication	2	1.23	3.43^{*}	0.06	0.84	2.12	3.62^{*}
Cultivar	1	0.01	5.95^{*}	12.96^{**}	9.13^{**}	6.78^{*}	6.21^{*}
Salt	3	55.92^{**}	16.66^{**}	9.55^{**}	13.08^{**}	22.63^{**}	20.15^{**}
Level	4	0.40	16.36^{**}	25.13^{**}	23.26^{**}	16.23^{**}	6.34^{**}
Cultivar*Salt	3	0.38	2.22	3.32	2.38	3.14^{*}	0.98
Cultivar*Level	4	0.33	0.06	0.30	0.49	1.06	0.02
Salt*Level	12	0.04	0.76	2.98^{**}	2.15	1.31	0.37
CV(%)		19.01	21.96	9.79	32.10	40.50	44.62

[†] RWC, relative water content.

[‡] SI, stress index.

[§] NDVI, normalized difference vegetation index.

[¶] QY, quantum yield of photosystem II (F_v/F_m).

[#] Chl, total chlorophyll content.

The only significant factor influencing RWC was salt type (Table 5.4). Sodium carbonate resulted most reduction of RWC, followed by NaCl and CaCl$_2$, while Na$_2$SO$_4$ had the least reduction compared to the untreated control (Table 5.6). Leaf firing followed

Table 5. 5 Physiological properties of two tall fescue cultivars affected by sodium chloride, sodium carbonate, sodium sulfate, and calcium chloride in six concentration levels from 0 to 250 m*M*. Data were pooled across salt types and concentrations.

Cultivars	RWC[†]	Leaf firing	SI[‡]	$NDVI_{775/675}$[§]	QY[¶]	Chl[#]
	........%........					....mg g^{-1}....
Tar Heel II	64.36a	85a	0.78a	0.36a	0.238a	4.80a
Wolfpack	64.73a	76b	0.73b	0.42b	0.298b	5.87b

[†] RWC, relative water content.
[‡] SI, stress index.
[§] NDVI, normalized difference vegetation index.
[¶] QY, quantum yield of photosystem II (F_v/F_m).
[#] Chl, total chlorophyll content.

similar trend as salt effects on RWC (Table 5.6). The most detrimental effects of Na_2CO_3 was also supported by the vegetation indices and quantum yield (F_v/F_m) (Table 5.6).

Table 5.6. Physiological properties of two tall fescue cultivars affected by sodium chloride, sodium carbonate, sodium sulfate, and calcium chloride in six concentration levels from 0 to 250 m*M*. Data were pooled across cultivars and salt concentrations.

Salt treatment	RWC[†]	Leaf firing	SI[‡]	$NDVI_{775/675}$[§]	QY[¶]	Chl[#]
	.........%...........					.mg g^{-1}.
Untreated	92.30a	13d	0.57d	0.68a	0.599a	9.33a
NaCl	76.47bc	71bc	0.71c	0.47b	0.403b	7.32b
Na_2SO_4	84.08b	69c	0.76b	0.37c	0.357b	6.03c
Na_2CO_3	25.51d	96a	0.80a	0.28d	0.079d	2.69d
$CaCl_2$	72.13c	80b	0.73bc	0.43bc	0.257c	5.31c

[†] RWC, relative water content.
[‡] SI, stress index.
[§] NDVI, normalized difference vegetation index.
[¶] QY, quantum yield of photosystem II (F_v/F_m).
[#] Chl, total chlorophyll content.

The reduction of shoot and root biomass only was significant for Na_2CO_3 among salt levels although the salt factor was significant. And this was shown by the interaction between salt and salt levels (Table 5.1). For this reason, only the biomass regression with Na_2CO_3 concentrations is shown for both cultivars (Fig. 5.2).

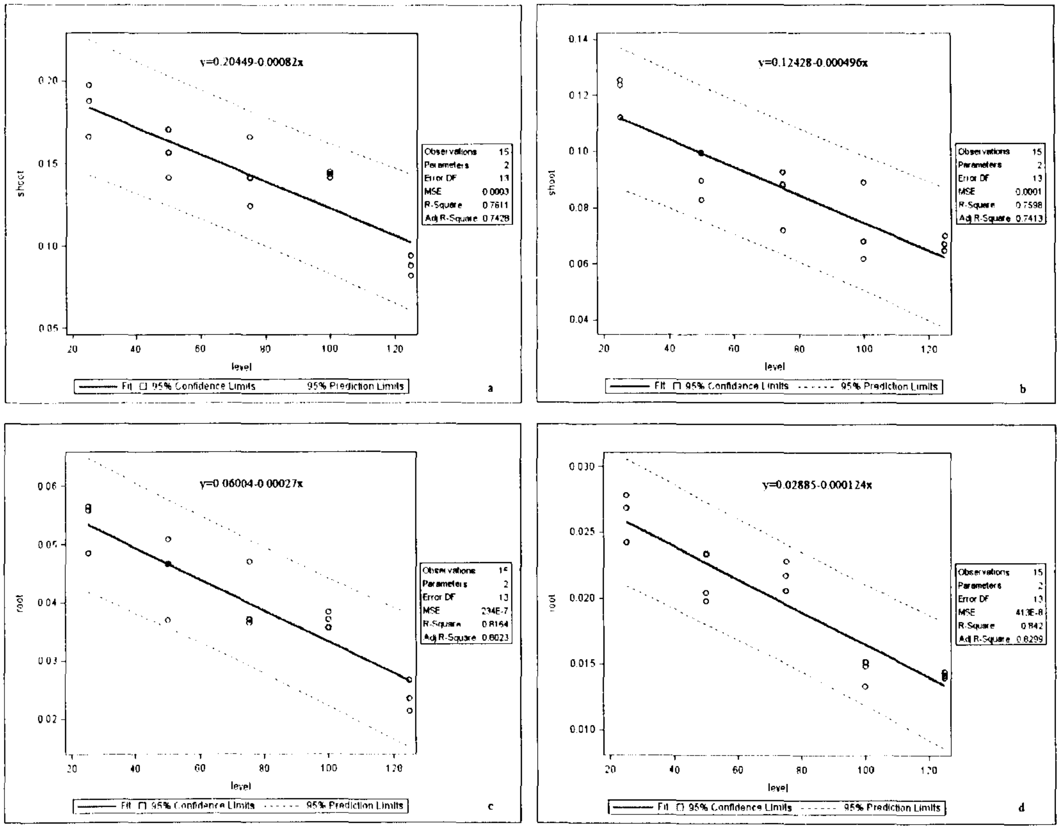

Figure 5.2. Tall fescue shoot and root biomass (g plant^{-1}) after four weeks of treatment with Na_2CO_3 at 0 to 125 m*M* at the 4th leaf stage applied with Hoagland solution. a) and c) are 'Tar Heel II'; b) and d) are 'Wolfpack'.

There was no interaction between cultivar and salt levels (Table 5.1). The biomass of shoot and root was in a negative linear relationship with salt levels with adjusted R^2 ranging from 0.74 to 0.83. Similarly, the plant height and tiller number only was significant for Na_2CO_3 among salt levels and the interaction between salt and salt levels were significant (Table 5.1). The percentage reduction of plant height and tiller number also decreased linearly with increasing salt levels (Fig. 5.3).

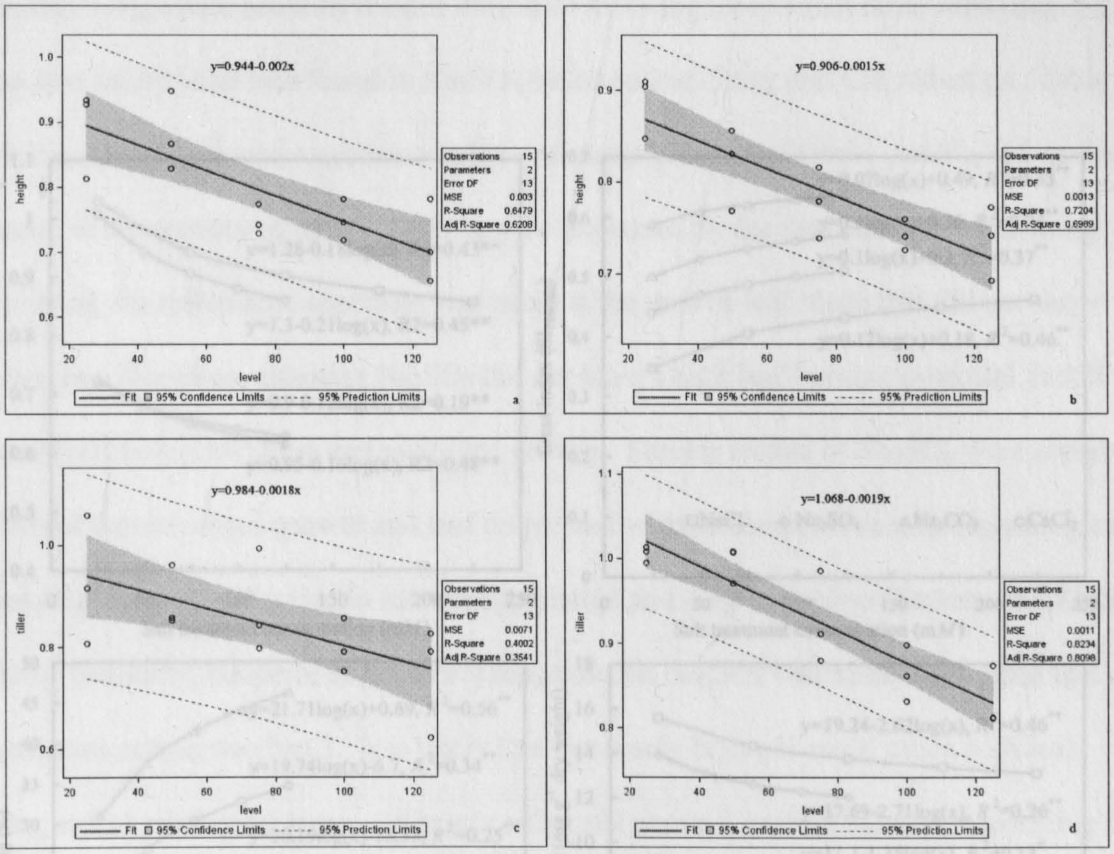

Figure 5.3. Tall fescue plant height and tiller percentage reduction after four weeks of treatment with Na_2CO_3 at 0 to 125 m*M* at the 4th leaf stage applied with Hoagland solution. a) and c) are 'Tar Heel II'; b) and d) are 'Wolfpack'.

One possible explanation for Na_2CO_3 as the only salt that showed different growth inhibition was that the other salts had a stimulating effect at low concentration and inhibition at high concentration. Within the tested salt levels, those salts therefore showed no effects. Bhivare and Nimbalkar (1984) reported that Na_2SO_4 at levels of 2.5 to 5 dS m^{-1} were stimulating growth of French beans (*Phaseolus vulgaris* L.).

Despite that only Na_2CO_3 resulted in different biomass and growth among different levels, vegetation indices and quantum yield (F_v/F_m) all showed differences for all four salts with different levels (Fig. 5.4).

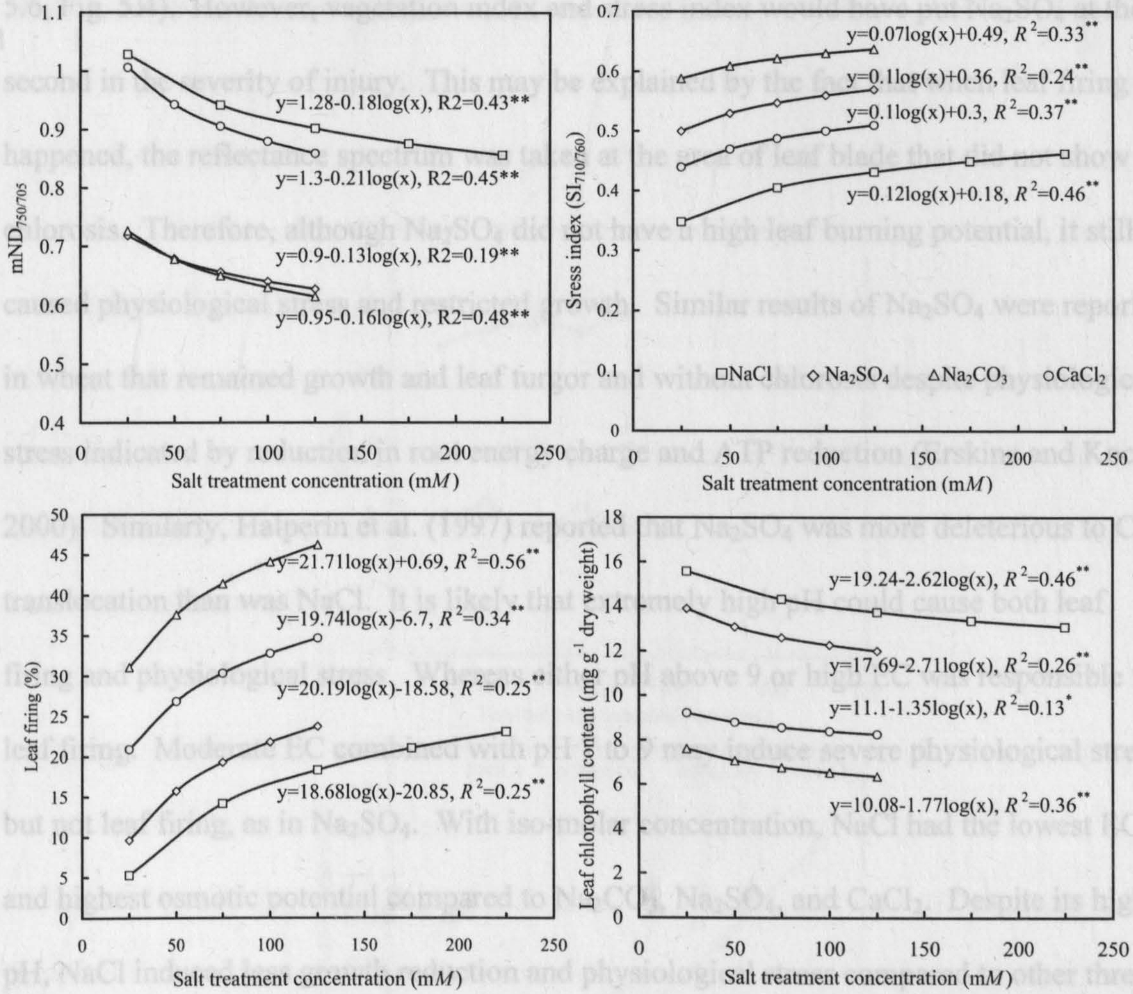

Figure 5.4. Physiological properties of two tall fescue cultivars affected by sodium chloride, sodium carbonate, sodium sulfate, and calcium chloride in six concentration levels from 0 to 250 m*M*. Data were pooled across cultivars.

Because Na_2CO_3 resulted in the most reduction in biomass, plant growth, and severity in physiological indices, it is probably related to its high alkalinity (Fig. 5.1). The second severity level of injury in terms of leaf firing and Chl reduction was found in $CaCl_2$ (Table 5.6, Fig. 5.4), which could have been attributed to its higher EC than other salts (Fig. 5.1). The next injury level was found in Na_2SO_4 based on leaf firing and Chl reduction (Table 5.6, Fig. 5.4). However, vegetation index and stress index would have put Na_2SO_4 at the second in the severity of injury. This may be explained by the fact that when leaf firing happened, the reflectance spectrum was taken at the area of leaf blade that did not show chlorosis. Therefore, although Na_2SO_4 did not have a high leaf burning potential, it still caused physiological stress and restricted growth. Similar results of Na_2SO_4 were reported in wheat that remained growth and leaf turgor and without chlorosis despite physiological stress indicated by reduction in root energy charge and ATP reduction (Erskine and Koch, 2000). Similarly, Halperin et al. (1997) reported that Na_2SO_4 was more deleterious to Ca translocation than was NaCl. It is likely that extremely high pH could cause both leaf firing and physiological stress. Whereas either pH above 9 or high EC was responsible for leaf firing. Moderate EC combined with pH 7 to 9 may induce severe physiological stress but not leaf firing, as in Na_2SO_4. With iso-molar concentration, NaCl had the lowest EC and highest osmotic potential compared to Na_2CO_3, Na_2SO_4, and $CaCl_2$. Despite its high pH, NaCl induced less growth reduction and physiological stress compared to other three salts. Datta et al. (1995) found that Na_2SO_4 caused more injury and growth reduction in wheat than an iso-osmotic NaCl. Therefore, osmotic potential did not seem to be a predominant factor in this study. The Chl content reduction found in this study agreed with the results in pea plants reported by El-Samad and Shaddad (1996) at salt levels with osmotic potentials higher than -0.9 MPa and the results in sunflower (*Helianthus annuus* L.), both showed growth reduction in the order of Na_2CO_3, Na_2SO_4, and NaCl.

At the end of experiment, the soil EC did not show significant difference between salts at the same molarity but the pH was highest for Na_2CO_3, followed by Na_2SO_4 and NaCl, and the lowest was found in $CaCl_2$ treatment (Fig. 5.5). The pH in $CaCl_2$ and Na_2SO_4 treated soil increased by 1 and 2, respectively.

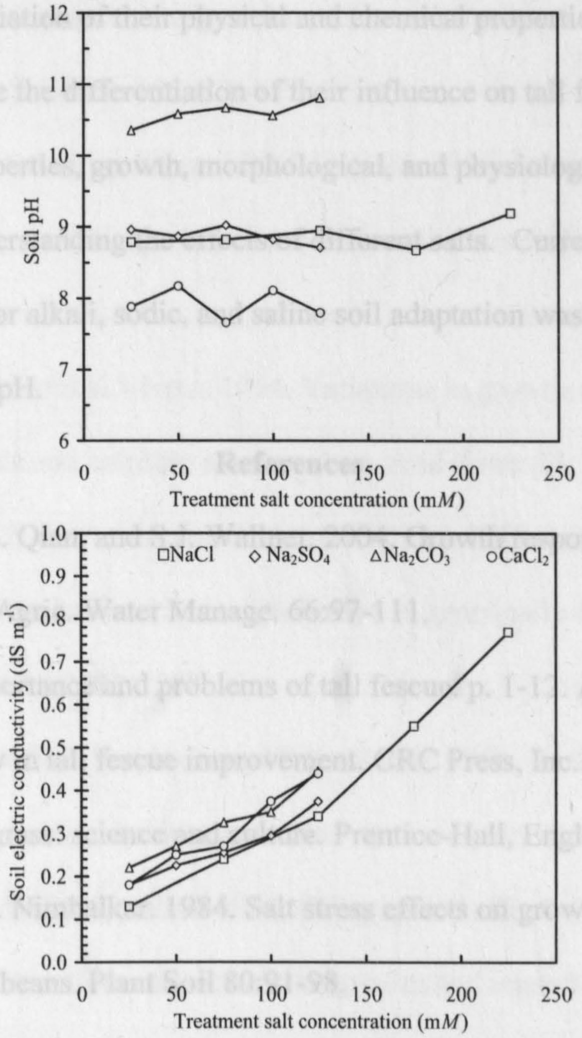

Figure 5.5. Soil properties of silica sand medium with tall fescue established in and after treatment with sodium chloride, sodium carbonate, sodium sulfate, and calcium chloride in six concentration levels from 0 to 250 m*M* weekly for 5 weeks.

Conclusions

Tall fescue leaf firing was mainly caused by an alkaline condition with pH higher than 9 as in Na_2CO_3, or moderate pH combined with high salinity (high EC) as in $CaCl_2$. Plant growth was more affected by sodicity which was high in Na_2CO_3 and Na_2SO_4. Osmotic adjustment also played an important role in tall fescue salinity stress in chlorides. Chloride effects were closely related to RWC of the leaves. The combination of different salts along with the variation of their physical and chemical properties, such as EC, pH, and osmotic potential, made the differentiation of their influence on tall fescue stress rather difficult. Multiple properties, growth, morphological, and physiological, should be measured to better understanding the effects of different salts. Current study showed that evaluating tall fescue for alkali, sodic, and saline soil adaptation was often complicated by interaction of ions and pH.

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GENERAL CONCLUSIONS

At least three indices calculated based on single leaf spectrum showed promise as sensitive means to differentiate salinity stress from untreated plants. Those indices also are strongly correlated to many physiological parameters that have been shown to be reliable measures of salinity tolerance in many plant species including tall fescue. Tall fescue leaf senescence was accelerated by salinity stress which was different from the aging process under shade. Tall fescue had several adaptation strategies in order to conserve water under salinity stress, while the plants under shade had adaptation mechanisms centered on light harvesting. Tall fescue growth responded to salinity stress differently from shade stress, a fact that has to be taken into consideration when selecting for stress tolerant traits, such as leaf length, root to shoot ratio. Leaf appearance rate may be different with stress tolerance levels and mediate the tiller number and shoot density. By comparing and contrasting the effects on nutrients uptake, it was shown that K, Ca, Mg may be very important in the ion balance and salinity stress tolerance. Tall fescue leaf firing was mainly caused by an alkaline condition with pH higher than 9 as in Na_2CO_3, or moderate pH combined with high salinity (high EC) as in $CaCl_2$. Plant growth was more affected by sodicity which was high in Na_2CO_3 and Na_2SO_4. Osmotic adjustment also played an important role in tall fescue salinity stress in chlorides. Chloride effects were closely related to RWC of the leaves. The combination of different salts along with the variation of their physical and chemical properties, such as EC, pH, and osmotic potential, made the differentiation of their influence on tall fescue stress rather difficult. Multiple properties should be measured to better understanding the effects of different salts.