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Physiological response of Kentucky bluegrass under salinity stress

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PHYSIOLOGICAL RESPONSE OF KENTUCKY BLUEGRASS UNDER SALINITY STRESS

by

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Approved:

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ABSTRACT

Physiological Response of Kentucky Bluegrass under Salinity Stress

by

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Utah State University, 2013

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Salinity is a major abiotic stress in plant agriculture which reduces seed germination, vegetative growth, and flowering, and limits crop productivity world-wide. Salinity causes water deficit, ion toxicity, and nutrient deficiency in plants, which can result in cellular damage, growth reduction, and even death. Kentucky bluegrass (*Poa pratensis* L.) is the most widely used cool-season species in cool-arid climates; however it has relatively poor salt-tolerance. Thus the development of Kentucky bluegrass genotypes with increased salt tolerance is of interest to turf breeders. One impediment to selection towards this goal is finding an efficient and accurate method to evaluate the salt tolerance. The objective of this study was to examine physiological responses to salt stress and to evaluate the genetic diversity among the accessions used in the research. Salt-tolerant accessions PI371768 (768) and PI440603 (603) and salt-sensitive varieties Midnight and Baron were exposed to four levels of salinity imposed by irrigating with salt solutions of 0 dS m^{-1} (control), 6 dS m^{-1}, 12 dS m^{-1}, and 18 dS m^{-1} or 24 dS m^{-1}. Soil salinity was measured using Acclima Digital TDT sensors and grass

response to the stress was measured using turf quality ratings, stomatal conductance, leaf water potential and electrolyte leakage. In general, turfgrass quality, stomatal conductance, and leaf water potential decreased while electrolyte leakage increased under salinity stress. Midnight and Baron exhibited greater changes in these measurements, indicating more sensitivity compared to 768 and 603. The 6 dS m^{-1} treatment had little effect on the salt-tolerant accessions. Salt tolerance of 603 and 768 was confirmed and likewise, salt sensitivity of Baron and Midnight was confirmed. The genetic similarity of all cultivars used in this study was very high.

All of the evaluation measurements were highly correlated, with water potential and electrolyte leakage being the most reliable and accurate methods due to the low standard deviations. Due to more repeatable methods and less user error, electrolyte leakage and turfgrass quality are recommended methods for screening salt tolerance of turfgrasses.

PUBLIC ABSTRACT

Physiological Response of Kentucky Bluegrass under Salinity Stress

Lijun Wang

Salinity is a significant stress for plants world-wide. In agriculture, salts reduce germination, overall growth, yield, and sometimes death in crop plants. Salinity similarly affects turfgrass in our urban landscapes. Kentucky bluegrass (*Poa pratensis* L.) is the most widely used cool-season grass in the northern part of the United States, including the cool-arid West, but generally is a salt sensitive species. The overall objectives of this study were to study the physiological responses of Kentucky bluegrass to salt stress and to evaluate the genetic similarity among the cultivars used in the research.

Four Kentucky bluegrass entries, two salt-tolerant and two salt-sensitive, were used in this research were exposed to four levels of salinity stress. Soil moisture sensors were used to measure soil salinity levels. Several measurements of plant health were used to evaluate stress responses including turf quality, stomatal conductance, water potential, and electrolyte leakage. Molecular methods were used to evaluate genetic diversity of the same Kentucky bluegrass accessions.

In general, turf quality, stomatal conductance, and water potential decreased while electrolyte leakage increased with the increase of salt concentration. Susceptible varieties Midnight and Baron showed greater changes in these measurements, indicating more sensitivity to salts than 768 and 603. The 6 dS m^{-1}treatment had little effect on the salt-tolerant entries and had effect on the salt-susceptible accessions. Among the measurements used, turfgrass quality, water potential, and electrolyte leakage were the

most accurate. In future studies to screen salt tolerant plants, electrolyte leakage and turf quality are recommended methods.

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LITERATURE REVIEW

According to the FAO Land and Plant Nutrition Management Service in 2010, over 6% of the world's land, or over 400 million ha, are salt-affected, which means affected by either salinity or sodicity and contain sufficient concentrations of soluble salts to reduce the growth of most plant species (Tester and Davenport, 2003). Much of the world's land is not cultivated, but a significant proportion of cultivated land is salt-affected. There are more than 65 million ha of such soil in Africa (Aubert, 1977), 50 million ha in Europe (Kovda et al., 1973; Szaboles, 1979), 17.4 million ha in Australia, and 77.5 million ha in North, Central, and South America (Massoud, 1974). Of the current 230 million ha of irrigated land, 45 million ha are salt-affected (19.5 %) and of the 1,500 million ha under dry land agriculture, 32 million are salt-affected to varying degrees (Abbas et al., 1994).

Much of the arid west of North America is salt-affected, particularly in Utah, Arizona, Texas, New Mexico, Nevada, and California (Szaboles, 1989). When annual rainfall is less than 15 inches (380 mm), salt affected soils are most prevalent because insufficient leaching occurs to remove salts that accumulate due to weathering of minerals and ground water (Pitman, 2002). The most common salts in arid and semi-arid climates are sodium and sulfate salts such as Na_2SO_4, K_2SO_4, $CaSO_4$ and $MgSO_4$. Irrigation water is another source of salts. Irrigation contributes to increased soil salinity through high evapotranspiration rates coupled with inadequate leaching, low quality irrigation water, and rising water tables that receive salts leached from the plant root (Carrow and Duncan, 1998). Saline soils also exist near sea coasts due to the tidal action

and airborne salt deposition, or where water tables are shallow and highly saline (Harivandli et al., 1992).

Other sources of landscape salinity include salts used for roadside de-icing and fertilizer application (Hutchinson, 1970). It is reported that the average application rates of salts on roadways are 8,000 to 14,000 kg km^{-1} (Hutchinson, 1970). The soil salt concentrations increase in adjacent areas by the brine flowing from the treated surface (Hanson and Oster, 1986). Many fertilizers, such as animal manure, sewage and sludge, may increase soil salinity (Langdale et al., 1973).

In addition to soil salinity, the availability of high quality irrigation water has become a serious concern for many communities, rural areas and farm lands in the Western United States. Surface-water supplies are fully appropriated, sometimes over appropriated, and many communities are dependent upon ground water (Lazarova and Asano, 2005). At the same time, urban areas in the western U.S. are growing rapidly and demanding more irrigation water for urban landscapes. Rather than meeting this demand by diverting water from agricultural production, one possible solution is the use of recycled wastewater or other low quality irrigation water sources for landscape irrigation (Lazarova et al., 2003).

Throughout the United States, large volumes of municipal recycled non-potable water are used for urban irrigation. For example, in the Denver, CO area, approximately 30-40 million gallons of recycled water is used for landscape irrigation every day during the growing season (Munns, 1993). In 2003, the National Golf Foundation (NGF) reported that 13% of golf courses (about 2000 golf courses) nationwide now use recycled

water for irrigation. However, such sources are generally higher in particulate matter and salinity which can lead to increased soil salinity levels (Marcum, 2008).

Many landscape plants are susceptible to damage from high salt levels (Alshammary et al., 2004). Governments have placed registrations on potable water use for landscape irrigation, and encourage or require use of secondary, saline water sources, such as recycled water (Butler et al., 1974; Aronson, 1989; Blits and Gallagher, 1991; Harivandi et al., 1992). Therefore, the need for salt-tolerant turfgrasses and management for these saline conditions has increased in recent decades and will continue to increase.

The issues of saline soils and low quality irrigation water are critically important because salinity is one of the most significant environmental factors limiting plant growth. Salinity may cause damage through various physiological effects such as water deficit, ion toxicity and nutrient deficiency, which result in cellular damage, growth reduction and plant death (Poss and Russell, 2010). In agronomic and horticultural crops, high salt concentrations are toxic to seed germination, vegetative growth, flowering, fruit set, and ultimately diminish economic yield and quality. High levels of salinity also adversely affect urban landscape plants, decreasing their ability to provide their environmental and cultural benefits. However, salt tolerant species exist among both C3 turfgrasses, and C4 turfgrasses (Butler et al., 1974; Horst and Beard, 1977; Harivandi et al., 1992). Some turfgrass cultivars have been developed and marketd for their salt tolerance performance (Carrow and Duncan, 2011).

The turfgrass salt tolerance mechanisms of salt-sensitive (glycophytes) and moderately salt-tolerant (mesophytes) are generally through osmotic adjustment, ionic regulation, and compartmentalization (Marcum, 2008). Turfgrass salinity tolerance has

been associated with osmotic adjustment and avoidance of 'physiological drought' (Harivandi et al., 1992). Grasses use osmotic adjustment to maintain cellular turgor and plant growth allowing them to take up water at lower soil water potentials to compensate for external osmotic stress, the process of osmoregulation, or osmotic adjustment (Levit, 1980; Hellebust, 1976). In saline soil, dissolved solutes in the root zone lower the soil water potential. The general water balance of plants is affected since leaves need to create even lower water potential to maintain a gradient of water potential between the soil and the leaves.

Salinity tolerant species can avoid ion toxicity by excluding specific ions from the xylem of the roots (Schubert and Läuchli, 1990). Salinity Mechanisms in some warm-season grass species such as zoysia (*Zoysia japonica*) and bermudagrass (*Cynodon dactylon*) are associated with shoot saline exclusion (Marcum and Murdoch, 1994; Pessarakli and Touchane, 2006). Shoot saline ion exclusion is also an important factor influencing intraspecies salinity tolerance, i.e., at the cultivar or accession level. Salt-sensitive accessions were found to have higher shoot Na^+ and Cl^- levels than saline-site, salt tolerant accessions in *Festuca rubra* (Hannon and Barber, 1972; Khan and Marshall, 1981), *Cynodon dactylon*, and *Agrostis* spp. (Wu, 1981). The accumulation of high concentration of Na in the shoots decreases the content of phospholipids in thylakoids, thus reducing the photosynthetic capacity and growth rate (Thomann and Muller, 1987). Energy is also spent on Na^+ exclusion by the plant as a defensive response, and this energy expenditure can reach a level of diminishing returns resulting to eventual tissue and cell death (Apse and Blumwald, 2007).

Plants that cannot exclude salt from the transpiration stream must have the ability to compartmentalize the salt in vacuoles, thereby protecting the cytoplasm from ion toxicity and avoiding buildup in the cell wall which would cause dehydration (Munns, 2005). With sequestration of Na^+ and Cl^- in the cell vacuoles, the organic solutes must be compatible with concentration and must accumulate in the cytosol and other organelles at sufficient levels to balance the osmotic pressure of those accumulated ions in the vacuole (Flowers et al., 1977; Wyn Jones et al., 1977; Hasegawa et al., 2000; Munns, 2005). Salt-tolerant plants that successfully accumulate ions for osmotic adjustment above concentrations of 100-200 mm do so by compartmentalizing them within the vacuole, which typically makes up 90-95% of a mature plant cell's volume (Flowers and Yeo, 1995).

Many salinity studies have been focused on Kentucky bluegrass due to its wide use among C3 turfgrass. Kentucky bluegrass possesses poor salinity tolerance (Flowers et al., 1977; Khan and Marshall, 1981; Marcum and Murdoch, 1994; Qian et al., 2000, 2001) or <3 dS m^{-1} (Maas and Hoffman, 1977). The relative salt tolerance rankings of turfgrass species were presented in Table 1.Yet, Kentucky bluegrass is the most widely used cool-season turf grass in the northern United States and throughout much of northern Europe and the temperate latitudes. Kentucky bluegrass possesses highly desirable turf quality and an ability to remain green throughout the growing season with supplemental irrigation in cool, arid climate areas (Huff, 2003).

Many cultivars of Kentucky bluegrass too numerous to mention here, have been developed over the past several decades. Kentucky bluegrass has relatively high irrigation needs compared to most warm-season grasses and some drought adapted

species due to its shallow root system (Leksungnoen et al., 2012). However, it survives extended droughts through summer dormancy or quiescence (Huff, 2003). Because of its poor salinity tolerance compared to many cool season turfgrasses (Marcum, 2008), the development of Kentucky bluegrass genotypes with increased salt tolerance is of interest to turf breeders.

Although as a species Kentucky bluegrass is salt sensitive, several studies have reported a wide range of tolerance among Kentucky bluegrass germplasm. According to Robins et al. (2009), NPGS Kentucky bluegrass accession, W6 19573, PI371768, PI 440603, and PI372472 entries showed high salt tolerance, Midnight had moderate salinity-tolerance, and Baron and Brilliant were salt-intolerant cultivars. This variation indicates potential to genetically improve salt tolerance in this species and may indicate variation in how accessions tolerate salt stress.

Leaf water potential, or plant turgor maintenance, appears to be an important estimate of turf growth and development (Turgeon, 2008) and has been attributed to differences in salt tolerance among Kentucky bluegrass cultivars (Qian et al., 2001). It was found that Kentucky bluegrass salt-tolerant cultivars had significantly higher relative water content compared to Kentucky bluegrass salt-intolerant cultivars after salt treatments (Koch et al., 2011). Salt-induced osmotic effects outside the roots immediately affect stomatal conductance, decreasing stomatal aperture (Fricke, 2004; Fricke et al., 2006). Salinity also affects photosynthesis through stomatal closure and damage to the photochemical system and subsequent reduced overall growth rate (Kaymakanova and Stoeva, 2008; Megdiche et al., 2009). Stomatal conductance is further reduced in response to a decline in leaf turgor, high vapor pressure deficit in the atmosphere, or root-

Table 1. Relative salinity tolerance rankings of turfgrass species

Common Name	Scientific Name	Salinity Tolerance	Grass Type
Seashore paspalum	*Paspalum vaginatum* O. Swartz.	VT-T	Warm
Alkaligrass	*Puccinellia* spp.	VT-T	Cool
Saltgrass	*Distichlis stricta*	T	Warm
Kikuyu	*Pennisetum clandestinum*	T	Warm
Fairway wheatgrass	*Agropyron cristatum*	T	Cool
Western wheatgrass	*Agropyron smithii*	T	Cool
St. Augustinegrass	*Stenotaphrum secundatum*	T	Warm
Tall fescue	*Festuca arundinacea*	T	Cool
Perennial Ryegrass	*Lolium perenne*	T	Cool
Slender creeping red fescue	*Festuca ruba* L. spp. Trichopylla	T	Cool
Buffalograss	*Buchloe dactyloides*	MT	Warm
Blue grama	*Bouteloua gracilis*	MT	Warm
Hard fescue	*Festuca longifolia*	MT	Cool
Creeping red fescue	*Festuca ruba* L. spp. ruba	MT	Cool
Common Bermudagrass	*Cynodon dactylon*	MT	Warm
Hybrid Bermudagrass spp.	*Cynodon*	MT	Warm
Creeping bentgrass	*Agrostis palustris*	MT	Cool
Kentucky bluegrass	*Poa pratensis*	MS	Cool
Zoysiagrass	*Zoysia* spp.	MS	Warm
Carpetgrass	*Axonopus* spp.	VS	Warm
Centipedegrass	*Eremochloa ophiuroides*	VS	War,
Annual bluegrass	*Poa annua*	VS	Cool
Colonial bentgrass	*Agrostis tenuis*	VS	Cool
Rough stalk bluegrass	*Poa trivialis*	VS	Cool

Source: Adapted from Carrow, R. N., and R. R. Duncan, Salt-Affected Turfgrass Sites: Assessment and Management, P.34, John Wiley, Hoboken, NJ, 1998.
VS: Very sensitive; MS (Moderately sensitive); MT (Moderately tolerate); T (Tolerant); VT (Very tolerant).

generated chemical signals, which is common to both drought and salinity (Liu et al., 2011).

Leksungnoen et al. (2012) used stomatal conductance and leaf water potential to measure the difference of salt tolerance of *P. pratensis*. Salt stress outside the roots immediately affected leaf water potential, evapotranspiration, stomatal conductance, electrolyte leakage, and decreasing stomatal aperture in plants (Aronson, 1989). Because of these responses, many studies have used these measurements to access tolerance in *P. pratensis* (Jiang and Huang, 2000; Poss and Russell, 2010; Liu et al., 2011).

Several research projects have investigated the physiological responses of Kentucky bluegrass under salinity stress with attempts to identify the mechanisms involved in the stress tolerance observed (Poss and Russell, 2010; Leksungnoen et al., 2012). However, these studies relate salt stress with the concentration of irrigation solutions rather than actual salinity stress present in the soil. Most of the studies of turfgrass salinity response were conducted in the greenhouse containers and hydroponic saltwater solutions, which were conducted in relatively controlled conditions of the greenhouse or laboratory (Alshammary et al., 2004; Koch and Bonos, 2010). Some studies use organic medium or sand culture to conduct the salt experiments for grass (Qian et al., 2000; Lee et al., 2002; Peel et al., 2004), which meant the saline soils were different from the actual field soil. To address this and to more accurately determine salt stress being experienced by the plants roots, soil sensors may be useful for monitoring. These are valid studies, but to better understand plant response in field conditions, more "field-like" conditions are important.

Successful cultivar development and breeding in Kentucky bluegrass is highly dependent on an understanding of its apomictic reproductive system. The analysis of apomictic levels of Kentucky bluegrass will promote the process of the screening of salt tolerant Kentucky bluegrass. As a facultative apomict, apomictic levels in *Poa pratensis* can vary between 25 and nearly 100% apomictic (Huff, 2003). Currently Kentucky bluegrass breeding utilizes plants from a population that are space planted in a field setting, and surveyed during anthesis the next year for phenotypic differences. Only obvious differences are detected, and when the parental phenotypes are similar the ability to assess the level of apomixis (similarity within plants of the same population) is not easy. One way to estimate if constituent plants of a population are similar, regardless of the limitations of phenotyping, is through the use of molecular markers (Glaszman et al., 1989; D'Hont et al., 1994).

Amplified fragment length polymorphisms (AFLPs), simple sequence repeats or microsatellites (SSRs), and other minor methods have been used for fingerprinting varieties, cultivars and clones of plants. Amplified fragment length polymorphisms is robust and repeatable (Hale and Miller, 2005), but primarily marks highly variable genomic regions such as centromeres. The use of SSRs targets expressed sequences if generated from cDNA rather than genomic DNA, and therefore is less variable. However, SSRs require several more procedures, such that the cost-benefit ratio warrants caution. Although little molecular information is available regarding Kentucky bluegrass, studies used AFLP markers to investigate the diversity within the species (Renganayaki and Fritz, 2001) and for genetic mapping (Porceddu et al., 2002).

Given the discovery of putative salt tolerant accessions of Kentucky bluegrass, and the physiological measurements that best describe their mode of tolerance, it was necessary to understand the apparent level of similarity within plants of the tolerant germplasm. This level of similarity within plants of the same populations is an indirect estimation of the level of apomixes, and similarity estimates would be preparatory to attempting hybridization with other elite Kentucky bluegrass cultivars.

PROJECT OBJECTIVES

The main purpose of my research was to characterize the physiological responses of putatively salinity tolerant and intolerant Kentucky bluegrass under salt stress. In particular, my objectives were to: (1) test common salt stress physiological measurements and determine the most repeatable and discriminatory, (2) validate previous greenhouse salt screening by testing putatively tolerant and intolerant germplasm, and (3) characterize the breeding system (level of apomixis) in tolerant germplasm.

MATERIALS AND METHODS

Experimental Design

Four accessions of Kentucky bluegrass were selected for this study to represent a range of tolerances to salinity. Accessions PI 371768 and PI 440603 were identified previously as salt tolerant by Robins et al. (2009). These plants were originally established by seed obtained from the National Plant Germplasm System and then grown in a field plot. Plants for this study were propagated vegetatively from this plot. Two cultivated varieties were also chosen. 'Midnight' (Meyer et al., 1984) has shown to have moderate salinity tolerance (Robins et al., 2009). 'Baron' was considered salt sensitive by (Hurley and Ghijsen, 1980). Both of these varieties were established from seed.

The study was conducted in a research greenhouse at Utah State University (Logan, UT). There were 16 pots (Diameter: 10 inches; Depth: 20 inches) filled with a 3:1 sand/ peat moss media. A fiberglass screen was put in the bottom of each pot to prevent the media from leaking out the drainage holes. During pot preparation, half the media was placed in the pot, an Acclima Digital TDT SD-12 moisture sensor (Acclima, Inc., Meridian, ID) was placed on the sand, and the pot was then filled completely. This placed the sensor at a depth of 10.2 cm. The pots were shaken slightly to settle the soil and watered to remove any air pockets around the sensors. In experiment 1, sensors were activated and data logged with DataSnap dataloggers (Acclima, Inc., Meridian, ID) and recorded using 'SnapView' software (Acclima, Inc.). In experiment 2 and 3, the sensors were connected to a CR1000x datalogger (Campbell Scientific, Inc., Logan, UT) and data was collected using LoggerNet software. Plants of each of the four Kentucky bluegrass entries were spaced equidistantly and randomized in each pot (Fig. 1). Plants were grown

under an 18-hr photoperiod and temperatures of 25±5/15±5°C (day/night). Photosynthetically active radiation (PAR) ranged from 350 to 500 umol/m^2.s Plants were irrigated with fertilizer (EC=802 us/cm) twice a week for 3 weeks after planting and prior to the start of salinity treatments. This ensured well-established and healthy, non-stressed plants at the start of the experiments. All plants were mowed weekly to a 10 cm height before imposing salt stress treatment.

The experimental design used was a split plot design with four replications (Fig. 2). Successive observations on the plants created a repeated measures design. The experiment was repeated three times from May to October in 2012. Salinity treatments were 0, 6, 12, and 18 dS m^{-1} and prepared as described in Table 1. These treatments were the whole plots and four Kentucky bluegrass varieties planted within each pot were sub plots. Salinity treatments were increased gradually at the start of the experiment (start of week four after planting). Salinity levels were increased 6 dS m^{-1} each week until the desired level was reached. Once all salinity levels were reached, plant measurements (see below) began and continued for 5 weeks. Pots were irrigated twice each week with the goal of flushing the soil to replace the solution within each pot.

Evaluation of Grasses

To evaluate stress on the plants imposed by the salinity treatments, turfgrass quality, leaf water potential, stomatal conductance, and leaf electrolyte leakage were evaluated and/or measured throughout the experiment. The turf quality was measured twice a week from 12PM-3PM, stomata conductance was measured twice a week from

Figure 1. Four entries in a pot

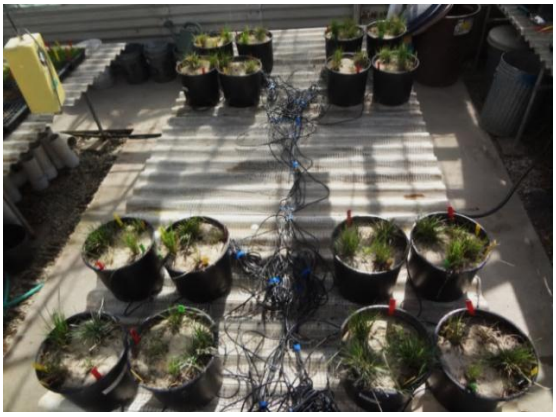

Figure 2. Split plot design with four replications

Table 2. Composition of salinizing salts in solutions

Electrical conductivity (EC)	NaCl	$CaCl_2$ (dehydrate)
dS m^{-1}	mmol l^{-1}	mmol l^{-1}
6	16.35	21.82
12	24.22	47.88
18	30.28	74.85

12PM-3PM, Leaf water potential was measured twice a week from 12PM-3 PM. Electrolyte leakage was measured once a week.

Visual rating

Turf quality was visually rated on a scale from 1 to 9, where a rating of 1 represented completely necrotic (brown) plants and where a rating of 9 represented healthy plants with dark green, turgid leaf blades, and a full turf canopy (Liu et al., 2011). Digital images were taken every week to analyze percent green cover and dark green color index (DGCI) (Karcher and Richardson, 2003). A light box (length:50cm, width:30cm, height:70cm) with 2 LED strips (Utilitech model 29123), inside was used to ensure consistent light conditions throughout the experiment. All digital images were taken with a Sony DSC-WX9 camera and saved in JEPG format, with a color depth of 16.7 million colors and an image size of 4608x3456 pixels. The turf quality was measured twice a week from 12PM-3PM. Digital images were taken once every week from 12pm-3pm.

Plant water potential

Leaf water potential was measured twice a week from 12pm to 3pm using a portable pressure chamber (Model 3005HGPL, Soil, Moisture Equipment Corp, Santa Barbara, CA, USA) (Liu et al, 2011; Leksungnoen et al., 2012). A fully developed grass stem including roots was collected, and then immediately wrapped in plastic wrap. Stems were cut slightly above the root and placed in the pressure chamber with the cut end sticking out of the chamber. Pressure was gradually increased in the chamber using

compressed nitrogen until plant sap was observed at the cut end of the stem. This pressure was recorded and considered the leaf water potential.

Stomatal conductance

Stomatal conductance measurements were made twice a week using a leaf porometer (Model SC-1, Decagon Devices, Inc., Pullman, WA, USA). The leaf porometer was calibrated prior to every data collection. Because leaves were narrower than the porometer chamber, four to five leaf blades were excised and arranged side by side with the adaxial side of the leaves facing the porometer chamber. This was done as quickly as possible, typically less than 5 s to prevent desiccation of the leaves which would cause stomatal closure.

Leaf temperature

Immediately after stomatal conductance measurements, the surface temperature of the leaves was recorded. We used an infrared temperature sensor (Model SI-111, Apogee Instruments, Inc., Logan, UT, USA) connected to a digital thermometer (Model 52-II Dual Input Digital Thermometer, Fluke Corporation, Everett, WA, USA). The sensor was held 2 cm from leaf canopy.

Electrolyte leakage

Leaf electrolyte leakage (EL) was measured similar to methods described by Blum and Ebercon (1981) and Marcum (1998). Five to ten leaf blades were cut and immediately put into the plastic bags, then 2 minutes later, 0.2g leaves were weighed and cut into 2-cm segments, rinsed three times with distilled deionized water, and put in 20 mL deionized water. This weighing and preparation process typically took 2 minutes.

Tubes were shaken at 120 rpm (Lab-Line Instruments Inc., Melrose Park, IL) for 24 h and then the solution was measured with a conductivity meter (Orion Star A112, conductivity meter, Thermo Scientific). This initial measurement is referred to as C1 and represents cell leakage due to stress on the plant. Leaves and solution were then autoclaved at 120°C, shaken again for 24 h, and again measured with the conductivity meter to extract all electrolytes from the cells. This measurement is referred to as C2 and represents the total electrolytes in the cells. Percentage of the total electrolytes that leaked from cells during stress treatments (EL) was calculated as EL = C1/C2 × 100. Lower EL indicated greater resistance to stresses. Electrolyte leakage was measured once a week.

Soil moisture

Soil moisture and soil salinity were measured in each pot throughout the experiments with the Acclima sensor described previously. These sensors measured volumetric water content, soil electrical conductivity, permittivity and soil temperature. In experiment 1, sensors were activated and data logged with DataSnap dataloggers (Acclima, Inc., Meridian, ID) and recorded using 'SnapView' software (Acclima, Inc.). In experiments 2 and 3, the sensors were activated and logged using a CR1000x datalogger. In all three experiments, measurement interval was 30 minutes.

Statistical Analysis

The experiment was a split plot design with four whole plots with four salinity treatments applied (0 dS m^{-1}, 6 dS m^{-1}, 12 dS m^{-1}, 18 dS m^{-1}). Successive observations on the plants created a repeated measures design. These treatments were the whole plots and four Kentucky bluegrass varieties planted within each pot were sub plots. Effects of

salinity treatments, cultivars, and treatments× cultivars interactions were analyzed by analysis of variance according to the Mixed procedure of SAS (version 9.0; SAS Institute, Cary, NC, USA). Mean differences were tested with least significant difference test at a probability level of 0.05.

Molecular Marker Genotyping

Four accessions of Kentucky bluegrass (PI371768, PI371742, PI371771, and PI440603) were found to be salt tolerant in Robins et al. (2009), and were compared with the cultivar Midnight. Four seeds were germinated from each of the four salt tolerant entries and leaf tissue was collected from each plant. Leaves were frozen at -80 °C and lyophilized for 48 hours. Genomic DNA was extracted using the Qiagen DNeasy Mini-Kits according to the manufacturer's instructions (Qiagen, Valencia, CA). Quantity and quality of DNA was assessed using spectrophotometry and agarose gel electrophoresis.

AFLP markers were generated using the method of Vos et al. (1995). Briefly, genomic DNA was digested with EcoRI and MseI restriction enzymes. Restricted genomic DNA fragments were ligated to EcoRI and MseI adapters. A 1:5 dilution of restricted and adapter-ligated DNA was prepared using Tris-EDTA (TE) buffer. Pre-amplification occurred from adaptor-ligated DNA, and products were diluted (1:20) using TE and as templates for selective amplification. For selective amplification, 2 combinations of primers were used: E-ACC+M-CTC and E-AGG+M-CAG. Technical and biological replicates were performed to remove non-reproducible markers. The AFLP fragments were resolved on an ABI3730 (Life Technologies, Foster City, CA) capillary genotyping instrument. Markers were scored for presence (1) and absence (0)

of bands; bands of different electrophoretic mobilities were assumed to be non-allelic, while bands of the same length were assumed allelic. Band calling used the Genographer software (Benham, 2001).

The SSR markers were derived from Expressed Sequence Tags of the variety 'Cabernet' (Bushman, personal communication). Fifty SSR markers were genotyped on the same plants used for AFLP genotyping. Band calling of SSR markers also used Genographer software.

Analyses included estimations of the average molecular similarity within accessions, and a cluster analysis of individual plants. Average similarity within accessions was obtained using the Dice similarity coefficient (Dice, 1945), and corrected standard errors were obtained following Leonard et al. (1999). Clustering of individual plants utilized a neighbor-joining genetic distance algorithm implemented in PAUP software (Swofford, 2002).

RESULTS

Turf quality, stomatal conductance, and water potential of all entries decreased with longer exposure time as salinity increased. Likewise, but in the opposite trend, electrolyte leakage increased in response to higher salinity levels and with longer exposure to salinity stress. These trends were observed for all four *Poa pratensis* entries. At high salinity levels, the salt-tolerant entries (768, 603) exhibited higher turf quality, higher stomatal conductance, higher water potential, and lower electrolyte leakage than the moderately salt-tolerant (Midnight) and susceptible entries (Baron).

The effects of salt treatment and the exposure time (date) on turf quality, water potential, electrolyte leakage, and stomatal conductance were significant in all three experiments (Tables 3, 4, and 5). The effects of entry on turf quality, water potential, electrolyte leakage, and stomatal conductance were significant in all experiments except on water potential in experiment 1 (Tables 3, 4, 5). The two-way interactions of salt and entry for the four measured traits were significant in all experiments except for water potential and stomatal conductance in experiment 1. The two-way interactions of salt and date were significant in all experiments. The two-way interactions of date and entry were significant in all experiments except for water potential and stomatal conductance in experiment-1 and stomatal conductance in experiment 3. The three-way interactions of date, salt and entry were significant in experiment 1 for turf quality and electrolyte leakage experiment 1 (Table 3). In experiment 2, the three-way interaction was significant for turf quality and water potential (Table 4). The three-way interaction in experiment 3 was significant for all traits but stomatal conductance (Table 5).

Table 3. ANOVA summary of turf quality, water potential, electrolyte leakage, and stomatal conductance in experiment 1

Effect	Turf Quality	Water Potential	Electrolyte leakage	Stomata conductance
Salt	<0.0001	<0.0001	<0.0001	<0.0001
Entry	<0.0001	0.1642	<0.0001	0.0208
Salt*Entry	<0.0001	0.6286	<0.0001	0.3290
Date	<0.0001	<0.0001	<0.0001	<0.0001
Date*Salt	<0.0001	<0.0001	<0.0001	<0.0001
Date*Entry	<0.0001	0.2573	<0.0001	0.3340
Date*Salt*Entry	<0.0001	0.7274	0.0003	0.9992

Table 4. ANOVA summary of turf quality, water potential, electrolyte leakage, and stomatal conductance in experiment 2

Effect	Turf Quality	Water Potential	Electrolyte Leakage	Stomata conductance
Salt	<0.0001	<0.0001	<0.0001	<0.0001
Entry	<0.0001	<0.0001	<0.0001	0.0002
Salt*Entry	0.0012	<0.0001	0.0045	0.0003
Date	<0.0001	<0.0001	<0.0001	<0.0001
Date*Salt	<0.0001	<0.0001	<0.0001	<0.0001
Date*Entry	<0.0001	<0.0001	0.0021	<0.0001
Date*Salt*Entry	<0.0001	<0.0001	0.1015	0.9505

Table 5. ANOVA summary of turf quality, water potential, electrolyte leakage, and stomatal conductance in experiment 3

Effect	Pr Turf Quality	Pr Water Potential	Pr Electrolyte Leakage	Pr Stomata conductance
Salt	<0.0001	<0.0001	<0.0001	<0.0001
Entry	<0.0001	<0.0001	<0.0001	<0.0001
Salt*Entry	0.0014	<0.0001	<0.0001	0.1016
Date	<0.0001	<0.0001	<0.0001	<0.0001
Date*Salt	<0.0001	<0.0001	<0.0001	<0.0001
Date*Entry	<0.0001	<0.0001	<0.0001	0.1293
Date*Salt*Entry	<0.0001	<0.0001	<0.0001	1.0000

Turf quality

Turf quality decreased over exposure time (date) within salinity levels and decreased as salinity levels increased. When averaged over entries, the turf quality of control and 6dS treatment were similar and higher than turf quality means for the 12dS m^{-1}, 18 dS $m^{-1}$and 24 dS m^{-1} treatments (Fig. 3). The 603 and 768 entries had higher turf quality than Midnight and Baron at all salinity levels, but were equal under control conditions. The four entries did not all respond similarly to salinity and exposure time to salinity, as indicated by the significant salt×entry and date×salt×entry interactions.

Experiment-1 was unique in this work because the cultivar Brilliant was used instead of Baron (Fig. 3). The use of Baron in experiments 2 and 3 was because Brilliant was too aggressive in spreading prior to the initiation of the experiment, and thus biased results. In experiment-1, turf quality eventually decreased in all four entries at 6 dS m^{-1}, 12 dS m^{-1}, 18 dS m^{-1} and 24 dS m^{-1} (Fig. 3). At 6 dS m^{-1}, Brilliant and 768 turf quality

ratings were similar to control ratings through the first five of the seven measurement dates while the other two entries showed reductions in quality beginning at the third measurement date. At 12 dS m^{-1} and 24 dS m^{-1}, all four entries had a reduction in turf quality; however, the reduction was less severe in the cultivar Brilliant. Turf quality was similar in the 12 dS m^{-1} treatment and 24 dS m^{-1} treatment for Midnight and Brilliant, however, for 603 and 768, turf quality was higher ($P<0.05$) in the 12 dS m^{-1} treatment compared to the 24 dS m^{-1} treatment (Fig. 3). Brilliant had the highest visual quality in the end of experiment at highest salinity level (24 dS m^{-1}) and significantly different from other entries ($P<0.0001$). Entries 768 and 603 had the similar turf quality value at highest salinity level (24 dS m^{-1}) ($P=1.000$. at the end of experiment. In contrast, Midnight had the lowest quality values at the end of experiment ($P<0.0001$).

In experiment 2, the 6 dS treatment reduced turf quality slightly for 603 and 768, but to a significantly greater extent for Midnight and Baron (Fig. 3). By the end of the experiment, Midnight turf quality sank below the rating of 6 in the 6 dS treatment, Baron approached a rating of 6, and the two tolerant entries remained between 7 and 8. At 12 dS m^{-1}, Midnight ratings were near zero, Baron and 768 were just below 4, and 603 was 6. Entry 603 also had significantly higher turf quality at 24 dS m^{-1} ($P<0.0001$, Fig. 3) compared to the other entries at the end of experiment.

Due to extreme salt stress and death in plants exposed to 24 dS m^{-1} levels in experiments 1 and 2, the highest salt level was reduced to 18 dS m^{-1} in experiment 3. In this experiment the 6 dS m^{-1} treatment reduced turf quality ratings to 6 (603), 5 (768), and 4 (Midnight and Baron) (Fig. 3, $P<0.01$) by the end of the experiment. The graphs of the 12 dS m^{-1} and 18 dS m^{-1} treatments had similar quality ratings for Midnight and Baron.

Entry 603 had highest turf quality at all salt treatments by the end of experiment (Fig. 3, $P<0.02$). Entry 768 had an intermediate rating at 12 dS m^{-1} by the end of the experiment, and was significantly higher than Midnight, and Baron ($P<0.008$) and significantly lower than 603 ($P=0.0013$). Midnight, Baron, and 768 had similar turf quality in the 18dS treatment in the end of experiment ($P>0.05$ Fig. 3).

Water potential

Main effects and interactions for water potential were significant except for experiment-1 (Tables. 3-5). Like turfgrass quality, water potential declined over time within salinity levels and decreased as salinity stress increased. When averaged over entries, water potential of control and 6dS treatment were similar and higher than for the 12 dS m^{-1}, 18 dS m^{-1} and 24 dS m^{-1} treatments (Fig. 4). The 603 and 768 entries had higher water potential than Midnight and Baron at all four salinity levels (6 dS m^{-1}, 12 dS m^{-1}, 18 dS m^{-1} and 24 dS m^{-1}). However, the entries did not all respond similarly, as indicated by the significant two-way and three-way interactions.

In experiment 1, the effect of salt was significant, but the effect of entry was not significant. Interactions of salt×entry, date×entry, and date×entry×salt were not significant (Table 3). The 6 dS m^{-1} treatment did not significantly affect water potential for every entry ($P>0.9$), but as the salinity levels increased to 12 dS m^{-1} and 24 dS m^{-1}, the water potential was quickly declined lower than that of control ($P<0.05$, Fig. 4). However, the water potential in 12 dS m^{-1} treatment and 24 dS m^{-1} treatment was similar for each entry ($P>0.2$). Interestingly, Brilliant had lowest water potential in the end of experiment at 24 dS/m^{-1} level ($P<0.0001$, Fig. 4) while 603 and 768 had highest water potential in the 24 dS/m^{-1} level at the end of experiment (Fig. 4).

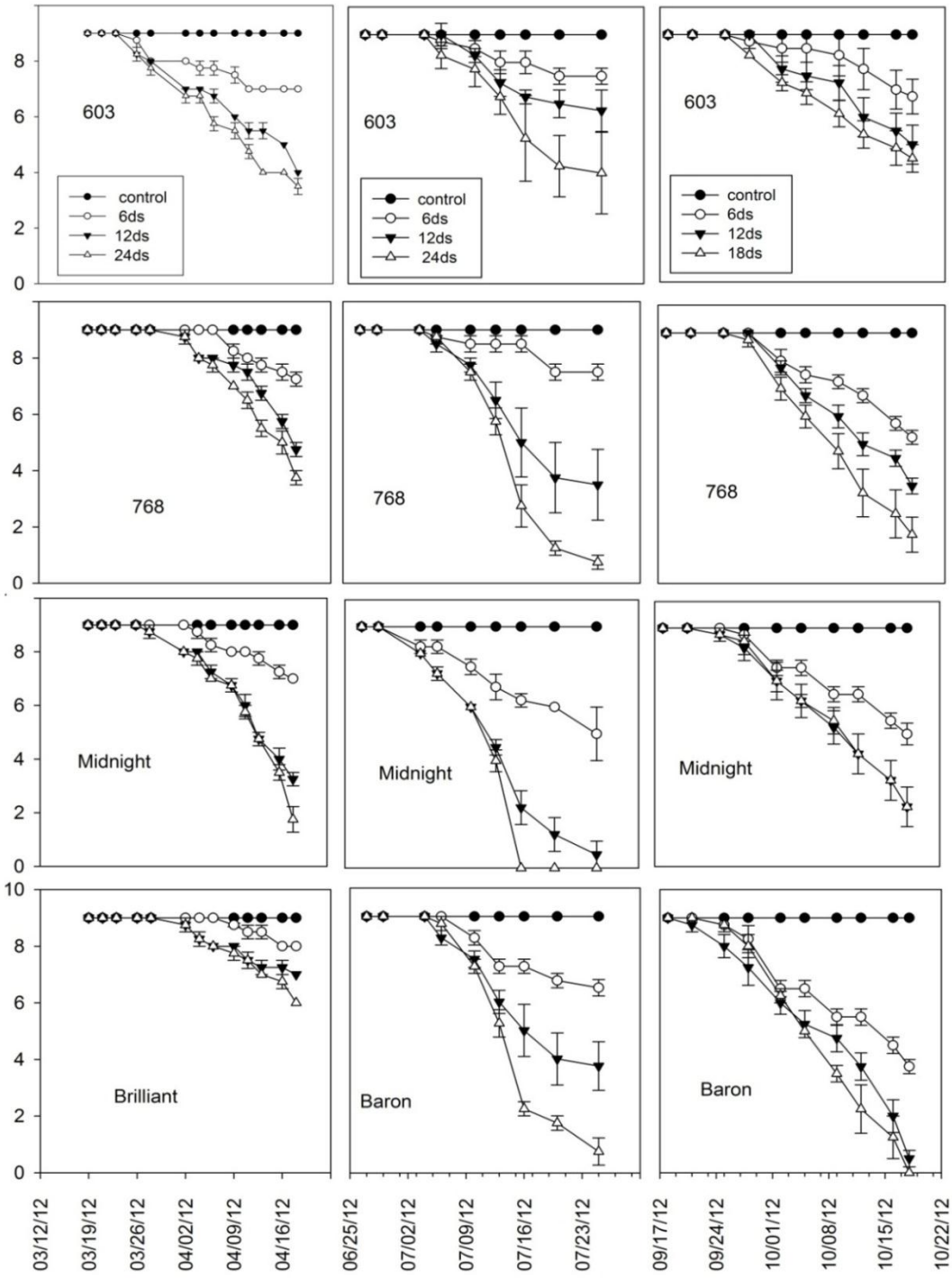

Figure 3. Turfgrass quality ratings of five Kentucky bluegrass entries at three salinity levels plus control during three experiments during 2012. Error bars represent standard error of the mean.

In experiment 2, the 6dS treatment did not affect water potential in three of the four entries, except for Baron ($p<0.0001$). Figure 4 shows the similar trends in 12 dS m^{-1} and 24 dS m^{-1} treatments for 603 ($P>0.9$). The water potential means of 12 dS m^{-1} and 24 dS m^{-1} treatment for 768, Midnight, and Baron were significantly different ($P<0.001$), with Midnight having the lowest values by the end of the experiment. When entries were compared at similar salt levels, the means of 603 and 768 were similar, and likewise Baron and Midnight ($P>0.1$). The 603 and 768 entries had the highest water potential by the end of experiment at 24 dS/m^{-1} level ($P<0.0001$).

In experiment 3, the trends of the 6 dS m^{-1} and control treatments were not significantly different (Fig. 4) such that 6dS treatment did not influence water potential for entries in the experiment ($P>0.8$). The values of water potential of 12 dS m^{-1} and 18 dS m^{-1} were also similar within each entry, except for Baron ($P<0.0001$). By the end of experiment at 18 dS m^{-1} level, the water potential of 603 was about -1.0 MPa, and Baron was about -1.36 MPa. 603 has significantly higher water potential than Midnight and Baron ($P<0.001$). By the end of experiment at 18 dS m^{-1} level, the water potential of 603 was about -1.0 MPa, Baron was about -1.36 MPa, and Midnight was about -1.15 MPa. Entry 603 had significantly higher water potential than Baron and Midnight ($P<0.001$). The 603 entry had the least reduction in water potential, followed by 768, Midnight, and then Baron (Fig. 4.).

Stomatal conductance

The stomatal conductance of all entries decreased as salt stress increased and with increasing time of exposure to salt stresses (Fig. 5). The main effects of salt, date, and

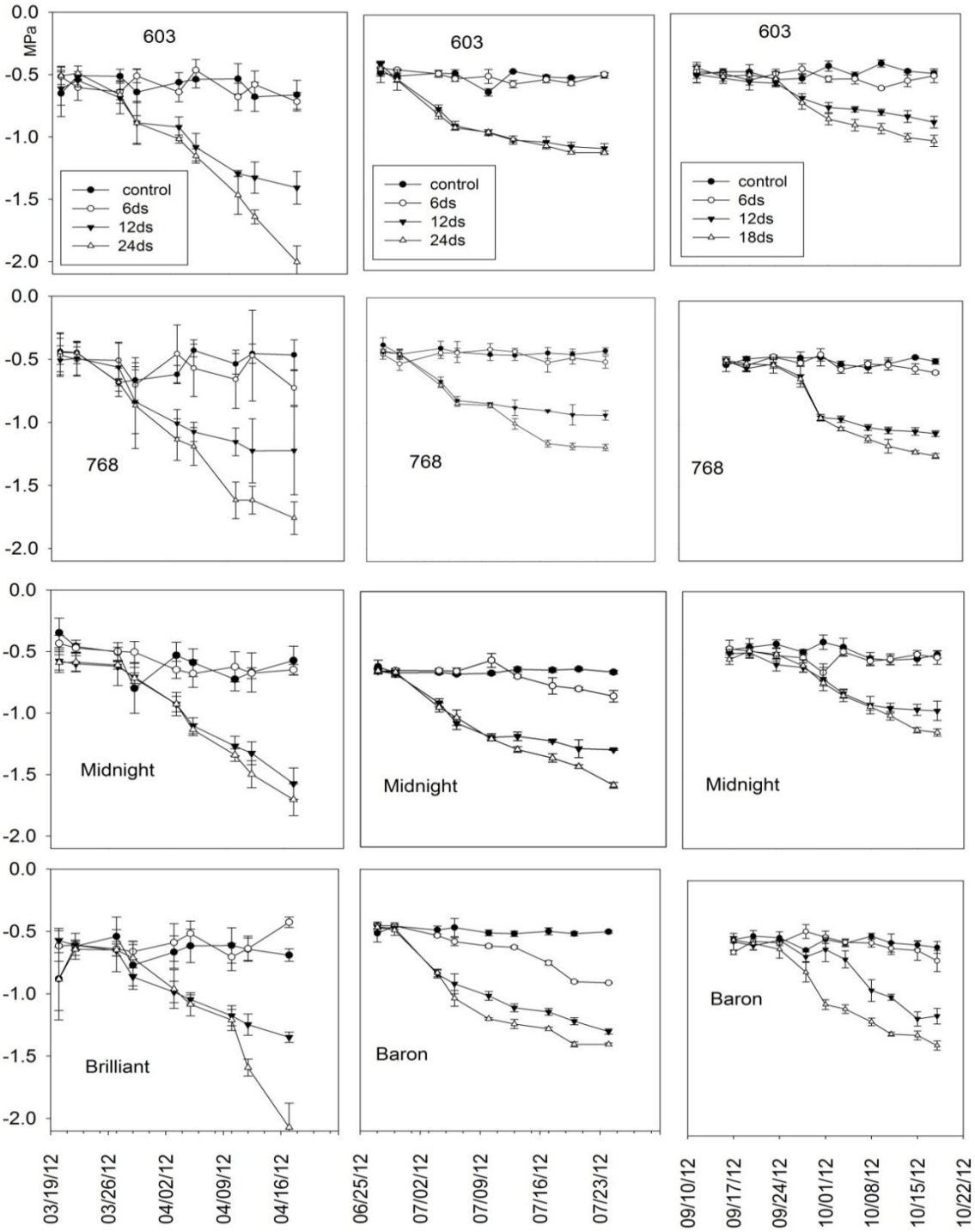

Figure 4. Water potential of five Kentucky bluegrass entries at three salinity levels plus control during three experiments during 2012. Error bars represent standard error of the mean.

their two-way interaction were significant in experiment 1. All main effects and two-way interactions were significant in experiment 2, and all main effects and the salt×date interaction were significant in experiment 3 (Table 3-5).

In experiment 1, the stomatal conductance decreased as exposure time (date) to salt increased and as salinity levels increased; however, the declines were sporadic (Fig. 5). Additionally, the 6 dS m^{-1} and 12 dS m^{-1} treatment did not affect stomatal conductance significantly for all entries ($P>0.05$) except for Baron at 12 dS m^{-1} level ($P<0.01$). The lack of significant entry effects (Table 2) is highlighted in Figure 5 where the entries also had no significant differences by the end of experiment at 24 dS m^{-1} level ($P>0.05$). Thus, stomatal conductance was not able to discriminate among the varieties and salt treatments in experiment 1.

In experiment 2, stomatal conductance gradually differentiated salt treatments by the end of the experiment and were much less sporadic. The overall means of stomatal conductance of 6 dS m^{-1} treatment and control were not significantly different for entries except Midnight ($P<0.05$) (Fig. 5), but the stomatal conductance in 12 dS m^{-1} and 24 dS m^{-1} treatments were significantly lower by the end of the experiment. The means at 12dS and 24dS m^{-1} treatment were also not significantly different within 603 and Midnight entries but higher in 12 dS m^{-1} compared to 24 dS m^{-1} treatment for 768 and Baron (Fig. 5). When comparing across entries, the means at 12 dS m^{-1} and 24 dS m^{-1} treatment were not significantly different ($P<0.05$). Entry 603 had the highest stomatal conductance by the end of experiment at 24 dS m^{-1} level ($P<0.005$).

In experiment 3, trends were apparent and consistent but separation between means was less than in experiment 2. The means of 603 and Midnight in the control and 6

dS m^{-1} are very similar, while the means of 768 and Baron in the 6 dS m^{-1} treatment declined over time compared to control (Fig. 5). The declining trends of all four entries for 12 dS m^{-1} and 18 dS m^{-1} treatments were similar, and showed 603 and Midnight declining to near 100, while 768 and Baron declined nearly to zero (Fig. 5). Entry 603 had the highest mean of stomatal conductance compared to other entries by the end of experiment within 18 dS m^{-1} level ($P<0.005$).

Electrolyte leakage (EL)

Unlike the other measurements where low values mean greater stress or response to stress, electrolyte leakage measurements increase in response to stress. Additionally, whereas other measures were made 4-6 times during each experiment, electrolyte leakage (EL) measurements were made only 3-4 times during each experiment. In general, EL increased as salinity levels increase and over time at a given salinity level (date) (Fig. 6). All the main effects and two-way interactions were significant; only the effect of date×salt×entry was not significant in experiment 2 (Tables 3-5).

In experiment 1, 6 dS m^{-1} treatment increased the EL for all entries ($P<0.0001$, Fig. 6). However, the means of 12 dS m^{-1} and 24 dS m^{-1} are very similar, the pattern of 12 dS m^{-1} and 24 dS m^{-1} treatments were not significantly different from each other ($P>0.05$). Brilliant and Midnight had similar values of EL by the end of experiment at 24 dS m^{-1} level ($P>0.05$), and those values were higher than for 603 and 768. 603 and 768 were not significantly different from each other ($P>0.05$)

In experiment 2, EL increased in all salt levels with comparison to the control treatment, but the increase in 603 was not always significant (Fig. 6). By the end of the experiment, all four treatments were significantly different for each entry. At 12 dS m^{-1},

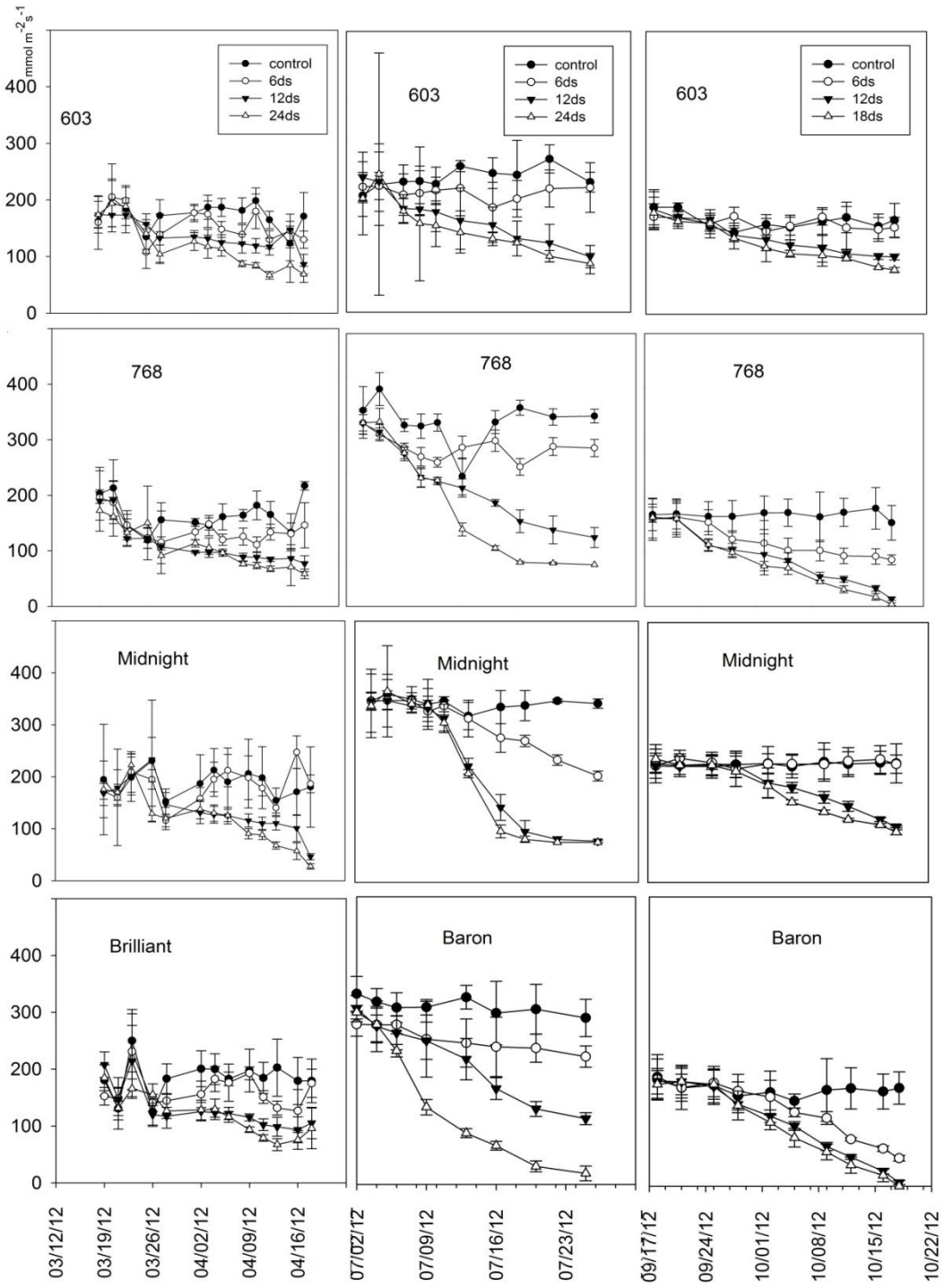

Figure 5. Stomatal conductance of five Kentucky bluegrass entries at three salinity levels plus control during three experiments during 2012. Error bars represent standard error of the mean.

Midnight had the highest EL and 603 the lowest. At 24 dS m^{-1}, Midnight and Baron had similar high EL values, followed by 768, and then 603 had the lowest EL (Fig. 6).

In experiment 3, the means of 6 dS m^{-1} and 12 dS m^{-1} salt treatments were not significant for 603 ($P>0.05$). However, the effect of the 6 dS m^{-1} treatment on EL was significant and much greater for other entries ($P<0.005$). At 12 dS m^{-1}, 768 and Baron had similar patterns of high EL values, followed by Midnight, and then followed by 603. The 18 dS m^{-1} trends were similar to those of 12 dS m^{-1}, as all the means of all entries in the 12 dS m^{-1} and 18 dS m^{-1} treatments were not significant ($P>0.05$). Entry 603 had the lowest EL among all entries in the end of experiment at 18 dS m^{-1} level ($P<0.0001$) while 768, Midnight and Baron had similar EL values ($P>0.05$).

Salinity and soil moisture parameters

During each experiment, salt solutions were applied from the surface as a drench with the intent to flush the soil solution with the applied salt solution (Fig. 7). The bulk conductivity increased as the pots were irrigated with the treatments, and then gradually decreased as soil moisture levels decreased until the next salt solution irrigation event. The bulk conductivity measurement after irrigation tended to increase at least until the middle of each experiment. However, salinity levels limited the ability of the soil sensors to measure soil moisture and salinity since the maximum EC_{bulk} measured by the sensor is 6.5 dS m^{-1}. In the 12dS m^{-1} and 24 dS m^{-1} treatments, this quickly exceeded those bulk conductivity limits and measurements were not obtained.

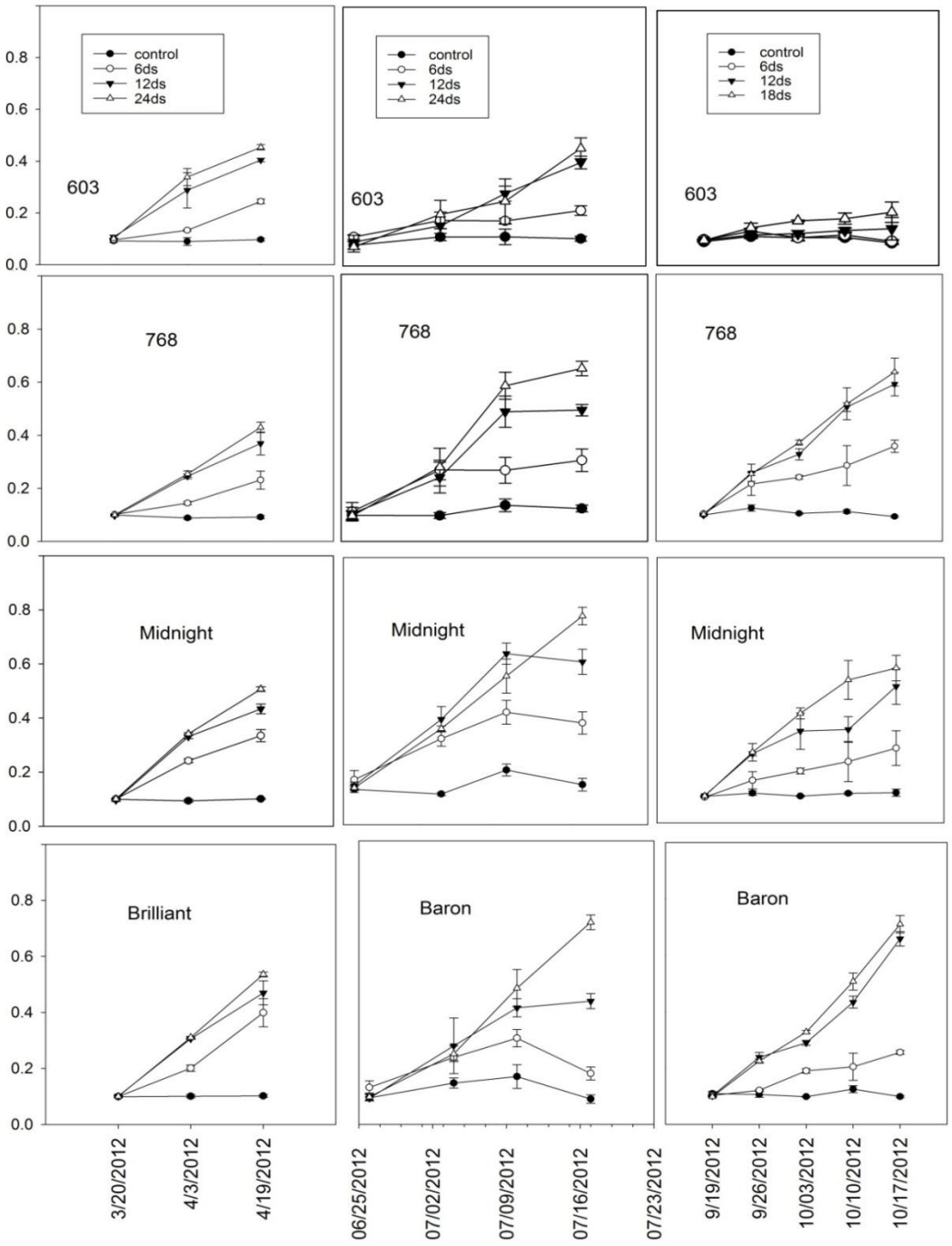

Figure 6. Electrolyte leakage of five Kentucky bluegrass entries at three salinity levels plus control during three experiments during 2012. Error bars represent standard error of the mean.

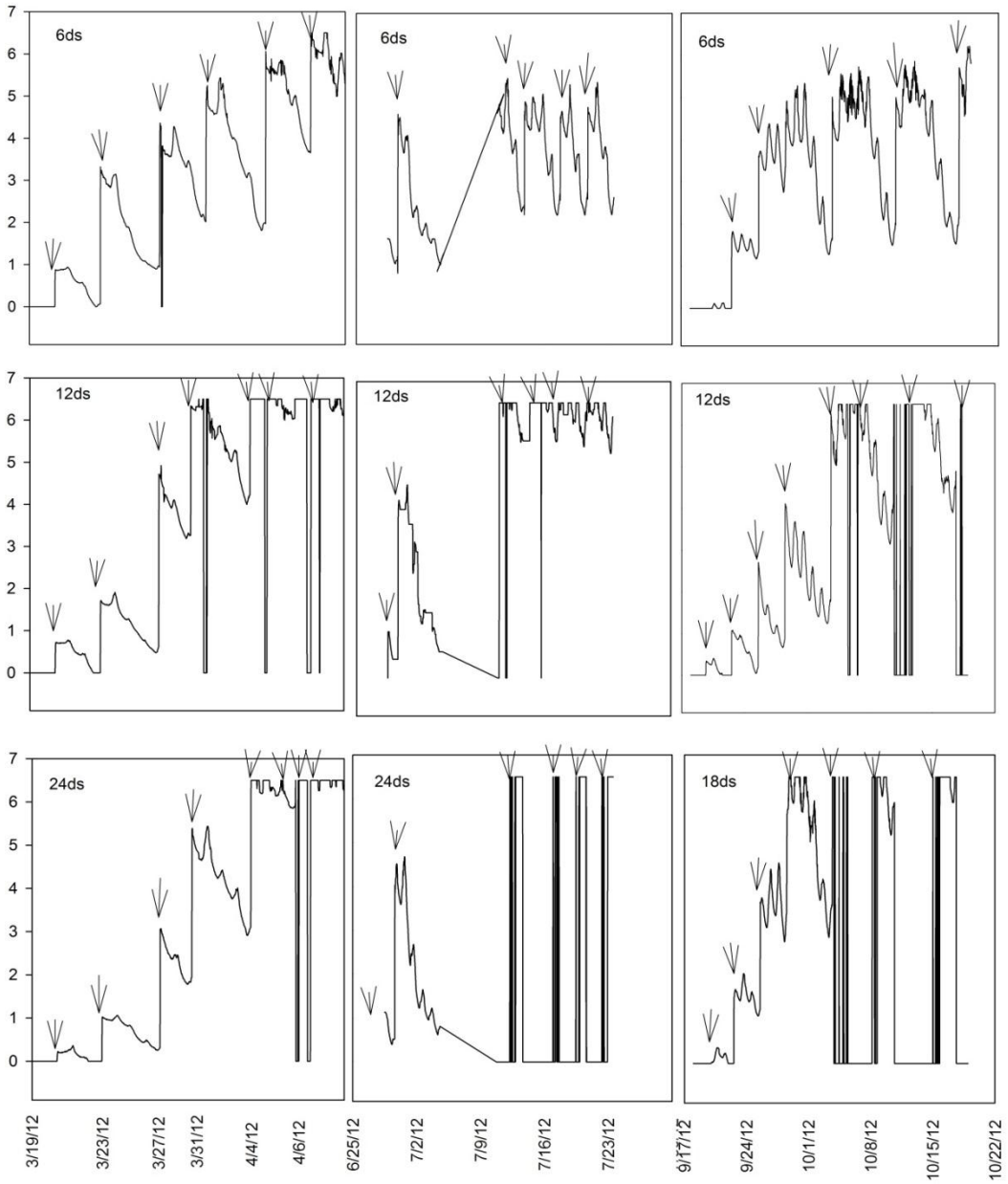

Figure 7. Bulk conductivity as measured by Acclima sensors at three salinity levels during three experiments during 2012.

Correlations

From Tables 6-8, the absolute value of the correlation coefficients are above 0.7, and all values are significant at $P<0.01$. This indicates a strong association between each pair of measurements. As turf quality decreased, water potential and stomatal conductance decreased and electrolyte leakage increased.

Table 6. Pearson correlation coefficients of electrolyte leakage, turf quality, water potential, and stomatal conductance measurements in experiment 1.

	Electrolyte Leakage	Turf Quality	Water Potential	Stomatal Conductance
Electrolyte Leakage	1	-0.81**	-0.86**	-0.84**
Turf Quality	-0.81**	1	0.84**	0.84**
Water Potential	-0.86**	0.84**	1	0.87**
Stomatal Conductance	-0.84**	0.84**	0.87**	1

Symbols *, ** are used to show significance at the $\alpha = 0.01$, and 0.001 levels

Table 7. Pearson correlation coefficients of experiment 2

	Electrolyte Leakage	Turf Quality	Water Potential	Stomatal Conductance
Electrolyte Leakage	1	-0.87**	-0.86**	-0.85**
Turf Quality	-0.87**	1	0.80**	0.83**
Water Potential	-0.86**	0.80**	1	0.74**
Stomatal Conductance	-0.86**	0.83**	0.74**	1

Symbols *, ** are used to show significance at the $\alpha = 0.01$, and 0.001 levels

Table 8. Pearson correlation coefficients of experiment 3

	Electrolyte Leakage	Turf Quality	Water Potential	Stomatal Conductance
Electrolyte Leakage	1	-0.86**	-0.85**	-0.88**
Turf Quality	-0.86**	1	0.82*	0.89**
Water Potential	-0.85**	0.82**	1	0.87**
Stomatal Conductance	-0.88**	0.89**	0.87**	1

Symbols *, ** are used to show significance at the $\alpha = 0.01$, and 0.001 levels

Molecular marker genotyping

As entry 603 has been shown to have the lowest EL, and the lowest declines in other measures, it is consistent with its characterization as a salt tolerant source of Kentucky bluegrass. Prior to its incorporation into a breeding line, or its introgression into elite Kentucky bluegrass lines, an assessment of apomixis is necessary to predict if it will remain true-to-type Apomixis can best be assessed using an average similarity of molecular marker bands within plants of the 603 accession. By comparing to other salt tolerant accessions and the cultivar Midnight (highly apomictic), its relative level of apomixis can be estimated.

The average similarity within the two accessions and the cultivar Midnight ranged from 0.937 to 0.995 (Table 9). The highest similarity within entries was for PI371768 using AFLP markers, while the lowest was for PI440603 using SSR markers. Both marker systems showed similar results, although AFLP result tended give slightly higher values. The similarity of 768 was so high with AFLP markers that the plants sampled from this accession were likely all identical from a molecular perspective. Midnight is considered highly apomictic and its similarity value was 0.94. Thus, the accessions that all had high average similarity values, including the two salt tolerant accessions included in the physiological studies above, would also be considered highly apomictic.

The plants within each accession clustered with bootstrap support (Fig. 7). The 768 and 742 plants also formed a subgroup with support. The horizontal distance between plants corresponds to the genetic difference between plants and entries. Thus, the most genetic distance lied between entries, as might be expected for an apomictic species. PI660603 had the lowest average similarity value as an entry, and also had the longest

branch lengths between its constituent plants. Conversely, PI371768 was the most similar and almost had no horizontal branch difference.

Table 9. Similarity of polymorphic bands in five *Poa* genotypes

Enter ID	N	Marker Type	Mean Similarity	SE
PI371768	4	AFLP	0.995	0.0029
PI371742	5	AFLP	0.986	0.0024
PI371771	8	AFLP	0.969	0.0051
PI440603	4	AFLP	0.937	0.0431
PI371771	4	SSR	0.956	0.0275
PI440603	4	SSR	0.966	0.0060
Midnight	4	SSR	0.964	0.0046

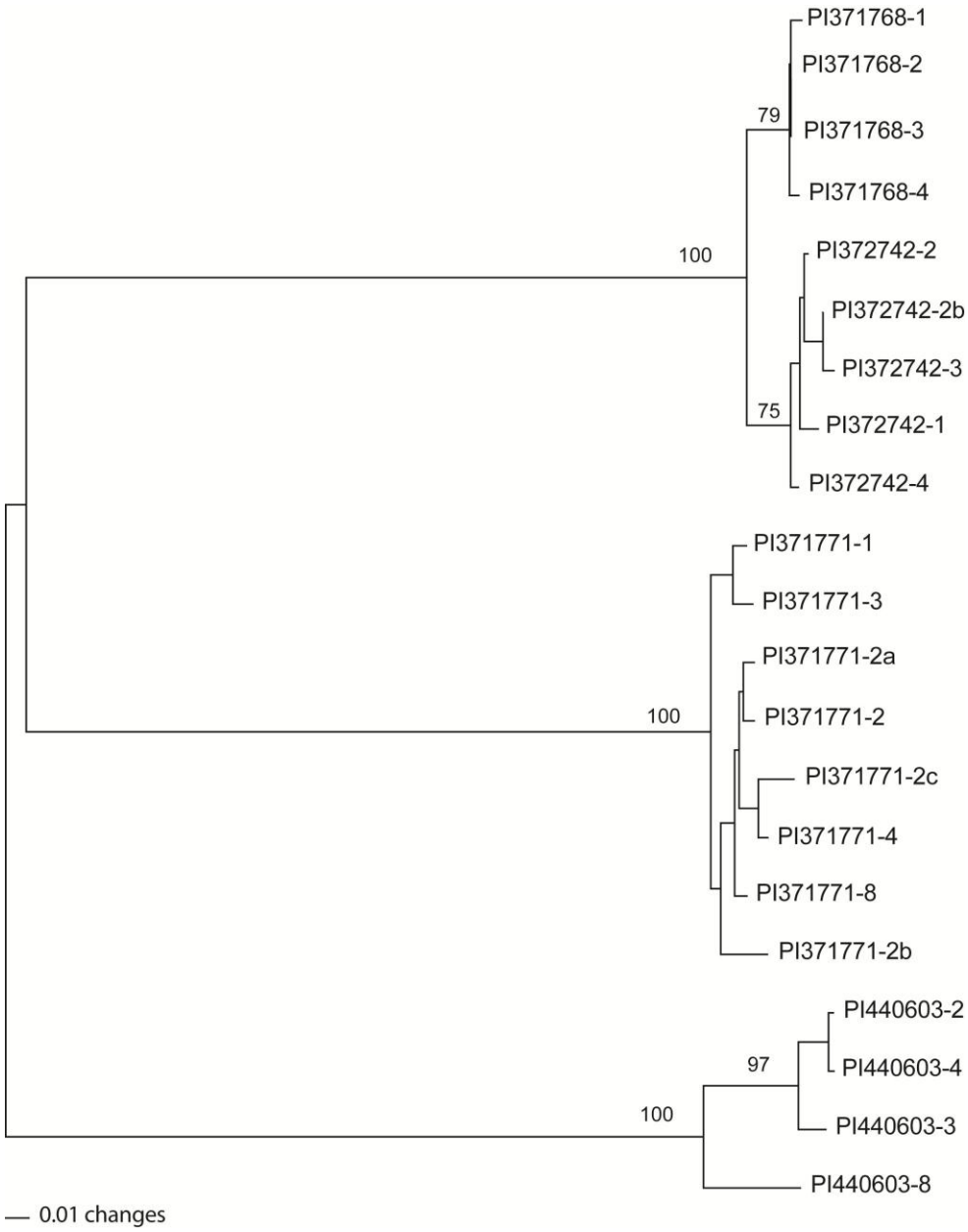

Figure 8. Neighbor-joining cluster analysis of four *Poa pratensis* accessions and the cultivar Midnight.

DISCUSSION

Salinity tolerance in Kentucky bluegrass is often studied in terms of morphological (turf quality) and physiological (leaf water potential, stomata conductance, electrolyte leakage) criteria. Like in other literature, relative water content and shoot growth of salt-sensitive turfgrass species was reported to decrease more quickly under salinity stress than for a salt-tolerant species (Suplick-Ploense, 2002; Alshammary et al., 2004; Liu et al., 2011). In Kentucky bluegrass cultivars (Midnight, PI372742, PI4368223), turf quality and relative water content and stomatal conductance declined with increasing salt concentration and the duration of salinity treatment (Leksungnoen et al., 2012).

In this study, water potential, stomata conductance and turf quality showed earlier and more severe decline, leaf EL showed an earlier and sharper increase (more leakage) in Midnight and Baron than that in 603 and 768 at the 12 dS m^{-1}, 18 dS m^{-1}, and 24 dS m^{-1} treatment levels (Figs. 3-6). Previously, 768 and 603 were reported to have more salinity tolerance, Brilliant and Baron were considered salt-susceptible, and Midnight was considered moderately salt-tolerant (Robins et al., 2009). Overall, our results confirmed the tolerance of 603 and 768, with 603 being the most tolerant, and found little differences among the less tolerant group of Midnight, Brilliant, and Baron.

Previous studies showed that variation among Kentucky bluegrass cultivars in salt tolerance existed (Marcum, 2008; Poss and Russell, 2010), but the responses or mechanisms used by the cultivars to tolerate the stress, and the general ranking of tolerance appear to differ. For example, Koch reported that Kentucky bluegrass salt-tolerant cultivar Diva had significantly higher relative water content compared to the salt-

sensitive P-105 under salinity stress in hydroponic environments (Koch et al., 2011). However, according to Leksungnoen et al. (2012), a susceptible Kentucky bluegrass had higher leaf water potential than other salt-tolerant entries at high salinity levels. The 768 and 603 lines in this study had higher leaf water potential than the susceptible Kentucky bluegrass varieties Brilliant and Baron when conducted salt solutions. Thus entry 768 and 603 have more capability to adjust water potential to survive in higher salt concentration conditions than Brilliant and Baron.

Previous studies have exhaustively showed variation among cultivars of salt tolerance in Kentucky bluegrass existed (Marcum, 2006; Poss and Russell, 2010). However, in one previous study, salinity tolerance rankings were reported as Baron>Brilliant>Eagleton>Cabernet>Midnight (Grieve et al., 2006), while in another study the tolerance of Midnight was higher than Baron and Brilliant (Torello and Symington, 1984). For this reason, we deemed it necessary to conduct salinity trials that included Midnight and Brilliant alongside our internal salt tolerant lines. Although the overall tolerance across experiments in our study was 603≥768>Brilliant>Midnight, Brilliant showed high turf quality and stomata conductance at high salinity levels (12 dS m^{-1} and 24 dS m^{-1}) in experiment-1. This apparent tolerance may have actually occurred because Brilliant had high plant vigor prior to initiation of the study, higher than the other entries because of different propagation techniques. This appeared to provide it with a competitive advantage by expanding and competing for space with the other entries. Because of this, we used Baron, another putatively salt intolerant variety in its place for experiments 2 and 3. Similar to Brilliant, Baron has also been included in salinity tolerance research and has shown variable results (Horst and Taylor, 1983; Torello and

Symington, 1984). According to the performance of turf quality, water potential, stomatal conductance and electrolyte leakage, in experiments 2 and 3, the salt-tolerance rank was 603≥768>Midnight>Baron as expected.

Stomatal conductance showed no significant differences between control and 6dS m^{-1} treatment, and no significant differences among 12 dS m^{-1}, 18 dS m^{-1} dS and 24 dS m^{-1} treatments. However, control and 6dS m^{-1} treatment were significantly different from 12, 18, and 24 dS m^{-1} treatments. This bifurcation indicated that there was no significant difference of effects on turfgrass between control and 6 dS m^{-1} treatment, and also between 12 dS m^{-1} to 24 dS m^{-1}. The salt tolerance of Kentucky bluegrass was already exceeded at 12dS m^{-1}, therefore no further stress responses were observed at 18 dS m^{-1}or 24 dS m^{-1}.

Plants open their stomata to create leaf a water potential gradient between the leaf and the atmosphere, which enables plant leaves to absorb and pull out water from soil (Taiz and Zeiger, 2006). Root water potential must be lower than the soil solution to absorb the water from soil. Salts reduce soil solution water potential, and as a result plants must lower their root water potential to create the gradient to absorb water from soil. In this study, water potential declined for all entries as the soil EC increased, or in other words, all varieties lowed their water potential to absorb water from soil. Midnight and Baron reduced their water potential much lower than 603 and 768 entries at salinity levels above 6 dS m^{-1}.

Electrolyte leakage (an indicator of cell membrane stability) and tissue yield (an indicator of overall vigor) has been widely used for screening tolerance of drought-related stresses in plants, including sorghum (*Sorghum bicolor L.*) (Sullivan and Ross,

1979), wheat (*Triticum aestivum L.*) (Blum and Ebrecon, 1981), tomato (*Lycopersicon esculentum M.*) (Chen et al., 1982), and turfgrasses (Marcum, 1998; Su et al., 2007). It was reported that significant correlations existed between EL and salt stress injury (relative water content, Na^+ and K^+ leaf content, and yield) in wheat (Farooq and Azam, 2006). In the current study, EL increased as salinity level increased, and as water potential, stomatal conductance and turf quality decreased (Fig. 6). These findings were similar to previous studies (Liu et al., 2011; Leksungnoen et al., 2012), which means that all the parameters can be effectively used to evaluate the relative salinity tolerance. Entries 603 and 768 had the lower EL than Midnight, Brilliant and Baron under all salinity levels in all experiments (Fig. 6).

The soil parameters of the root zone were effectively measured by the Acclima sensors, but levels of EC_{bulk} is limited as well as interpretation of EC_e. One major limitation was the threshold of electrical conductivity measurable by these probes. The bulk conductivity was limited by a maximum EC_{bulk} of 6.5 dS m^{-1} in these probes, which prevented us from obtaining bulk conductivity measurements in excess of 6.5 dS m^{-1}. In this experiment, our control, EC6, and EC12 solution treatments were measurable within this threshold, but higher treatments were not. As a result, parameters such as water content, bulk conductivity, and temperature in the soil were not able to be measured when the bulk conductivity increased above 6.5 dS m^{-1}.

Cultivars showing increased salinity tolerance by way of turf quality and stomatal conductance coincided with plants that had higher water potential and lower EL measurements. The results showed that 603 and 768 were the salt-tolerant entries among KBG, which is consistent with the previous studies (Robins et al., 2009), and that 603

was more tolerant than 768. The salt-tolerance of Baron, previously described as salt sensitive, were similar to Midnight, which was previously described as moderately tolerant. These different responses of cultivars compared to previous work could be explained in a number of ways. One difference is in experimental methods. Previous studies have used hydroponic methods compared to our overhead irrigated methods. The hydroponic method may cause less stress resulting in higher percent green ratings than the overhead-irrigated methods (Koch and Bonos, 2011).

Other possible reasons of different results could be due to environment, such as temperature and the salts used in the salinity treatments. Even if the experiments were all conducted in greenhouse, the temperature, day length, humidity, light intensity can be different. And also the discrepancy between runs can be partially attributable to the plants being at different stages of vegetative growth before initiation of the experiment, or due to the time of year of the experiments. In our work, experiment-1 was conducted in the spring when plants were exposed to initially lower light intensity but increasing closer to summer. Experiment 2 was conducted in summer, as the plants were subjected, high light intensity, higher air and leaf temperatures despite good climate control, and lower relative humidity. Experiment 3 was conducted in the fall as the plants were exposed to lower light intensities and cooler temperatures. Despite this issue, genotype responses were relatively consistent across the experiments.

Among four measurements: turf quality, water potential, stomatal conductance, and electrolyte leakage used in the research, all measurements were highly correlated due to the high coefficients (Tables 6-8). Turf quality is the easiest and most efficient way to measure the responses, however it is very subjective. However, because of convenience

and efficiency, turf quality should be still used in future work. The stomatal conductance measurements are difficult in part because grass leaves are narrow, requiring several leaves to be arranged across the instrument's measurement chamber. This creates experimental error. In addition, light conditions influence stomatal conductance measurements to a great extent. Because of these sources of variation in the measurement, the standard deviation of these measurements is very high compared to water potential and electrolyte leakage measurements (Figs. 3-6). Water potential and electrolyte leakage are very reliable and accurate methods due to the low standard deviations (Figs. 3-6). The electrolyte leakage methods are less affected by environment.

CONCLUSION

The four salt tolerant accessions all had high similarity among plants within accessions. This high similarity indicates high levels of apomixis. Both AFLP and SSR markers were similar.

In general, the salt tolerance of KBG cultivars in this study can be divided into two groups: salt-tolerant and salt-sensitive. Entries 603 and 768 are salt-tolerant entries, Midnight and Baron are salt-sensitive cultivars. These groupings are based on evaluations of turf quality, water potential, electrolyte leakage and stomatal conductance.

Each of the measurements used to evaluate salinity response was effective, however the standard deviation of stomatal conductance is large and the data was not as reliable at other measurement methods. Among all parameters evaluating the salt tolerance of turfgrass, electrolyte leakage and water potential are the most accurate and reliable parameter. Among all the measurements, measuring visual quality is the fastest and direct way to evaluate the salt tolerance of Kentucky bluegrass cultivars. Measuring water potential can be time consuming, but the measurements are accurate. Electrolyte leakage measurements are highly accurate, but time to collect samples is relatively high. In the future field study, electrolyte leakage is highly recommended for screening salt tolerance of turfgrasses.

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