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Salinity Tolerance in Kentucky Bluegrass Hybrids

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SALINITY TOLERANCE IN KENTUCKY BLUEGRASS HYBRIDS

by

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ABSTRACT

Salinity Tolerance in Kentucky Bluegrass Hybrids

by

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Diminishing water sources in the Intermountain West have led to increased use of alternative sources of water. These sources, such as reclaimed water, generally have elevated salinity levels that may stress turfgrasses. Kentucky bluegrass (*Poa pratensis* L.) is sensitive to salinity stress, but is otherwise very well adapted to many turfgrass areas because of its dark color, durability, ability to recover from wear, and soft texture. Because of these positive traits, it has been the subject of selection for salinity tolerance. However, Kentucky bluegrass is a polyploid plant that can exhibit dosage differences upon hybridization. Furthermore, Kentucky bluegrass is a facultative apomictic, with hybridization occurring at a low level, that is difficult to detect. This study was designed to evaluate the differences in salinity tolerance among parental lines of Kentucky bluegrass that were reported to vary in salt tolerance and hybrids among them. My hypothesis was that hybrids between salt tolerant and susceptible parent lines would have mid-parent salt tolerances.

Thirty one Kentucky bluegrass parent and hybrid plants established in 6.4×25.5

cm containers with silica sand were irrigated with increasing salinity levels. Plants were irrigated 1.25 cm every-other-day with an automated boom irrigation system. Treatments began at 3 dS m^{-1} for two weeks then increased to 6 dS m^{-1} for six weeks. Electrolyte leakage was measured to quantify salt stress along with visual ratings of plant health. The experiment was replicated 4 times over the course of 3 years.

There was significant variation in salinity tolerance among the different parents and hybrids. Entries that tended to have low electrolyte leakage ratios also tended to have higher visual turf quality ratings, but this was not always the case. Grasses that performed well in both areas were parent lines 768, 827, and the cultivar North Star, and hybrids (NS × 768)-21, (NS × 768)-22, (827 × 768)-32, (827 × 768)-36, (557 × 603)-51, and (557 × 603)-53. Eleven hybrids showed mid-parent or better salt tolerance, while 14 hybrids had less than mid-parent salt tolerance in either turf quality or electrolyte leakage

I concluded that some Kentucky bluegrass hybrids had mid-parent salinity tolerance and have potential for use in environments with elevated salinity levels. The large numbers of hybrids with less than mid-parent salt tolerances indicate the need to individually test hybrids for traits of interest in this complex turfgrass species.

(85 pages)

PUBLIC ABSTRACT

Salinity Tolerance in Kentucky Bluegrass Hybrids

Paul G. Harris

Diminishing water sources in the Intermountain West have led to increased use of alternative sources of water. These sources, such as reclaimed water, generally have elevated salinity levels that may slow growth, and cause a decline in turfgrass quality. Kentucky bluegrass (*Poa pratensis* L.) is sensitive to salt stress, but is otherwise very well adapted to many turfgrass areas because of its dark color, durability, ability to recover from wear, and soft texture. Because of these positive traits, it has been the subject of selection for salt tolerance. This study was designed to evaluate the salt tolerance of hybrids from parents that had previously recorded higher salt tolerance, and parents with higher quality traits. My hypothesis was that hybrids from these parent would have mid-parent salt tolerances.

Thirty-one Kentucky bluegrass entries were included in this experiment, ten parents and twenty-one hybrids. Parents and hybrid plants were irrigated with increasing salinity levels. Plants were irrigated every-other-day with an automated boom irrigation system. Treatments began at a lower salinity level (3 dS m^{-1}) for two weeks then increased to a higher salinity level (6 dS m^{-1}) for the remainder of the eight-week experiment. Electrolyte leakage was measured to quantify salt stress along with visual quality ratings of plant health. The experiment was replicated 4 times over the course of 3 years.

There was significant variation in salt tolerance among the different parents and hybrids. Grasses demonstrating higher salt tolerance generally did so during all four replications of the experiment. Of the hybrids that were evaluated, six demonstrated improved salt tolerance. The majority of these hybrids were offspring of parents: 768, ‘North Star’, 827, and 603. The numbered parents are breeding lines in the USDA-USU bluegrass program. I concluded that some Kentucky bluegrass hybrids have potential for use in environments with elevated salinity levels.

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INTRODUCTION

Population Growth

Many western states are growing rapidly, at a rate of 1.6 to 2.0% annually (U.S. Census Bureau 2016). In the case of the state of Utah, that could mean increasing its population by 30% in as little as 15 years. Along with this rapid urban development comes an increased strain on clean water sources, especially in an area with relatively little annual precipitation. Population growth coupled with unpredictable weather patterns have raised an awareness of increasing water restrictions in agriculture and urban landscapes irrigation. To help conserve water in the urban landscapes, turfgrass removal is being recommended in some locations and 'more drought tolerant' or 'adapted' plants are being considered. Despite misconceptions surrounding turfgrass water use, this class of plant material continues to play an important role where green spaces are needed in parks and safe playing surfaces are needed for athletic events. And while there are benefits to having green spaces, large irrigated areas of turfgrass may put a substantial demand on water supplies. For example, golf courses can use between 250,000 and 1 million gallons of water per day during the summer months depending on location (Huck et al., 2000). In an effort to conserve the highest quality water sources for human consumption, alternative, (lower quality) water sources may be considered for these areas that require a functional stand of turfgrass.

Maintaining healthy turfgrass has many benefits. Aside from being aesthetically pleasing, there are social benefits to having access to turfgrass areas. Turfgrass provides a space to recreate and gather. As we spend more time in green spaces our physical and

mental health improve, whether is playing soccer or enjoying a barefoot stroll to clear your mind (Beard and Green, 1994). There are also several environmental benefits of turfgrass. Turfgrass improves air quality by absorbing atmospheric pollutants (Stier et al., 2013). Ground water is recharged as a result of turfgrass reducing runoff (Gross et al., 1991). As water percolates through the turfgrass/soil system it is filtered and cleansed by microorganisms that degrade organic chemicals and pollutants (Beard and Green, 1994).

While healthy turfgrasses provide these benefits, the increase of poor quality or reclaimed irrigation water may threaten this functionality. Reclaimed water often has elevated salinity levels that are detrimental to Kentucky bluegrass (KBG), which is the most common grass used in parks and athletic fields in the Intermountain West. The objective of this project was to evaluate salinity tolerance of KBG hybrids bred from salt-tolerant parents.

Wastewater

Along with an increased demand for freshwater resources from growing populations comes an increased volume of waste water generated from sewage treatment systems. It's estimated that the average person in the United States uses roughly 100 gallons per day (USEPA, 2008; Kenny et al., 2017). A city with 100,000 residents could, therefore, produce ten million gallons of wastewater daily, not including contributions from other commercial or industrial sites. According to the city of Logan Utah, their water treatment facility, which services most of Cache County, receives an average of 14 million gallons of wastewater daily.

The degree of wastewater treatment depends on its intended application, such as industrial or agricultural purposes (Harivandi 2012; Cassanit et al., 2012). Recycled water for turfgrass irrigation must be treated to at least a secondary level, meaning it has received treatments such as oxidation, activated sludge, and filtration, as well as disinfection by UV lights or chlorine (Qian and Harivandi 2007; Haering et al 2009).

Because of its increasing abundance, and as a way to maximize existing urban water resources, some municipalities have begun utilizing wastewater, or reclaimed water, for turfgrass and landscape irrigation (Koch and Bonos, 2011). With stricter wastewater discharge standards, the use of reclaimed water is becoming increasingly attractive (Qian and Harivandi 2007). In the United States it's estimated that 12 to 15% of golf courses us reclaimed water. While 35% of golf courses from the southwestern states use reclaimed water (Harivandi, 2011; Throssell et al., 2009). As drought becomes more common in western states an increase of reclaimed water use is expected.

Economically, the use of reclaimed water can provide a huge cost savings for turfgrass managers. Golf courses in arid western states may expect to spend from $100,000 to $1,000,000 annually on potable water for irrigation purposes. At a savings of 80% compared to potable water, the use of reclaimed water can become an appealing alternative (Huck et al., 2000). Despite economic advantages, irrigating with reclaimed water can result in negative effects on turfgrass health and quality. Because it is derived from domestic waste water, reclaimed water may have poor quality due to elevated amounts of dissolved salts from food processing, water softening and soaps or detergents. These added contaminants cannot be removed during the treatment process and contribute to elevated salinity levels. Water salinity is most commonly measured by

electrical conductivity (EC_{iw}), and is reported in units of decisiemens per meter (dS m^{-1}). Increased salinity can create challenges for turfgrass managers (Qian and Harivandi, 2007). Salinity mimics drought conditions in plants, and sodium degrades soil structure (Munns, 2002; Morugán-Coronado et al., 2011). The use of poor quality irrigation water can also degrade soil structure with increased sodium adsorption ratios (SARs) (Qian and Harivandi, 2007). While elevated salinity and sodicity are concerns for turfgrass managers, this research focuses on the effects of salinity.

Plant Response to Salinity

The increasingly commonplace use of reclaimed water and its elevated salt concentrations present a number of challenges for turfgrasses as well as other landscapes plants, as salinity levels increase in the soil over time (Shani and Dudley, 2001). A major problem that results from saline conditions is physiological drought (Munns, 2002; Carrow and Duncan, 1998). The introduction of salt to the soil solution leads to an increase in osmotic pressure. Water moves from a solution with low osmotic pressure into a solution with higher osmotic pressure. As the osmotic pressure in the soil approaches and increases beyond the pressure of the cells in the plant, water entry is restricted into the plant leading to drought symptoms (Munns and Tester, 2008; Marcum, and Murdoch, 1994).

Other problems associated with saline soils are ion toxicity and ion imbalance. Ion toxicity is cause by specific ions that have detrimental effects on plant root or shoot tissues. Of these, most dominant toxic ions are Na^{+} and Cl^{-}. Accumulation of Cl^{-} can lead

to leaf burn and desiccation, this damage to leaf tissue can lead to a reduction in photosynthesis (Carrow and Duncan, 1998). Ion imbalance from increased Na^+ and Cl^- can also lead to nutrient imbalances that lead to an overall decline in turfgrass vigor (Pace and Johnson, 2002; Rusan et al., 2007). Specific reductions in ion uptake include, Ca^{+2}, K^+, and NO_3^-, Mg^{+2}, Mn, and P (Carrow and Duncan, 1998; Grattan and Grieve, 1998; Lauchli and Luttge, 2011).

Added Maintenance

Using reclaimed water; rather than potable water; for irrigation may cause problems with plant growth. However, using reclaimed water can result in reduced irrigation costs. While the apparent monetary gain from using reclaimed water is significant, just as noteworthy is the added expense needed to remedy the harmful effects of salinity stress on turfgrass. These expenses come in the form of added management practices. One of the most important resulting practices is regular flushing of the soil profile (Pace and Johnson, 2002) to remove salts. In order to achieve this adequate flushing, proper drainage is paramount. Frequent mechanical cultivation or aeration is needed to allow salts in the soil to be flushed below the root zone of the turfgrass (Carrow and Duncan, 2011). Root zone modification may even be necessary in heavier soils that are more prone to compaction and reduced drainage. Dual plumbing may also be considered to irrigate high value, more sensitive, areas with nonsaline water such as golf course putting greens (Qian and Harivandi, 2007). In addition to improvements in drainage and irrigation systems, other management practices may include increased soil

and water tests. This allows the turf manager to monitor deficient elements and make amendments to fertility programs. These practices result in added equipment and labor expenses (Huck et al., 2000), and often increased irrigation.

Primary and Secondary Salinization

While the use of reclaimed water is a major contributor to saline conditions for plants, fresh water supplies can also have elevated salinity levels. Saline environments can be attributed to both to natural processes (primary salinization) and anthropogenic causes (secondary salinization) (Cañedo-Argüelles et al., 2013). Natural processes of salinization are more common in arid and semi-arid climates and the geology of a region has much to do with potential salinity (Miller et al., 1986). For example the Rocky Mountains are mainly comprised of material resistant to erosion and weathering, and contain few soluble salts. These materials include granite, schists, gneisses, lava, and other sedimentary rocks. In contrast, areas downstream and surrounding the Rocky Mountains in the Great Basin area have a different geological make up that contains more soluble salts that are attributed to thick layers of sediment deposited in seas (Ghassemi et al., 1995). Johnson and Winger (2003) presented a case study of turf quality being negatively affected by saline parent material at the Carbon County softball fields in Price, Utah. As the fields were being constructed, top soil was removed and never replaced. The resulting soil profile contained Mancos shale, which is high in soluble salts. As the overlain turfgrass was irrigated, salts were released and percolated upward in the soil profile. Before improvements were made, soil tests indicated that two of the four fields

had salinity levels measuring 7.2 and 19 dS m^{-1}. The resulting high salinity coupled with poor drainage lead to unplayable field conditions.

Through natural weathering of oxidized mineral crystals, runoff containing these oxidized minerals collects in low lying areas. As the surface water evaporates, salts can be left behind (Pillsbury, 1981). This deposition becomes more problematic in climates where there is not sufficient annual precipitation to flush the salts through the soil profile (Pitman and Läuchli 2002; Anning et al., 2007).

Arid and semi-arid regions, with more soluble salts in the soil, are prone to secondary salinization when the ground is cleared of native vegetation and then irrigated for crop production. The added irrigation can leach the accumulated salts through the soil profile down to groundwater, or the groundwater may rise into a direct contact with saline soils and dissolve the salts contained in soil pores (Suarez; 1989; Barica, 1972). Additional application of fertilizers can also increase soil salinity and ground water contamination with nitrates (Darwish et al., 2005).

Water that percolates through the soil, whether from irrigation or natural precipitation, transports salts into the groundwater which can return to rivers and increase salinity (Ghassemi et al., 1995). A good example is the Colorado River. At its headwaters in the mountains of Colorado, the salinity of the Colorado River is 30 mg L^{-1}. At Lees Ferry in Arizona, the addition of soluble salts have increased the salinity of the Colorado River to more than 500 mg L^{-1} and to nearly 800 mg L^{-1} by the time it reaches the Hoover Dam (Ghassemi et al., 1995).

Turfgrass Salinity Tolerance

Due to increased use of reclaimed water for landscape irrigation and the resulting, gradual decline in groundwater quality, the need for more salt tolerant plants, especially turfgrasses, is increasing. Warm season grasses have adapted well to saline conditions that are often found in arid climates. Butler et al. (1985) noted that some bermudagrass varieties tolerated conditions of up to EC_e 18 dS m^{-1}. While Lee et al. (2004) noted that seashore paspalum may survive conditions up to EC_e 24 dS m^{-1}. While not as salt-tolerant as many warm season turfgrasses, some cool season turfgrasses are better adapted to saline conditions than others. Tall fescue and perennial ryegrass offer moderate salt tolerance, while KBG is relatively sensitive to salt stress. Zhang et al., (2013) evaluated salinity induced stress in tall fescue and KBG. As salinity levels were increased, tall fescue maintained higher visual quality and higher root dry weight than KBG. In a similar study Alshammary et al., (2004) showed that 50% shoot and root reduction in KBG occurred at a salinity levels of 4.9 and 5.8 dS m^{-1} respectively, where as 50% growth reduction of tall fescue occurred at 10.0 dS m^{-1} for shoots and 19.6 dS m^{-1} for roots. Visual quality of KBG dropped to unacceptable levels when salinity reached 4.7 dS m^{-1}, whereas TF maintained minimal acceptable quality even at 9.9 dS m^{-1}. A study including perennial ryegrass and KBG concluded that perennial ryegrass had the highest average turf quality score compared to KBG when subjected to saline conditions of 11 millimoles per cm (Gibeault et al., 1977). Despite their reasonable salt tolerance, tall fescue and perennial ryegrass have their limitations. While moderately drought tolerant, the bunch type growth habit of tall fescue requires more time to recover from injury (Christians, 2011). Perennial ryegrass has a soft texture and attractive color, but also has

limited recovery due to its bunch type growth habit

While there are cool season grasses that are more salt tolerant, the overall focus is turf quality. However, pursuing improvements in grasses for improved recovery and softer texture is a slow process. A more expedient option would is to improve the salinity tolerance of grasses that already possess desirable characteristics.

Kentucky Bluegrass Salinity Research

Kentucky bluegrass has several traits that make it a popular choice where a high quality turfgrass is needed. It is widely used because of its dark color, durability, soft texture, and rhizomatous growth habit. This rhizomatous growth habit makes recuperative potential quite good, making it well adapted for use on golf course tees and fairways, and athletic fields that are subject to frequent damage (Beard, 1972; Christians et al., 2016; Cockerham, 2007). Kentucky bluegrass is also able to undergo quiescence during prolonged drought (Wang and Huang, 2004). Leaf tissue and root loss can occur during drought conditions, but the crown and rhizomes can live for several months without water; regrowth occurs when water is once again available (Christians et al., 2016). Prior to the 1970s, all KBG cultivars were derived from naturalized stands in the Midwest that were prone to disease when maintained at close mowing heights. The 'Merion' collection was the first cultivar of KBG possessing a low growth habit and exhibiting improved resistance to disease (Casler, 2003). Since then, KBG has become one of the most popular turfgrasses in use today. In addition to its desirable quality characteristics, its extensive rhizome production allows KBG to develop into a dense sod

with high tensile strength when harvested, making it a valuable commercial crop in the northern United States (Huff et al., 2003).

Despite its good traits, KBG is more prone to salt stress than other grasses. Research and breeding programs are ongoing to find improvements in KBG that can match the salinity tolerance of perennial ryegrass and tall fescue while still maintaining its desirable characteristics. In a field study, Koch and Bonos (2011) observed varying levels of salinity tolerance amongst KBG cultivars. After exposure to a 10 dS m^{-1} Irrigation solution for 12 weeks, ‘Liberator’, ‘Eagleton’, ‘Diva’, and ‘Rhythm’ maintained 65-73% green coverage. The grasses that performed the poorest were ‘Julia’ and A03-84, with A03-TB676, RSP, ‘Aura’, and ‘Midnight’ tying for 3rd to last. These poorer performing grasses had percent green cover ranging from 38 to 48%. Friell et al., (2013) looked at salinity tolerance of 74 turfgrass cultivars. Among the entries were varieties of tall fescue, perennial ryegrass, creeping bentgrass, fine fescue, and 13 varieties of KBG. Grasses were suspended and partially submerged in a saline solution. Digital imagery was used to quantify percent green tissue. Kentucky bluegrass varieties ‘Park’ and ‘Diva’ exhibited green tissues percentages above 50% after being subjected to a solution measuring 14 dS m^{-1}, comparable to several perennial ryegrass varieties. Tall fescue varieties consistently maintained green tissue percentages above 75%. The experiment continued, increasing the conductivity of the salinity solution until it reached 24 dS m^{-1}. At this salinity level all KBG and perennial ryegrass varieties fell below 25% green tissue and tall fescue varieties stayed close to 50%.

The United States Department of Agriculture (USDA), working in conjunction with Utah State University, began studying salinity tolerance in KBG accessions in 2006.

Robins et al., (2009) observed 5 accessions of KBG (PI 371768, PI 440603, PI 372742, PI 371771, PI 371775) with comparable LD 50's to 'Matador' tall fescue and 'Brightstar' II perennial ryegrass when immersed in a nutrient solution with an EC as high as 48 dS m^{-1}. These findings were further confirmed by Bushman et al., (2016). Wang (2013) also observed greater salinity tolerance in PI 371768 and PI 440603 than in 'Midnight', which has been identified as moderately salt tolerant by Robins et al (2009). The observations from these studies indicate that KBG can potentially have comparable salinity tolerance to other more salt tolerant cool season grasses.

Measures of Turfgrass Stress

Among other things, turfgrass is used as an ornamental plant in landscapes, and a safe playing surface for athletic events. In general, high value is placed on its visual appearance, unlike agricultural crops that may evaluated based on yield or nutritive value (Morris and Shearman, 1998). To visually rate turfgrass performance, a 1-9 rating scale is commonly used where '1' indicates poor performance, such as dead or nearly dead grass. A '9' rating is an indicator of a healthy, unblemished, turfgrass. A rating of 6 or above in considered acceptable (Skogley and Sawyer, 1992). This 1-9 rating method dates back more than 50 years and can be used in several areas of turfgrass research. Areas such as: shade tolerance (Beard 1965), salinity tolerance (Marcum, and Murdoch, 1994), cultural practices (Salaiz et al., 1995), and drought stress tolerance (Qian and Engelke, 1999). The National Turfgrass Evaluation Program (NTEP) sponsors several variety trials throughout the United States and has adapted the 1-9 rating scale in several

aspects of general plant aesthetics. These aspects include, among others, spring green up, winter color, texture, percent living ground cover, genetic color, density, and general quality. These ratings, collected throughout the country, have been a valuable source of information for turfgrass breeders, seed companies, parks departments, golf courses, sod growers and sport turf managers. The interest of these users has made NTEP data the standard in the turfgrass industry in the United States (Morris and Shearman, 1998). While visual ratings are a quick method for collecting data on large trials, the method has come under scrutiny for being subjective. Horst et al., (1984) concluded that visual evaluations are inadequate. This conclusions was made after comparing the results of 10 trained turfgrass researchers and finding more variation was associated with the individual evaluator than the grasses being evaluated.

Digital image analysis (DIA) is a popular method of collecting data and is continually improving as new technologies have evolved. Using DIA, researchers may collect objective data in a short amount of time with little training. Early DIA was able to determine color and fertility differences in corn (Ewing and Horton, 1999), as well as canopy coverage in soybeans (Purcell, 2000). Richardson et al. (2001) began using DIA to evaluate turfgrass cover and concluded that DIA can be an effective, and more accurate, way to estimate green turf coverage as compared to visual quality ratings. DIA is now a common part of many experiments to quantify percent green coverage and is used to assess turfgrass injury due to drought stress (Karcher et al., 2008), salinity firing (Wang, 2013), or stand loss from disease (Kopp and Harris, 2017). Using a light box, a tool that that allows for consistent lighting and field of view, hundreds of photos can be taken in a relatively short amount of time. Newer software can determine percent green

cover, texture, and give overall turf quality ratings in a matter of seconds (http://turfanalyzer.com) (Karcher et al., 2017).

Electrolyte leakage (EL) is another objective method used to quantify stress in turfgrasses. This practice dates back several decades to Dexter and Totingham (1930) who evaluated cold hardiness of alfalfa by measuring the electrolyte leakage of cold stressed roots. Cell membranes are one of the first areas of the plant to degrade when under stress and as the cell membrane degrades, it becomes more permeable and “leaks” electrolytes. More leakage corresponds to higher levels of stress. This leakage is measured as dissolved solids in a solution. Measuring EL is a desirable method because it requires readily available equipment and is not destructive to the whole plant. The method may also be used for several different plant materials and is suitable for analyzing large sample numbers (Baiji et al., 2001). This technique can also be applied to measure various abiotic stressors such as drought (Huang et al., 1997; Fu et al., 2004), cold weather damage (Ebdon et al., 2002; Webster and Ebdon, 2005), and salinity (Wang, 2013; Esmaeili et al., 2015). The specific methods for measuring EL may vary slightly from researcher to researcher, but they all contain similar steps. Generally, a sample of plant tissue is excised and allowed to leak solutes in a bathing solution of distilled or deionized water for 12 to 24 hours. After, which the conductivity of the bathing solution is measured. Following the first measurement, plant samples are destroyed, allowing remaining electrolytes to leak from the plant cells. This may be accomplished through autoclaving, boiling, or rapid freezing by liquid nitrogen. After rupturing the cell, the conductivity of the bathing solution is measured a second time. The first and second measurements are presented as a ratio (Wang and Huang., 2004).

Measuring water potential and stomatal conductance are other methods for quantifying plant stress. Water potential measurements involve placing an excised leaf blade into a rubber compression gland in the lid of a pressure chamber. Pressure is then increased in the chamber until water is forced from the cut end of the leaf blade (Scholander et al., 1964). In general more pressure required to force water from a leaf blade corresponds to higher drought or salinity stress (Aronson et al., 1987). Stomatal conductance estimates the rate of gas exchange and transpiration through the leaf stomata. This is measured using a leaf porometer commonly in units of mmol m^{-2} s^{-1} (Latrach et al., 2014; Leksungnoen, 2012). Plants under stress generally have lower porometer readings (Leksungnoen, 2012). Wang (2013) used both stomatal conductance and water potential readings to measure plant stress. She found water potential readings to be an accurate method for measuring plant stress but felt it was rater time consuming. Stomatal conductance readings made with a porometer, however, were not time consuming, but showed no significant differences between control and grasses treated with a 6 dS m^{-1} salinity solution. Significant differences in the control and treated grasses weren't noted until treatment solutions were at, or above, 12 dS m^{-1}.

Polyploidy, Apomixis, and Plant Improvement.

Kentucky bluegrass lines have been identified with greater salinity tolerance (Robbins et al., 2009). However, combining salinity tolerance with the many other necessary traits for a superior turfgrass into a commercially viable variety has proven difficult (Casler, 2003; Funk, 2000; Johnson et al., 2003). The primary reason for this

challenge is the high polyploidy present in KBG: ranging from 8-14× and with rampant aneuploidy (Bushman et al., 2018; Huff and Bara, 1993). The nature of KBG's polyploidy is unclear, but is likely a mixture of auto- and allo-polyploidy (Bushman, 2018), with unknown number an identity of ancestral diploid genomes (Soreng et al., 2010). With this high and variable polyploidy comes a dosage effect for genes; where heterozygous loci can have few or many alleles, and more than two alleles can be present (Haldane, 1930). Without an ability to estimate the dosage of alleles in two parents, the hybrid progeny of a cross may have many dosage options for salt tolerant genes of interest.

Kentucky bluegrass is also a facultative apomict, meaning that the vast majority of the offspring are identical to the mother (Albertini et al., 2001; Bushman et al., 2018). Apomixis is an excellent means of maintaining genetic purity of a cultivar from one generation to the next. However, apomixis also makes crossing and selection of KBG difficult (Huff et al., 2003). There are varying levels of apomixis in this facultative species from cultivar to cultivar. Meyer (1982) reported that the cultivar 'Merion' had a level of apomixis at 96% or higher, making it ideal for stable seed production. The cultivar 'A-20' on the other hand had an apomixis level near 25%, meaning its high level of sexuality made vegetative propagation the best method for production.

Offspring that do not go through apomixis are genetically different from the maternal parent and can be categorized into four apomictic offtypes. The definition of the off-types results from either meiosis or apomeosis, and either fertilization or parthenogenesis. Of the four off-types, two might be considered true hybrids in that they involve both meiosis and fertilization. B_{II} hybrids go through meiosis and receive equal

amounts of gametes from the mother and father in fertilization. B_{III} hybrids are reduced during meiosis in only one of the two parents while the other undergoes apomeosis, such that fertilization lead to a 50% increase in the amount of gametes provided from the female than the male after fertilization. Of the other two off-types, polyhaploids go through meiosis but not fertilization and B_{IV} hybrids go through fertilization but not meiosis.

Plant improvement through breeding requires Mendelian selection, where hybrids are made and those with improved alleles are selected for future generations. Given the difficulty of combining traits due to polyploidy and apomixis in KBG, hybrids can often only be identified through molecular markers (Bushman et al. 2018). While hybridization can be confirmed, the dosage of desirable traits cannot. The objective of this research is to test differences in salinity tolerance among parental and hybrid progeny of KBG. These grasses include 21 hybrids from five paired crosses (Table 1). I hypothesized that a portion of these hybrids would have mid-parent or better salinity tolerance.

MATERIALS AND METHODS

This experiment quantified the salinity stress imposed on hybrid Kentucky bluegrasses and their parents. Based on Robins et al. (2009) and Bushman et al. (2016), seven cultivars or accessions were used as parents: PI 371768, PI 440603, PI 499557, PI 578827, the cultivar 'Washington', a breeding line 'Border', and the cultivar 'North Star'. The former two were highly salt tolerant while the other entries were previously reported as drought tolerant, without any information regarding their response to salt stress. Previously, parents were cross-pollinated, and hybrids were differentiated from apomictic progeny using molecular markers (Bushman et al., 2018). Of the 1152 progeny plants, only 21 were identified as hybrids. Parental crosses and hybrid offspring are identified in Table 1. For simplicity, during the experiment accessions: PI 371768, PI 440603, PI 499557, and PI 578827 were abbreviated and called 768, 603, 557, and 827, respectively. Parents 768a and 768b were split clones from the same plant, as were 603a and 603b. These plants were originally split for breeding purposes and have been kept separated since.

Table 1. Parent crosses and hybrid offspring

Mother **Father**	Washington x 603b	North Star x 768a	827 x 768b	Border x 557	*557 x 603a x 603b
Hybrid Offspring	(W × 603)-11	(NS × 768)-21	(827 × 768)-31	(B × 557)-41	(557 × 603)-51
	(W × 603)-12	(NS × 768)-22	(827 × 768)-32	(B × 557)-42	(557 × 603)-52
	(W × 603)-13		(827 × 768)-33	(B × 557)-43	(557 × 603)-53
	(W × 603)-14		(827 × 768)-34		
	(W × 603)-15		(827 × 768)-35		
	(W × 603)-16		(827 × 768)-36		
	(W × 603)-17				

*Hybrid progeny grouped from two separate crosses.

The experiment included four experimental runs over the course of three years (Table 2).

Table 2 Dates and duration for all 4 runs of the experiment.

Run	Year	Start Date	End Date	Duration (days)
1	2015	May 4	July 2	58
2	2015	August 3	September 30	58
3	2016	May 11	July 6	56
4	2018	May 2	June 27	56

Grasses used in the experiment were propagated from clones of an existing plant that had been grown from seed. Six clones from each entry, both parents and hybrids, were planted in Deepot Cells (Stuewe and Sons, Tangent, Oregon) measuring 25.4 cm deep × 6.3 cm wide. Silica sand was used as the growth media to minimize the accumulation of salt across time. The 70-grit particle size was used because courser grits did not hold sufficient moisture (Peel et al., 2004). The fine texture media held moisture to the point that it behaved similarly to a hydroponic system. In a hydroponic system, the growth solution can be easily exchanged or altered to fit the needs of the plant. A silica sand media has similar capabilities. In this experiment, each irrigation essentially replaced the solution from the previous irrigation, allowing the electrical conductivity of the media to remain consistent. Fabric was also placed in the bottom of each container to keep the fine sand from leaching out after each irrigation. Before treatments began, plants were allowed to establish for six weeks.

Grasses were irrigated with a nutrient solution consisting of Peters Excel soluble fertilizer (Everris NA Inc., Dublin, Ohio) with an analysis of 21-5-20, mixed at a nitrogen (N) concentration of 100 ppm. Irrigation was applied automatically using a Quantum irrigation controller (McConkey Co., Sumner, Washington) (figure 1). In this system, a

boom, guided by an overhead track system, was programmed to irrigate the plants every-other-day with the nutrient solution pumped from a 200 L tank.

Greenhouse conditions, during establishment and throughout the experiment, included day a temperature of 24° C and a set night temperature of 13° C. During experimental runs in the summer months, a 60% shade cloth covered the green house to help maintain a 24° C daytime temperature. No supplemental lighting was used in the green house as none was available. Greenhouse conditions for runs 1, 3, and 4 were similar as far as external conditions were concerned. The amount of sunny vs. cloudy days was similar, and outside temperatures increased as the experiment progressed. Run 2 was different in that it started later in the summer. The number of sunny and cloudy days was similar to the other three runs, however outside temperatures declined the final two weeks of the experiment.

Figure 1. Greenhouse arrangement of entries with the boom sprayer that applied control and salt irrigation treatments.

Once the experiment began, the grasses were divided into a split plot design, each with salinity treatment as the whole plot treatment and entries as the split plot treatment. The three replicates for all entries were randomized within each split plot. The whole plots were bordered by pots of perennial ryegrass to minimize edge effects (Figure 2). The experiment was replicated four times, but not all grasses were included in each of the four runs. During the process of cloning out grasses to begin a new run of the experiment, some of the grasses did not have enough biomass to divide out for the three replications for each of the two treatments.

Control

P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
P	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	P
P	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	P
P	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	P
P	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	P
P	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	P
P	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	P
P	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	P
P	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	P
P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

Salt Treated

P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
P	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	P
P	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	P
P	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	P
P	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	P
P	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	P
P	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	P
P	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	P
P	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	P
P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

Figure 2. Completely randomized split plot design of control and salt treated plots. C = untreated control plants. S = salt treated plants. P = perennial ryegrass border plants.

The control plot was irrigated with the same Peters soluble fertilizer solution but reduced to a concentration of 50 ppm. The treated plot was irrigated with a saline solution pumped from a separate 200 liter tank. This saline solution also included the base nutrient solution, the same as that used in the control, mixed with an additional 0.5 grams sodium chloride/L and 0.95 grams of calcium chloride/L of tap water to achieve a solution that measured 3 dS m^{-1}. Plants were irrigated at this level of saline irrigation water for two weeks then increased to 6 dS $m^{-1}$for six weeks. The eight week duration for the experiment was chosen to allow sufficient time for the grasses to show responses to the

salinity treatments, but not so long as kill off the majority of the entries. To achieve 6 dS m^{-1}, sodium chloride was increased to 1 g/L and calcium was increased to 2.9 g/L (Table 2). The reason the plants were treated at a lower salinity solution at the beginning of the experiment was to avoid physiological shock to the plants when the EC of the salinity solution was increased to 6 dS m^{-1} (Richards, 1954). Volumes used for the salinity solution were adapted from a table originally provided by Dr. Lynn Dudley of USU. The amounts of calcium chloride were added to offset calcium deficiencies that result from the introduction of sodium chloride. The calcium chloride also helped maintain a sodium absorption ratio of 4, as we were mainly looking at the effects of salt, not sodium. To ensure a solution of 3 or 6 dS m^{-1} a conductivity meter (Orion Star A112, conductivity meter, Thermo Scientific, Inc.) was used weekly to measure the electrical conductivity of solution. Additional sodium chloride and calcium chloride was added as needed to maintain EC levels. Both control and salt treated plots were irrigated approximately 1.6 cm every-other-day. This amount of irrigation was needed to leach out any salt accumulation that may have occurred through evaporation.

Table 3. Amounts of NaCl and $CaCl_2$, and fertilizer (Peters 21-5-20) used for the salt treatment

Solution EC (dS m^{-1})	NaCl (g/L)	CaCl (g/L)	Peters 21-5-20 (g/L)	Duration
-	-	-	0.46	6 weeks (establishment)
-	-	-	0.23 (control)	8 weeks
3	0.5	0.95	0.23	2 weeks
6	1	2.9	0.23	6 weeks

Evaluation of Grasses

Two methods were used to evaluate stress of the plants from the salinity treatments; visual turf quality ratings, or turf quality (TQ), and electrolyte leakage (EL). Water potential and stomatal conductance were eliminated as means for measuring stress based on previous studies that showed excessive variation when applied to many samples. Additionally water potential was not measured due to the time requirement to collect data, and stomatal conductance because salinity treatments weren't to exceed 6 dS m^{-1}. As stated earlier, Wang (2013) didn't notice significant differences between porometer measurements between untreated control grasses and those treated with a salinity solution measuring 6 dS m^{-1}.

Visual TQ ratings were recorded to evaluate plant health. Ratings began two weeks after the experiment began on day 14, and were recorded every two weeks thereafter on days 28, 42 and 56. The 1-9 rating scale (Skogley and Sawyer, 1992) used by the National Turfgrass Evaluation Program (NTEP) was used weekly to evaluate the amount of leaf firing from salinity stress. In this experiment a plant that rated a "9" had no evidence of leaf firing from salt stress. A plant with a "1" rating displayed severe salinity stress and was dead or nearly dead (Figure 3). Because this rating system was developed for larger stands of turfgrass, adaptations had to be made for smaller plants that were rated from a close distance. The lateral spread of the plant was not considered for evaluation as some plants had more vigorous rhizomes than others. While that is an important attribute in KBG, the focus of this study was salinity stress.

Figure 3. Representation of visual quality from ‘9’ on the left to ‘1’ on the right.

Electrolyte leakage was measured 2 weeks after the experiment began on day 14, and every two weeks thereafter on day 28, 42 and 56. To measure EL for this experiment we followed a similar process describe by Lutts et al., (1996), Wang and Huang (2004), and Dionisio and Tabita (1998). Leaf tissue from each plant was excised and 0.2 grams was weighed out. The 0.2 gram samples were then washed free of any sand or salt particles using deionized water. The washed clippings from each sample were cut into 1.25 to 1.5 cm pieces and placed into 50 ml centrifuge tube (VWR, Aurora, Colorado). The tubes were then filled with 20 ml of deionized water and placed on a platform shaker (Innova 2100 platform shaker, New Brunswick Scientific, Inc.) for 20 hours at 150 rpm. After 20 hours, the EC of each sample was measured. The first measurement was referred to as the “before” measurement because it was taken before the autoclave cycle. Once the “before” measurements were taken, samples were autoclaved to rupture the remaining cells of the plants. The autoclave cycle (Sterivap 669, MMM Group) was run for 15 minutes at 121.0° C. Samples were allowed to cool to room temperature, approximately

22° C. The EC of the samples were then measured a second time. This second measurement was referred to as the “after” measurement because it was taken after the autoclave cycle. The EC measurement before the autoclave cycle measured only the electrolytes that had leaked from salinity stress. The EC measurement after the autoclave cycle measured all the electrolytes from the ruptured cells. The “before” and “after” EC measurements were divided and then multiplied by 100: (Before/After) x 100, to determine EL ratio.

The methods for all runs were identical, however the fourth run utilized larger pots (Stuewe and Sons, Tangent, Oregon), measuring 10.2 cm × 10.2 cm and 34.3 cm tall. The larger pots allowed for a larger and more developed rootzone.

Data from the four experimental runs was analyzed with a mixed model in R Studio version 3.5.1, with packages: ‘data.table’, ‘ggplot2’, ‘dplyr’, ‘agricolae’, and ‘knitr’. In the model, experimental runs and replication were the random variables while entry, treatment, and day (the four collection dates within each experimental run) were fixed variables.

Treatment comparisons are made as they related to the overall average of the entries in both TQ and EL. The term ‘significantly above/below average’ refers to entries that are statistically higher/lower than the average based off of LSD. The term ‘trended below/above average’ refers to entries that were below or above the average though not statistically significant.

RESULTS

At the conclusion of each experimental run, the health of all entries subjected to the salinity treatments had declined when compared to the untreated control. Turfgrass quality ratings of salt treated entries, in general, decreased more during the beginning weeks of the experiment than the later weeks (Figure 4). At the conclusion of the experiment, treated entries exhibited a range of tolerance with TQ ratings from 2 to 7 (Figure 4). Untreated control entries maintained more consistency with the majority of the entries with a TQ rating between 7 and 8 (Figure 4). Electrolyte leakage measurements demonstrated an overall increase, with a sharper rise during the first two weeks and then a more gradual increase the remaining weeks of the experiment. This was consistent with the TQ ratings. Treated entries exhibited a wide range of responses, with ratios ranging from 14 to 72 (Figure 5). Untreated (control) entries maintained consistent ratios throughout the experiment with EL ratios ranging from 5 to 15 during the entire experiment (Figure 5). By the end of the experiment, most parental crosses produced at least one hybrid with above average TQ ratings and EL ratios. Of the five hybrids with above average TQ ratings at the end of the experiment, three had 768 as a parent. Considering EL ratios, two of the three hybrids that were below average (more favorable), at the end of the experiment, were also progeny of 768. Hybrids that had achieved mid-parent salinity tolerance were also identified. Four of the five crosses produced at least one hybrid that performed better than at least one parent in terms of TQ or EL.

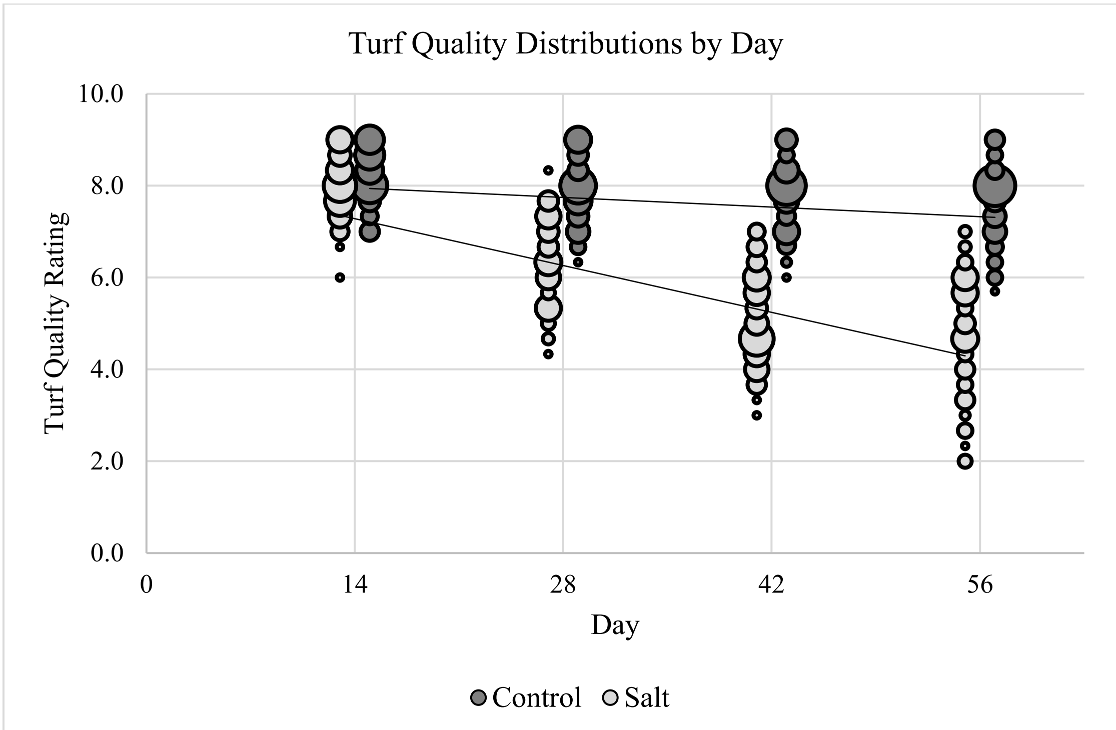

Figure 4. Distribution of turfgrass quality ratings between the control and salt treated entries as the experiment progressed. Data was taken from averages of all 4 runs. Larger circles indicate that more entries had the same rating.

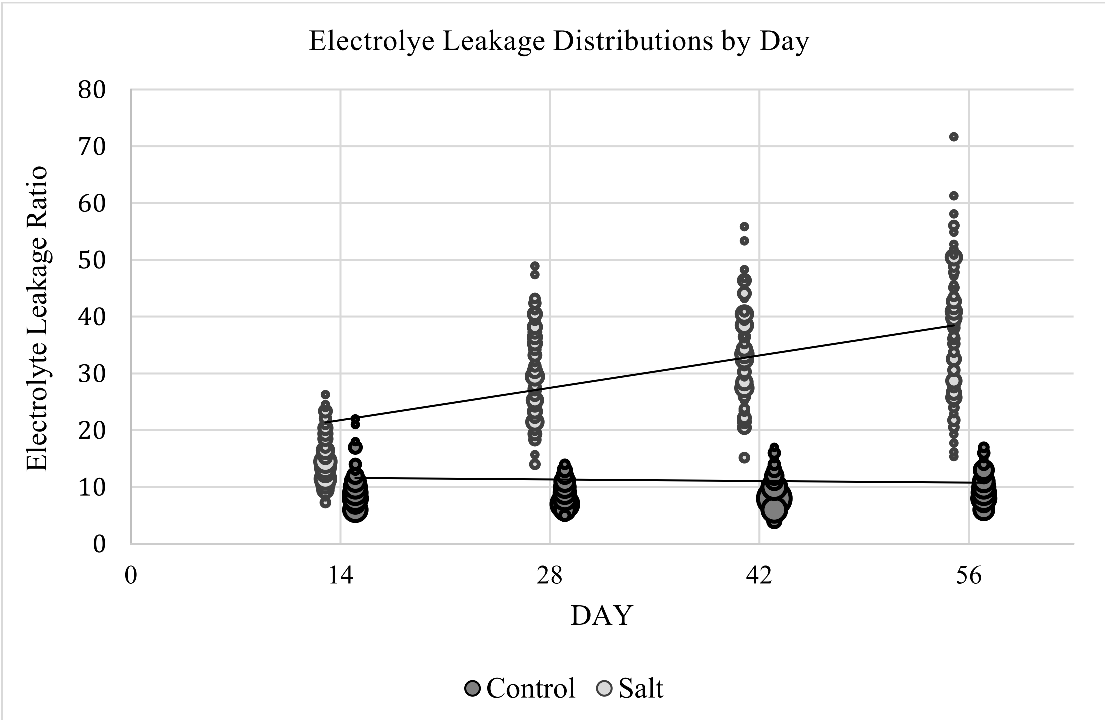

Figure 5. Distribution of electrolyte leakage between the control and salt treated entries as the experiment progressed. Data was taken from averages of all four runs. Larger circles indicate that more entries had the same rating.

As previously described, when stress was imposed upon the treated plants, EL increased and TQ decreased, resulting in a negative correlation. The spearman rank correlation of EL and TQ across the four experimental runs was $r_s = -.51$ ($P < 0.01$). The general trend highlighting this correlation is shown in Figure 6.

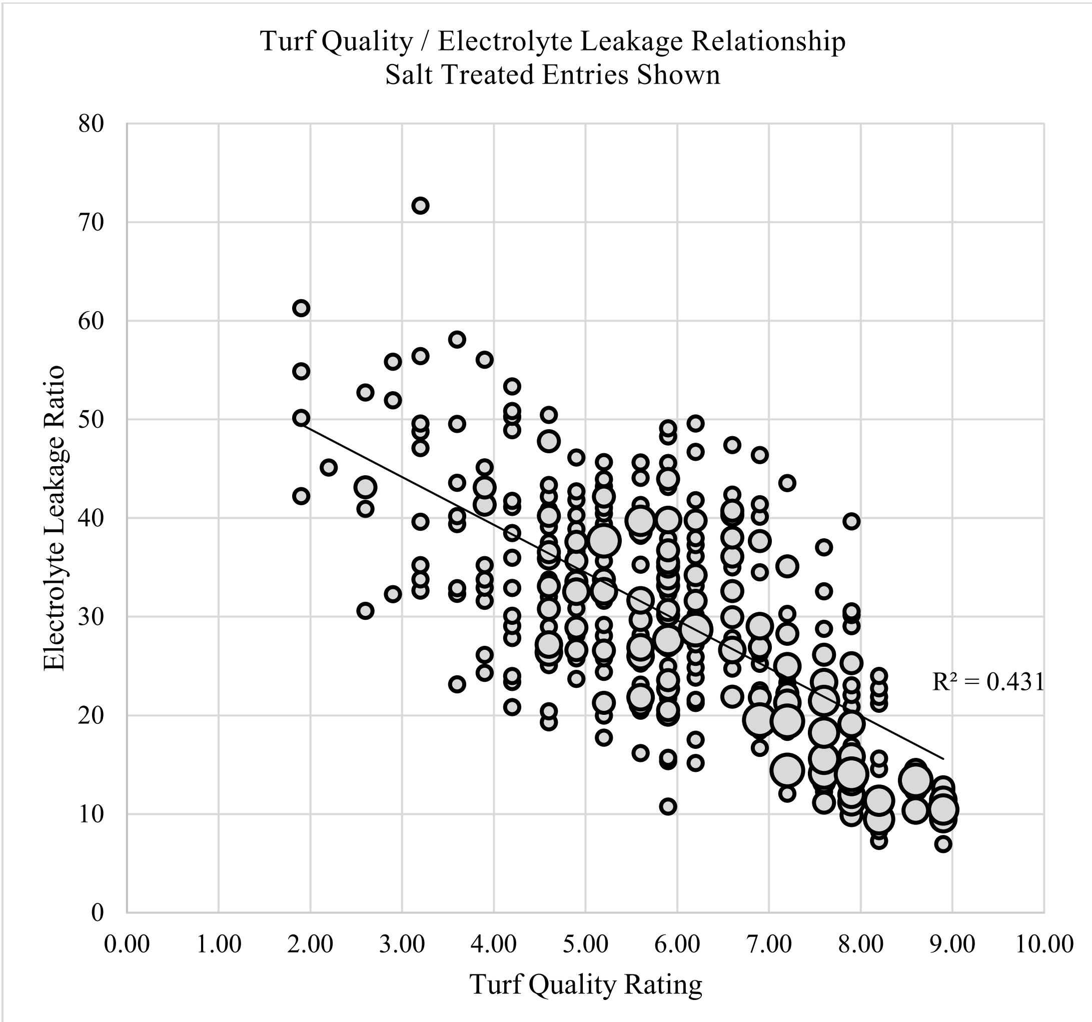

Figure 6. Relationship between increasing electrolyte leakage ratios and declining turfgrass quality of salt treated entries. Data shown is from all four runs across days 14, 28, 42, and 56. Larger circles indicate that more entries had the same rating.

Tables 4 & 5 illustrate sources of variation for the TQ and EL responses. When all effects were included in the analysis, all main effects and two way interactions were significant but three-way interactions were significant only in EL measurements (Tables 3 and 4). With the four collection dates over eight weeks the Day effect was significant along with its interactions with Entry and Treatment. Due to significant interactions, the different sampling date results were best analyzed and understood separately.

Table 4. Type 3 tests of fixed effects for electrolyte leakage.

Effect	Num DF	Den DF	F Value	Pr > F
Entry	30	563	13.03	<.0001
Treat	1	11	813.41	<.0001
Entry*Treat	30	563	7.69	<.0001
Day	3	1759	403.16	<.0001
Entry*Day	90	1759	1.63	0.0002
Treat*Day	3	1759	424.07	<.0001
Entry*Treat*Day	90	1759	1.37	0.0144

Table 5. Type 3 tests of fixed effects for turfgrass quality.

Effect	Num DF	Den DF	F Value	Pr > F
Entry	30	563	22.7	<.0001
Treat	1	11	190.35	<.0001
Entry*Treat	30	563	3.34	<.0001
Day	3	1783	1113.89	<.0001
Entry*Day	90	1783	2.06	<.0001
Treat*Day	3	1783	583.13	<.0001
Entry*Treat*Day	90	1783	1.08	0.2801

Considering the interest in salt tolerant KBG germplasm, confirming the performance of parents was an objective of this study, along with the hybrid progeny. The parents originally selected for salt tolerance in previous salinity studies were 768 and 603 and 557. The 557 accession, however, was not tested in this experiment due to earlier greenhouse mortality. The other parents selected as crosses with the salt tolerant parents were ‘Border’, ‘Washington’, 827, and ‘North Star’; which were chosen because of their drought tolerance and other turf quality characteristics (e.g. spring greenup). It was projected that their hybrid progeny might inherit some of the drought tolerance or value-added traits in addition to salt tolerance traits. As previously mentioned parents 768a and 768b were clones from the same plant but had been split for breeding purposes and were kept separated. Since these clones had similar responses to salinity stress, for purpose of

clarity, they will here and subsequently be referred to 768. Parents 603a and 603b are also clones that performed similarly and will here and subsequently be referred to as 603 the remainder of the paper.

Results from day 14 are not reported because the full strength treatment solution had not yet been applied and the entries were relatively unstressed, and recording few significant differences. Parents that had above average TQ ratings at day 28 of the experiment generally had above average TQ ratings at the end of the experiment as well. For the (Washington × 603) cross, Washington exhibited statistically above average TQ ratings on day 28 and 56 and trended above average TQ on day 42. Entry 603, however, exhibited statistically below average TQ, or trended below average TQ, throughout the experiment. For the (North Star × 768) cross, North Star exhibited statistically above average TQ throughout the experiment and 768 trended above average TQ throughout the experiment. For the cross (827 × 768), 827 was another parent exhibiting TQ ratings statistically above average throughout the experiment. For the (Border × 557) cross, Border exhibited at or above average TQ throughout the experiment while 557 (as mentioned previously) was not included in the experiment. For the (557 × 603) cross, 557 was not included in the experiment. For the 603 parent clone, TQ trended below average or was significantly below average for much of the experiment. Parent and hybrid comparisons of TQ ratings were also made (Table 6). Turf Quality ratings under control conditions showed few significant differences. Despite few changes under control conditions, both parents and hybrids with higher TQ under control conditions generally had above average TQ ratings under saline conditions. (Table 7).

Despite TQ ratings of some parents not being significantly above average, some of their offspring were transgressive in their TQ ratings under stress. The majority of the (Washington × 603) hybrids trended below average TQ ratings, and hybrids (W × 603)-12 and (W × 603)-14 exhibited significantly below average TQ and performed below that of either parent. Hybrid (NS × 768)-21 from (North Star × 768) exhibited significantly above average TQ and was also the best overall performing entry throughout the experiment. Several of the hybrids from (827 × 768) performed poorly exhibiting TQ ratings below both their parents. These hybrids were: (827 × 768)-31, (827 × 768)-33, (827 × 768)-34, and (827 × 768)-35. Hybrid (827 × 768)-36 exhibited TQ ratings significantly above average throughout much of the experiment but declined significantly toward the end. All hybrids from (Border × 557) exhibited TQ ratings below their parents, all of which generally trended below average. Cross (557 × 603) had one hybrid with TQ significantly above average at the end of the experiment, (557 × 603)-53. Conversely hybrid (557 × 603)-52 exhibited TQ below its 603 paternal parent throughout the experiment. Hybrid comparisons of all crosses for TQ ratings were also made (Table 6).

When evaluating the parent's performance for EL, those with above average TQ ratings under treatment conditions did not necessarily have below average (favorable) EL ratios. Similar to TQ ratings, parents that performed well (or poorly) on day 28 generally continued that trend throughout the remainder of the experiment. In cross (Washington × 603), Washington EL ratios trended above average (unfavorable) throughout the experiment while 603 consistently showed close to average ratios. In cross (North Star × 768), North Star trended above average on days 28 and 42 and was significantly below

average (favorable) at the end of the experiment. The 768 clone exhibited EL ratios significantly below average through the experiment. For (827× 768), parent 827 was significantly below average on days 28 and 42, and trended below average on day 56. In cross (Border × 557), Border had an early decline and was significantly above average on day 28 but, eventually trended with the average on day 56. In cross (557 × 603) the 603 clone, as mentioned previously, exhibited EL close to the average throughout the experiment.

Hybrids with favorable TQ also did not necessarily have favorable EL ratios. Hybrids from (Washington × 603) spanned a diverse range of EL ratios. Hybrids (W × 603)-14, (W × 603)-15 and (W × 603)-16 exhibited EL at or better than the 603 parent which was the parent with the lower EL ratio. Hybrid (W × 603)-17 was the worst progeny, with EL ratios consistently higher than both parents. Cross (North Star × 768), produced hybrids (NS × 768)-21 and (NS × 768)-22 exhibiting EL ratios higher than both parents throughout much of the experiment. However, as both parents had lower than average EL ratios, these hybrid progeny still trended below average (favorable). Hybrids from (827 × 768) also exhibited a diverse range of EL and several hybrids had EL ratios significantly below average throughout the experiment. Hybrids (827 × 768)-32 and (827 × 768)-33 exhibited EL ratios significantly below average (favorable) at the end of the experiment, while hybrids (827 × 768)-31 and (827 × 768)-34 exhibited EL ratios higher than both parents throughout the experiment. Hybrids from (Border × 557) all exhibited higher EL ratios than Border and were significantly above average the majority of the experiment. In cross (557 × 603) hybrid (557 × 603)-51 exhibited significantly below average EL throughout the experiment and hybrids (557 × 603)-51 and (557 × 603)-53

both exhibited EL ratios that were below parent 603. Parent and hybrid comparisons of EL ratios were also made (Table 8). Significant differences in EL ratios from the average under control conditions were nearly nonexistent. (Table 9).

Table 6. Turfgrass quality sorted from highest to lowest on the 4 days data was collected for the salt treatment. Cells highlighted in green are significantly above average for turf quality based on least significant difference comparisons. Similarly, cells highlighted in orange are significantly below average for turfgrass quality based on least significant difference comparison.

Above/Below Average Turf Quality Means by Day – Salt Treated							
Day 14		**Day 28**		**Day 42**		**Day 56**	
(NS×768)-21	8.8	(NS×768)-21	7.8	(NS×768)-21	6.8	(NS×768)-21	6.1
Washington-b	8.7	North Star	7.4	Washington-b	6.6	North Star	6
Border	8.6	827	7.4	(NS×768)-22	6.3	827	5.8
North Star	8.5	(NS×768)-22	7.3	827	6.3	(557 × 603)-53	5.6
(NS×768)-22	8.5	Washington-a	7.2	(827 × 768)-36	6.3	Washington-b	5.5
(827 × 768)-36	8.5	(827 × 768)-36	7.2	North Star	6.1	Washington-a	5.3
Washington-a	8.4	Washington-b	7.1	768b	6.1	(W × 603)-13	5.3
827	8.4	(W × 603)-13	7	(W × 603)-17	6	(NS×768)-22	5.2
768a	8.3	768b	7	Washington-a	5.9	(827 × 768)-32	5.2
(827 × 768)-31	8.3	768a	6.8	(827 × 768)-32	5.9	Border	5.2
(827 × 768)-35	8.3	(827 × 768)-32	6.8	(557 × 603)-53	5.9	768a	5.1
827-Z	8.3	(827 × 768)-33	6.8	(W × 603)-13	5.8	768b	5
(W × 603)-17	8.2	Border	6.8	(827 × 768)-33	5.7	(827 × 768)-36	5
768b	8.2	(827 × 768)-31	6.7	827-Z	5.7	(557 × 603)-51	5
(827 × 768)-32	8.2	(557 × 603)-53	6.7	Border	5.6	(827 × 768)-34	4.9
(557 × 603)-53	8.2	(827 × 768)-35	6.6	(W × 603)-11	5.5	(827 × 768)-35	4.9
(W × 603)-11	8	(W × 603)-17	6.5	(W × 603)-15	5.4	(W × 603)-15	4.8
(827 × 768)-33	8	(W × 603)-11	6.4	768a	5.4	(W × 603)-11	4.7
603a	8	(W × 603)-15	6.3	(827 × 768)-35	5.4	(W × 603)-17	4.7
(B × 557)-41	7.9	(B × 557)-41	6.3	(B × 557)-41	5.4	827-Z	4.7
(B × 557)-42	7.9	827-Z	6.2	(827 × 768)-31	5.3	(B × 557)-41	4.6
(B × 557)-43	7.9	(W × 603)-16	6.1	(B × 557)-42	5.3	(B × 557)-42	4.6
(557 × 603)-51	7.9	(W × 603)-14	5.9	(W × 603)-16	5.2	(827 × 768)-33	4.5
(W × 603)-13	7.8	603b	5.9	(827 × 768)-34	5.2	(W × 603)-16	4.3
(W × 603)-14	7.8	(557 × 603)-51	5.9	603b	4.9	(827 × 768)-31	4.3
(W × 603)-15	7.8	(827 × 768)-34	5.8	(557 × 603)-51	4.9	603a	4.2
603b	7.8	(B × 557)-42	5.8	603a	4.8	(B × 557)-43	4
(557 × 603)-52	7.8	603a	5.8	(557 × 603)-52	4.7	603b	3.9
(W × 603)-16	7.6	(557 × 603)-52	5.7	(W × 603)-14	4.6	(W × 603)-12	3.8
(W × 603)-12	7.3	(W × 603)-12	5.5	(W × 603)-12	4.4	(W × 603)-14	3.7
(827 × 768)-34	7	(B × 557)-43	5.5	(B × 557)-43	4.4	(557 × 603)-52	3.7
Day 14		**Day 28**		**Day 42**		**Day 56**	
Mean	8.1	**Mean**	6.5	**Mean**	5.5	**Mean**	4.8
Median	8.2	**Median**	6.6	**Median**	5.5	**Median**	4.9
Min	7	**Min**	5.5	**Min**	4.4	**Min**	3.7
Max	8.8	**Max**	7.8	**Max**	6.8	**Max**	6.1
LSD (0.05)	0.55	**LSD (0.05)**	0.6	**LSD (0.05)**	0.6	**LSD (0.05)**	0.72

Table 7. Turfgrass quality sorted from highest to lowest on the 4 days data was collected for the control treatment. Cells highlighted in blue are significantly above average for turf quality based on least significant difference comparisons. Similarly, cells highlighted in orange are significantly below average for turfgrass quality based on least significant difference comparison.

Above/Below Average Turf Quality Means by Day – Control							
Day 14		**Day 28**		**Day 42**		**Day 56**	
827	9	827	8.8	827	8.8	827	8.7
(NS ×768)-21	8.8	(827 × 768)-36	8.6	(NS×768)-21	8.5	Washington-a	8.3
Washington-b	8.8	(NS×768)-21	8.5	Washington-a	8.4	North Star	8.3
Washington-a	8.7	North Star	8.4	North Star	8.3	(NS×768)-21	8.3
North Star	8.7	Washington-a	8.3	(W × 603)-11	8.2	(557 × 603)-53	8.2
(827 × 768)-36	8.7	Washington-b	8.3	(W × 603)-13	8.2	Washington-b	8.2
(W × 603)-11	8.6	(W × 603)-13	8.2	Border	8.2	(827 × 768)-31	8.1
Border	8.6	(827 × 768)-35	8.2	(557 × 603)-53	8.2	827-Z	8.1
(827 × 768)-31	8.5	Border	8.2	Washington-b	8.2	(827 × 768)-35	8
(827 × 768)-35	8.5	(NS×768)-22	8.1	(827 × 768)-31	8.1	Border	8
827-Z	8.5	(827 × 768)-31	8.1	(827 × 768)-35	8.1	(B × 557)-42	8
768a	8.3	(827 × 768)-33	8.1	(827 × 768)-36	8.1	(W × 603)-11	7.9
(827 × 768)-32	8.3	827-Z	8.1	827-Z	8.1	(W × 603)-15	7.9
(557 × 603)-53	8.3	(W × 603)-11	8	(B × 557)-42	7.9	(B × 557)-41	7.9
(W × 603)-15	8.2	768a	8	(W × 603)-15	7.8	(W × 603)-13	7.8
(NS×768)-22	8.2	(827 × 768)-32	8	(W × 603)-17	7.8	(557 × 603)-51	7.8
(827 × 768)-33	8.2	(557 × 603)-52	8	(B × 557)-41	7.8	(557 × 603)-52	7.8
(B × 557)-41	8.2	(557 × 603)-53	8	(557 × 603)-51	7.8	(B × 557)-43	7.7
(B × 557)-42	8.2	(W × 603)-15	7.8	(557 × 603)-52	7.8	(W × 603)-17	7.6
(557 × 603)-52	8.2	(W × 603)-17	7.8	768a	7.7	(827 × 768)-36	7.6
(W × 603)-13	8.1	768b	7.8	(827 × 768)-32	7.7	768a	7.5
(B × 557)-43	8.1	(B × 557)-41	7.8	(B × 557)-43	7.7	(NS×768)-22	7.2
(W × 603)-17	8	(B × 557)-42	7.8	(NS×768)-22	7.6	(827 × 768)-32	7.2
768b	8	(B × 557)-43	7.5	768b	7.3	(827 × 768)-34	7.2
603b	7.9	603a	7.5	(827 × 768)-34	7.3	(W × 603)-12	7.1
603a	7.8	(557 × 603)-51	7.5	603a	7.3	768b	7.1
(557 × 603)-51	7.8	(827 × 768)-34	7.4	(W × 603)-14	7.2	603a	7
(W × 603)-14	7.6	(W × 603)-12	7.3	(W × 603)-16	7.2	603b	7
(W × 603)-16	7.5	(W × 603)-16	7.2	(827 × 768)-33	7.2	(W × 603)-14	6.9
(827 × 768)-34	7.5	603b	7.2	603b	7.1	(W × 603)-16	6.8
(W × 603)-12	7.4	(W × 603)-14	7.1	(W × 603)-12	6.8	(827 × 768)-33	6.8
Day 14		**Day 28**		**Day 42**		**Day 56**	
Mean	8.2	**Mean**	7.9	**Mean**	7.8	**Mean**	7.7
Median	8.2	**Median**	8	**Median**	7.8	**Median**	7.8
Min	7.4	**Min**	7.1	**Min**	6.8	**Min**	6.8
Max	9	**Max**	8.8	**Max**	8.8	**Max**	8.7
LSD (0.05)	0.55	**LSD (0.05)**	0.6	**LSD (0.05)**	0.6	**LSD (0.05)**	0.72

Table 8. Electrolyte leakage ratios sorted from highest to lowest in the 4 days data was collected for the salt treatment. Cells highlighted in green are significantly above average for electrolyte leakage ratios based on least significant difference comparisons. Similarly, cells highlighted in orange are significantly below average for electrolyte leakage ratios based on least significant difference comparison.

Above/Below Average Electrolyte Leakage Means by Day – Salt Treated							
Day 14		**Day 28**		**Day 42**		**Day 56**	
827	10	768a	19.9	768a	21.8	768a	25
(827 × 768)-35	10.7	(827 × 768)-32	20.2	(827 × 768)-32	22.1	(827 × 768)-32	25.6
(NS×768)-21	11	(827 × 768)-33	21	(557 × 603)-51	24.5	768b	27.1
(827 × 768)-33	11	827	23.2	768b	26	(557 × 603)-51	29.4
(827 × 768)-32	11.1	(NS×768)-22	23.4	(827 × 768)-35	26.6	North Star	29.8
North Star	11.6	(827 × 768)-36	24.5	827	26.7	(827 × 768)-33	29.9
768a	11.7	(557 × 603)-51	24.5	North Star	28	(557 × 603)-53	32.6
(827 × 768)-36	12.2	(827 × 768)-35	26.1	(NS×768)-21	28	(827 × 768)-36	33.2
(557 × 603)-53	12.5	(W × 603)-15	26.9	(W × 603)-14	28.4	827	33.6
(NS×768)-22	12.6	North Star	27.1	(827 × 768)-36	28.6	603a	33.8
Washington-b	12.6	(W × 603)-14	27.7	(557 × 603)-53	29.7	(827 × 768)-35	34.2
Washington-a	13	(NS×768)-21	27.7	(W × 603)-16	30	(NS×768)-21	34.3
(W × 603)-17	13	(827 × 768)-34	27.7	(NS×768)-22	30.3	(W × 603)-16	35.6
827-Z	13.1	603a	28.6	(827 × 768)-33	30.9	(557 × 603)-52	35.6
(W × 603)-15	14	(Washington-b	28.6	603a	30.9	(NS×768)-22	35.8
(557 × 603)-51	14.1	(W × 603)-16	29	(557 × 603)-52	32.9	(W × 603)-14	35.9
768b	14.3	(557 × 603)-53	29.5	(827 × 768)-34	33	603b	36.3
(W × 603)-13	16	768b	30.3	(W × 603)-15	33.3	(W × 603)-15	36.4
(557 × 603)-52	16.1	(557 × 603)-52	31.1	603b	33.5	Border	36.4
(827 × 768)-34	16.2	(W × 603)-12	33.4	(W × 603)-13	36.8	Washington-b	37.2
(W × 603)-14	16.3	(B × 557)-41	33.5	(W × 603)-12	37.3	(W × 603)-13	37.7
(827 × 768)-31	16.4	Washington-a	33.6	Washington-a	37.5	(827 × 768)-34	39.1
(W × 603)-12	16.6	(W × 603)-13	33.6	(W × 603)-17	38	(827 × 768)-31	39.9
Border	16.6	603b	34.4	Border	38.1	(W × 603)-17	40.3
(B × 557)-42	16.7	(W × 603)-11	35.1	(827 × 768)-31	38.3	(W × 603)-12	40.6
(B × 557)-43	17.2	(B × 557)-43	35.1	Washington-b	38.4	827-Z	42.1
603a	17.6	(W × 603)-17	36.1	827-Z	38.9	Washington-a	42.4
(W × 603)-11	17.8	(827 × 768-31	36.4	(B × 557)-41	41.9	(W × 603-11)	43.7
(B × 557)-41	17.9	Border	37.8	(B × 557)-43	42.3	(B × 557-41)	44.1
(W × 603)-16	18.1	827-Z	41.1	(W × 603)-11	42.6	(B × 557-43)	44.5
603b	20.2	(B × 557)-42	42.7	(B × 557)-42	46.3	(B × 557-42)	52.4
Day 14		**Day 28**		**Day 42**		**Day 56**	
Mean	14.5	**Mean**	30	**Mean**	33	**Mean**	36.3
Median	14.1	**Median**	29	**Median**	32.9	**Median**	35.9
Min	10	**Min**	19.9	**Min**	21.8	**Min**	25
Max	20.2	**Max**	42.7	**Max**	46.3	**Max**	52.4
LSD (0.05)	5.02	**LSD (0.05)**	5.25	**LSD (0.05)**	5.15	**LSD (0.05)**	5.53

Table 9. Electrolyte leakage ratios sorted from highest to lowest in the 4 days data was collected for the salt treatment. Cells highlighted in orange are significantly below average for electrolyte leakage ratios based on least significant difference comparison.

Above/Below Average Electrolyte Leakage Means by Day – Control							
Day 14		Day 28		Day 42		Day 56	
(W × 603)-17	6	(W × 603)-17	5.4	(NS×768)-21	6.8	(W × 603)-17	5.7
(NS×768)-22	6.7	(NS×768)-21	6.6	Washington-b	6.6	(NS×768)-21	7.3
(NS×768)-21	6.8	827	6.6	(NS×768)-22	6.3	(827 × 768)-36	7.4
(827 × 768)-32	7.2	North Star	6.8	827	6.3	North Star	7.7
(827 × 768)-35	7.2	(827 × 768)-35	7	(827 × 768)-36	6.3	(827 × 768)-35	7.9
Washington-a	7.3	(827 × 768)-33	7.2	North Star	6.1	Washington-b	7.9
827	7.4	(827 × 768)-36	7.4	768b	6.1	768b	8
North Star	7.5	768a	8.1	(W × 603)-17	6	(W × 603)-15	8.2
(827 × 768)-36	7.5	(NS×768)-22	8.1	Washington-a	5.9	768a	8.2
(W × 603)-15	8.6	(W × 603)-15	8.3	(827 × 768)-32	5.9	(NS×768)-22	8.4
(557 × 603)-53	8.6	Washington-b	8.3	(557 × 603)-53	5.9	(827 × 768)-31	8.7
827-Z	8.6	Washington-a	8.4	(W × 603)-13	5.8	Washington-a	8.9
(827 × 768)-33	8.8	768b	8.4	(827 × 768)-33	5.7	827	9
Washington-b	8.8	(557 × 603)-52	8.5	827-Z	5.7	(827 × 768)-32	9
768a	9	(827 × 768)-32	8.6	Border	5.6	827-Z	9.4
(W × 603)-13	9.1	(827 × 768)-31	8.9	(W × 603)-11	5.5	(557 × 603)-53	9.7
(827 × 768)-34	9.3	(W × 603)-11	9	(W × 603)-15	5.4	(B × 557)-43	9.8
(B × 557)-41	9.3	(557 × 603)-51	9	768a	5.4	(W × 603)-11	10.1
Border	9.8	(827 × 768)-34	9.3	(827 × 768)-35	5.4	603a	10.4
(557 × 603)-52	10.2	(B × 557)-41	9.3	(B × 557)-41	5.4	(557 × 603)-51	10.4
768b	10.4	(W × 603)-12	9.4	(827 × 768)-31	5.3	(B × 557)-42	10.5
(B × 557)-42	10.5	(B × 557-42	9.5	(B × 557)-42	5.3	(827 × 768)-34	10.7
(W × 603)-11	10.7	(557 × 603)-53	9.7	(W × 603)-16	5.2	(W × 603)-16	11.2
(W × 603)-12	10.8	(W × 603)-13	9.8	(827 × 768)-34	5.2	(557 × 603)-52	11.4
(827 × 768)-31	11.1	827-Z	9.8	603b	4.9	Border	11.6
(W × 603)-14	12.2	(W × 603)-14	10.2	(557 × 603)-51	4.9	(W × 603)-13	11.7
(W × 603)-16	12.2	603a	10.3	603a	4.8	(827 × 768)-33	11.7
(557 × 603)-51	12.2	Border	10.4	(557 × 603)-52	4.7	(B × 557)-41	11.7
(B × 557)-43	13	603b	10.5	(W × 603)-14	4.6	(W × 603)-12	12.1
603b	16.1	(B × 557)-43	11.2	(W × 603)-12	4.4	603b	12.2
603a	18.8	(W × 603)-16	11.3	(B × 557)-43	4.4	(W × 603)-14	13.2
Day 14		Day 28		Day 42		Day 56	
Mean	9.7	**Mean**	8.8	**Mean**	9.4	**Mean**	9.7
Median	9.1	**Median**	8.9	**Median**	9.5	**Median**	9.7
Min	6	**Min**	5.4	**Min**	6	**Min**	5.7
Max	18.8	**Max**	11.3	**Max**	12.1	**Max**	13.2
LSD (0.05)	5.02	**LSD (0.05)**	5.25	**LSD (0.05)**	5.15	**LSD (0.05)**	**5.53**

For the purposes of the experiment, it was important to evaluate the overall performance of parents and hybrids, but equally important to evaluate how the hybrids performed within their individual crosses for the purpose of determining how effectively salinity tolerance could be bred into polyploid KBG. Although the specific genes present in the five parent crosses for salinity tolerance are unknown, my hypothesis was that with independent assortment of these genes, the hybrid progeny would have salt tolerance comparable to mid-parent salt tolerance. Because trends persisted from day 28 through the end of each experiment (day 56), results from the different crosses will be presented as they were recorded on the final day of the experiment.

For cross (Washington × 603) seven hybrids were produced. Under salt treated conditions, Washington (maternal parent) exhibited significantly higher TQ ratings than 603 (paternal parent) throughout the duration of the experiment (Figure 7). In addition, under treated conditions, entries from this cross, saw a generally greater drop in TQ during the early weeks of the experiment and less of a decrease the later weeks. Hybrids exhibited mid-parent or higher TQ were (W × 603)-11, (W × 603)-15, and (W × 603)-17. Hybrid (W × 603)-13 exhibited TQ equal to parent Washington. Electrolyte leakage values for this cross also exhibited a sharper increase during the early weeks of the experiment followed by a more gradual increase during the remainder of the experiment. Unlike TQ, for which Washington exhibited more favorable TQ ratings, 603 exhibited more favorable EL ratios (Figure 7). Hybrids (W × 603)-14, (W × 603)-15, and (W × 603)-16 exhibited EL ratios equal to or less than those of the lowest performing parent, 603. Hybrid (W × 603)-13 did not exhibit EL ratios as low as its parent, but was still able to achieve EL ratios equal to mid-parent status. Under control conditions, these seven

hybrids saw little change in either TQ or EL ratios over time.

The cross (North Star × 768) produced two hybrids. Under salt treatment conditions, North Star (maternal parent) showed significantly higher TQ ratings than 768 (paternal parent). Turfgrass quality of the two hybrids from this cross (NS × 768)-21 and (NS × 768)-22 declined consistently throughout the experiment, where TQ of the two parents declined the most at the beginning of the experiment and less toward the end (Figure 8). Hybrid (NS × 768)-21 was the better performing, higher quality hybrid and was better than mid-parent, exhibiting a slightly higher TQ than North Star at the end of the experiment. Electrolyte leakage ratios for this cross, like others, saw a sharper increase at the beginning of the experiment and a slower increase toward the end of the experiment. While North Star had a higher TQ rating, 768 had a more favorable EL ratio, though not significantly different from North Star (Figure 8). Both hybrids from this cross did not achieve mid-parent EL ratios at the end of the experiment, but the two parents had relatively low EL ratios (not significantly different) and, both progeny also had favorable EL ratios.

The cross (827 × 768) produced six hybrids. Under salt treatment conditions, 827 (maternal parent) exhibited significantly higher TQ than 768 (paternal parent) (Figure 9). Turfgrass quality of entries from this cross declined consistently throughout the experiment with the exception of 827 and (827 × 768)-34. Turfgrass quality of those entries declined more in the early weeks of the experiment and less toward the end. Hybrids from this cross, in general, exhibited TQ below that of their parents, and none of the hybrids exhibited TQ equivalent to mid-parents at the end of the experiment. Electrolyte leakage ratios for this cross exhibited a similar trend to previous crosses, with

an initial sharp increase in EL ratios in the beginning weeks and a slower increase during the latter weeks of the experiment. The exception to this trend was 768 for which EL ratios declined mid experiment and increased only slightly during the final weeks. Similar to the previous cross, 768 had a significantly lower EL ratio than 827 (Figure 9). Hybrid (827 × 768)-32 exhibited EL ratios that were better than mid parent ratios, having a lower EL ratio than both its parents. Hybrid (827 × 768)-33 was also a notable hybrid in that it exhibited mid-parent EL ratios.

The cross (Border × 557) produced three hybrids. Turfgrass quality of the parents and hybrids from this cross declined sharply at the beginning of the experiment then tapered gradually toward the end of the experiment. Border had the highest TQ ratings and none of the hybrids exhibited TQ equivalent to mid-parent ratings at the end of the experiment. Electrolyte leakage ratios for this cross followed the pattern of other crosses in the experiment with sharp increases in the beginning slower increases as the experiment progressed. Border was an exception and exhibited similar ratios to parent 768 from the previous cross with declining EL ratios after an initial sharp increase. Border EL ratios were more favorable than its hybrid progeny, resulting in none of the hybrid progeny attaining mid-parent El ratios.

The cross (557 × 603) produced three hybrids. The 603 clone performed poorly compared to its progeny, in that its TQ was significantly lower than all three hybrids. All entries for this cross, like the previous cross, experienced an initial sharp decline in TQ during the beginning weeks and a more gradual decline the latter weeks (Figure 11). Hybrids (557 × 603)-51 and (557 × 603)-53 exhibited better than mid-parent TQ ratings at the end of the experiment. Electrolyte leakage ratios for this cross increased sharply in

the beginning of the experiment and slowed to a gradual increase for the remainder of the experiment. Electrolyte leakage ratios for parents and hybrids trended together throughout the experiment. Hybrids (557 × 603)-51 and (557 × 603)-53 exhibited lower EL ratios than their parents and also had EL ratios lower than mid-parents (Figure 11).

The general objective was to identify to hybrids that achieved mid-parent salinity tolerance. Considering TQ, hybrids (W × 603)-11, (W × 603)-13, (W × 603)-15, (W × 603)-17, (NS × 768)-21, (557 × 603)-51 and (557 × 603)-53 achieved mid-parent or better ratings. Considering EL, hybrids (W × 603)-13, (W × 603)-14, (W × 603)-15, (W × 603)-16, (827 × 768)-32, (827 × 768)-33, (557 × 603)-51 and (557 × 603)-53 achieved mid-parent or better EL ratios. In addition to the hybrids that performed well, we also noted those that performed poorly under salt stressed conditions. The transgressive hybrids for TQ were: (W × 603)-12, (W × 603)-14, (827 × 768)-31, (827 × 768)-33, (827 × 768)-34, (827 × 768)-35, (B × 557)-41, (B × 557)-42, (B × 557)-43, and (557 × 603)-52. The transgressive hybrids for EL were: (W × 603)-11, (NS × 768)-21, (NS × 768)-22, (827 × 768)-31, (827 × 768)-34, (827 × 768)-35, (B × 557)-41, (B × 557)-42, and (B × 557)-43. A general trend that was observed was that parents and hybrids with more favorable TQ ratings under control treatment also had more favorable TQ ratings under the salt treatment, with-in their own crosses. Likewise, those parents and hybrids with less favorable TQ ratings under the control treatment also had less favorable TQ ratings under the salt treatment. Hybrids (827 × 768)-31, (B × 557)-43, and (557 × 603)-52 were the exception, exhibiting poor TQ despite having favorable TQ ratings under control conditions (Figures 9, 10, 11).

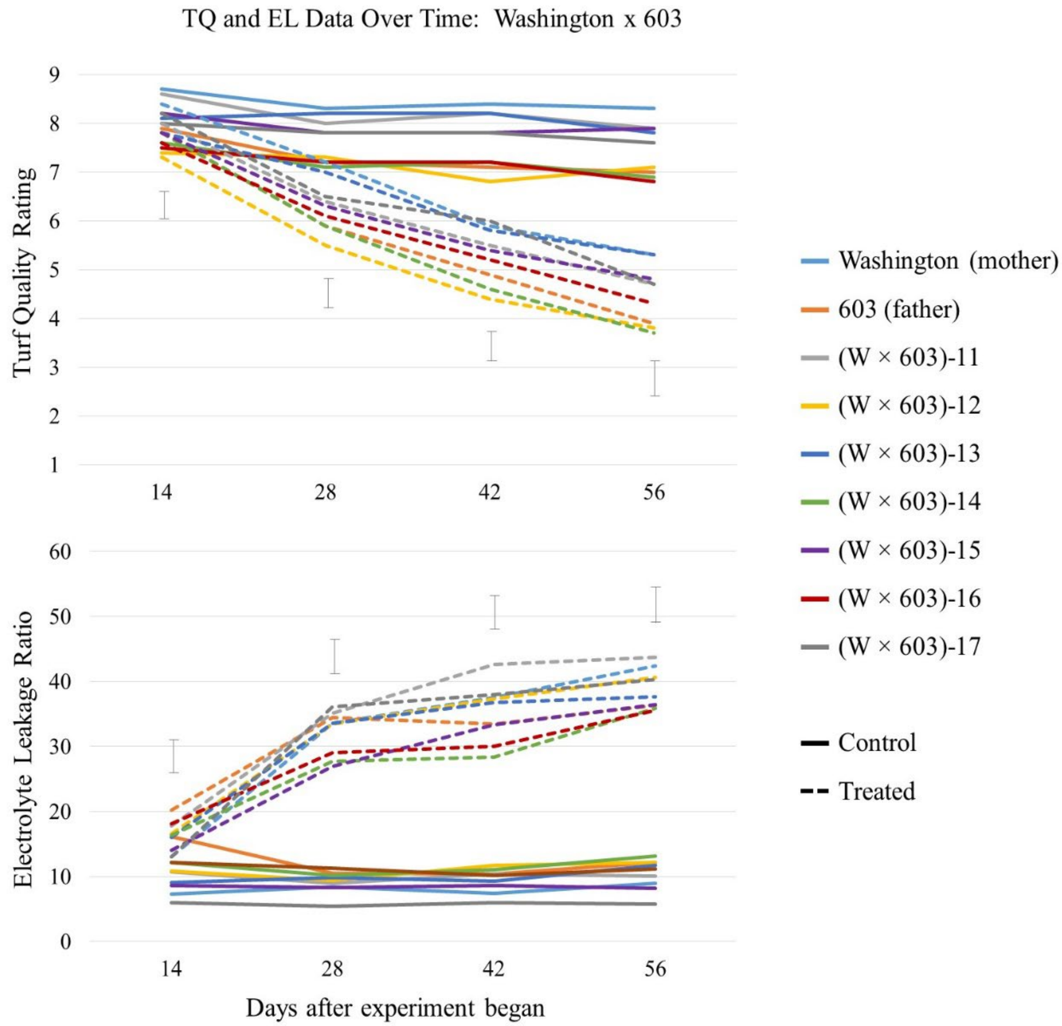

Figure 7. Turfgrass quality ratings and electrolyte leakage ratios from parents Washington × 603b and their resulting hybrids.

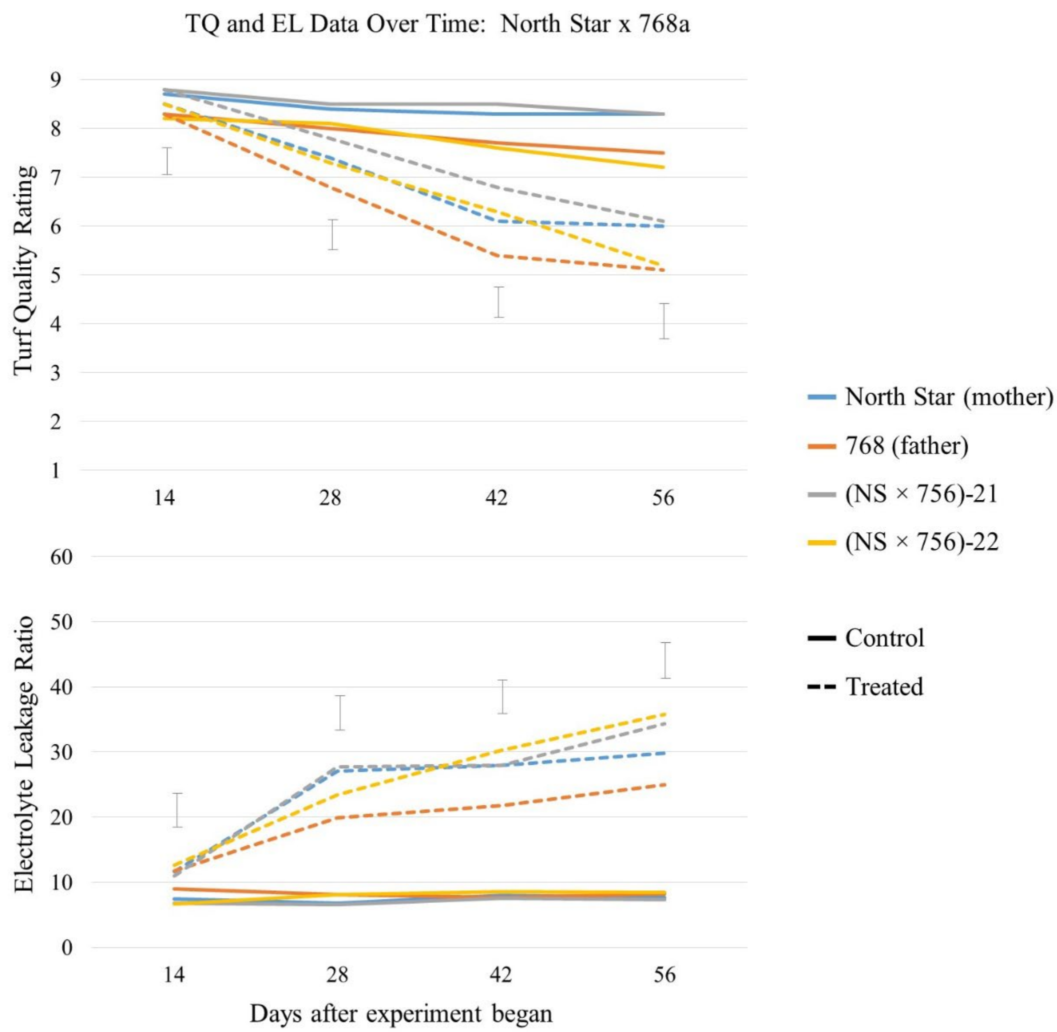

Figure 8. Turfgrass quality ratings and electrolyte leakage ratios from parents North Star × 768a and their resulting hybrids.

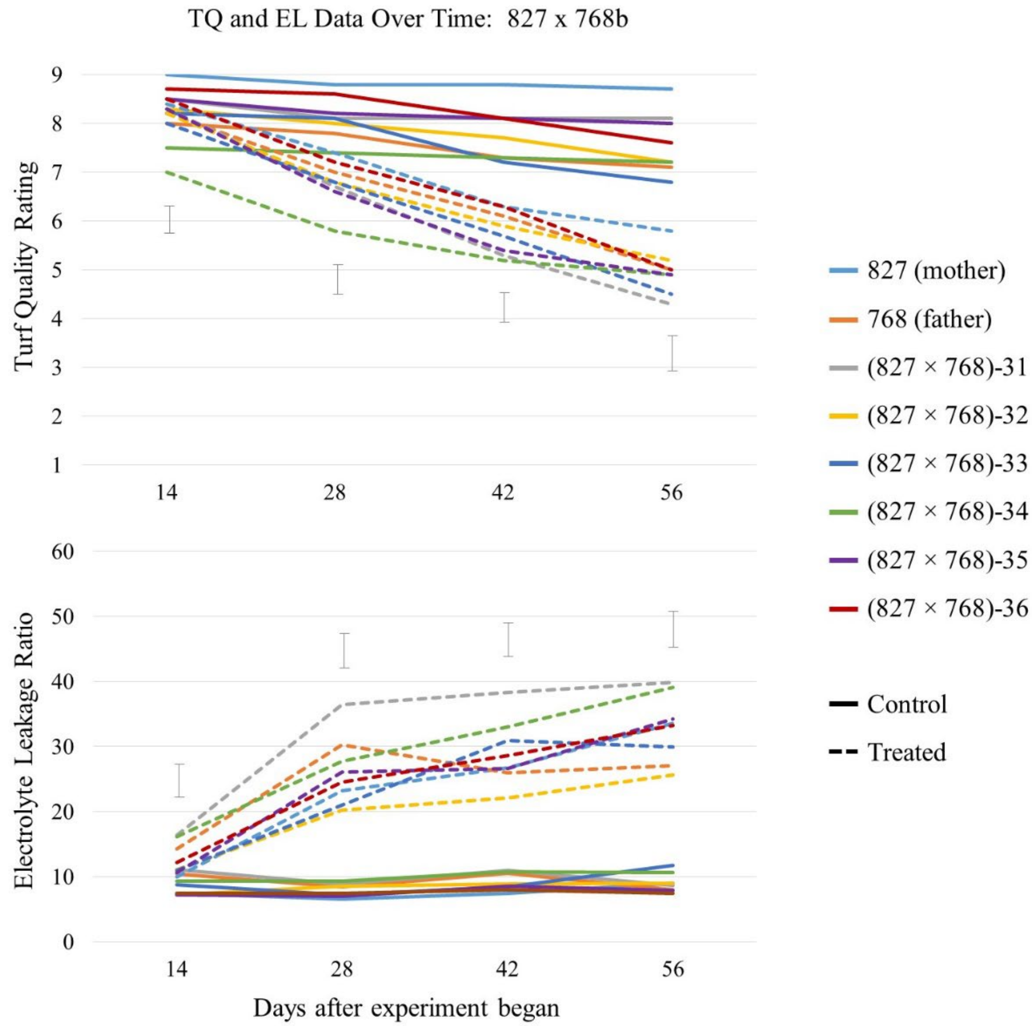

Figure 9. Turfgrass quality ratings and electrolyte leakage ratios from parents 827 × 768 and their resulting hybrids.

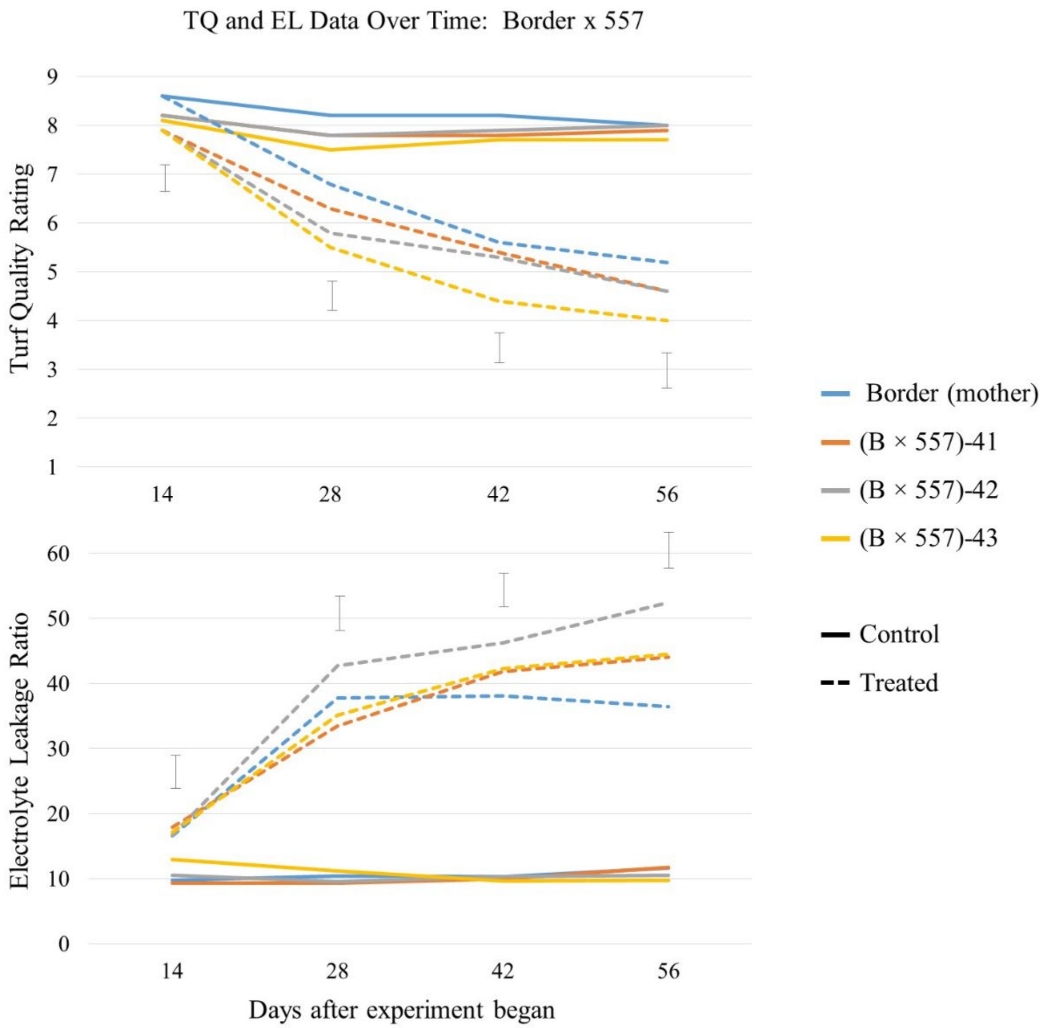

Figure 10. Turfgrass quality ratings and electrolyte leakage ratios from parents Border × 557and their resulting hybrids.

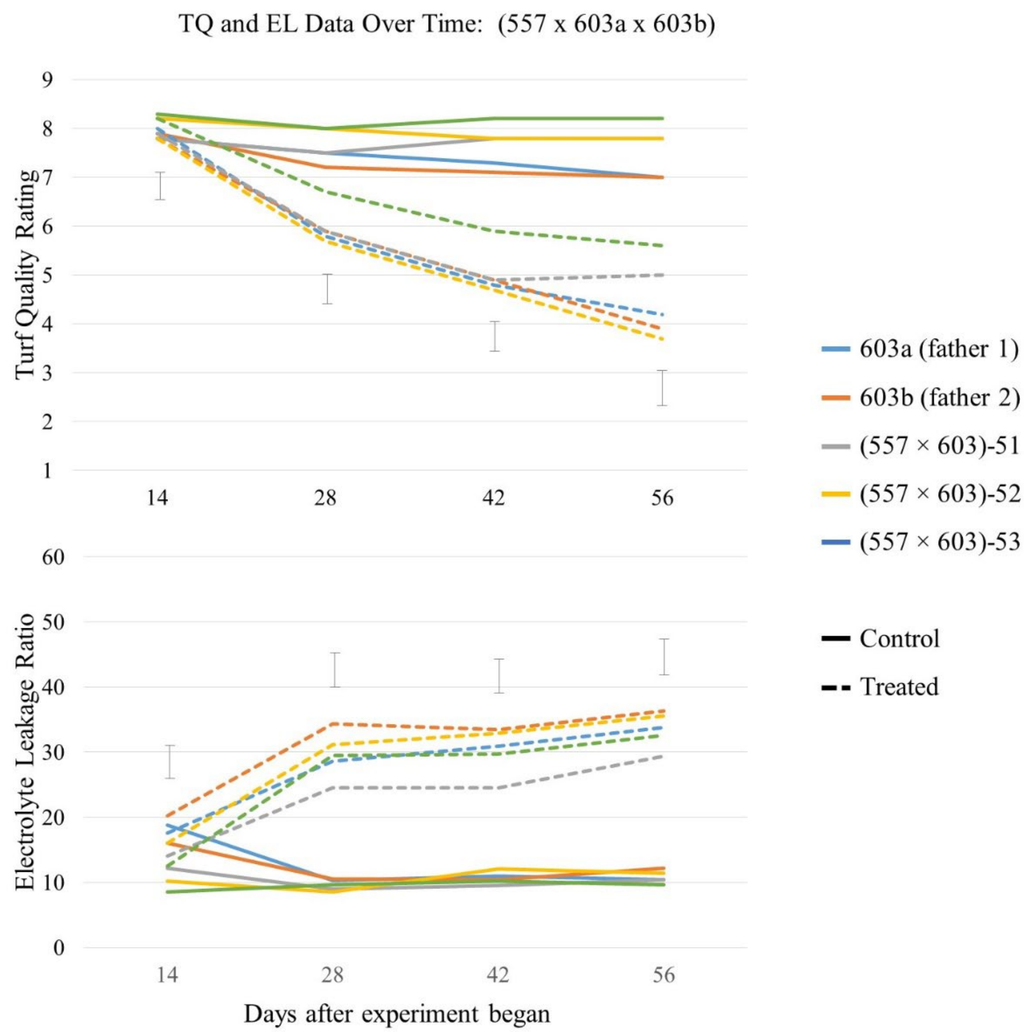

Figure 11. Turfgrass quality ratings and electrolyte leakage ratios from parents 557 × 603a × 603b and their resulting hybrids.

Discussion

This experiment was conceived of and developed due to the increased use of poor quality irrigation water and its negative effects on KBG. For KBG to thrive when irrigated with poor quality water, salinity tolerance must be added as a desirable characteristic. The breeding process that led to this experiment focused on improving salinity tolerance by crossing salt tolerant germplasm to cultivars or germplasm that already had desirable characteristics such as high canopy density, dark color, and drought tolerance. In particular, the accessions 603 and 768 were identified as salt tolerant, but were raw collections with little understanding of their TQ, seed production, or other desirable characteristics. Conversely, 827, North Star and Washington were elite cultivars with desirable qualities, but little understanding of their salt tolerance. This experiment was able to assess the likelihood that the rare hybrids of facultative apomictic KBG would inherit the salt tolerance trait. Additionally, the experiment was able to confirm the salt tolerance results of past experiments and characterize new potential parent lines for salt tolerance breeding.

Accession 768 has had exceptional salinity tolerance amongst KBG varieties, and was first identified by Robins et al. (2009), and confirmed by Wang (2013). In this experiment 768 trended above average in TQ and exhibited significantly below average (favorable) EL ratios (Tables 5 and 7). The other accession that exhibited salinity tolerance for Robins et al. (2009) and Wang (2013) was accession 603. This accession, however, delivered less than satisfactory results in our experiment, exhibiting poor TQ

throughout the experiment and average EL ratios (Tables 5 and 7). The poor TQ ratings were not unexpected, as the line has a less-dense, waxy blueish hue that rarely scores well compared to darker green entries. With an accession that has been reported to be salt tolerant, we are unclear why 603 did not exhibit lower EL ratios. One explanation for the average performance from 603 may be that Robins et al. (2009), and Wang (2013) compared the salinity tolerance of 603 to commercial varieties and accessions of unknown salinity tolerance. Commercial varieties are mainly selected for traits such as color, density, or drought tolerance rather than salinity tolerance. In this experiment, 603 was tested alongside known abiotic stress tolerant parents and their progeny. While the salinity tolerance of the hybrids was unknown, they were progeny of tolerant parents, giving them an expected advantage. Another possibility for the average performance of 603 is it was collected from semi-arid conditions and exhibits drought tolerance as well. Wang (2013) found that 603 only exhibited the highest salt tolerance metrics when tested in summer seasons, compared to cooler fall or winter seasons. As these experiments were conducted under well-watered and controlled conditions in the green house, the true poteltial of 603 may not have been able to be expressed.

In some instances, we noticed inconsistencies between TQ and EL, such as for 603 and (NS × 768)-21. Generally a negative correlation is expected when comparing TQ and EL. In other words an entry with a high TQ would be expected to have a low EL ratio (Figure 6). Accession 603, as stated earlier, had average EL ratios and poor TQ ratings. Hybrid 827 × 768-33 was another entry with poor TQ ratings but had a more favorable EL ratio. (NS × 768)-21 and Washington were unlike 603 and 827 × 768-33 in that they had high TQ ratings, but (NS × 768)-21 exhibited average EL ratios and Washington had

EL ratios that trended above the average under saline conditions. These seemingly inconsistent results may be attributed to inherent errors in the data collection methods and the physical characteristics of the entries in a greenhouse pot. When recording TQ, grasses that are darker in color or have a higher shoot density will naturally rate higher, like (NS × 768)-21, as opposed to lighter colored, low density, and narrow-leaved entries. Accession 603, on the other hand falls into the category of "less dense" and also has a glaucous leaf that results in lower green color ratings, such that it even exhibited lower TQ ratings under control conditions. Less dense entries, like 603, also displayed more visibly dead leaf tissue, simply because normal leaf senescence isn't covered by a living green leaves, while (NS × 768)-21 is able to hide salinity damage in a denser canopy, resulting in a higher TQ rating. These examples highlight the challenges of TQ ratings in greenhouse studies, and allowed us to better characterize promising parent lines and hybrid progeny for their salt tolerance.

Even though visual ratings are subjective, they are necessary because the health of turfgrass is largely based on aesthetics and is difficult to replace with quantitative measures. One consideration that can influence the aesthetic perception of a KBG entry is proximity and scale. Smaller pots in our greenhouse setting were evaluated from 0.5 to 1.0 m away, at close to eye level and more details of the plant can be seen in smaller plants at close proximity. In the field, evaluations on larger swards of grass are done from a distance of 2.0 to 2.5 m away, looking down at an angle. At this distance, imperfections may be concealed by the canopy. For experiments that can only be conducted in a greenhouse setting, larger pots would help to resolve this problem. Thus, in the experiment, our greenhouse TQ ratings were more meticulous than field-based TQ

ratings, and may possibly have provided different information compared to TQ ratings in field plots.

The difficulty of evaluating salt tolerance on plants for EL in a greenhouse setting is further complicated with the possible inconsistencies of the EL measuring process. Measuring EL follows a series of mechanical steps in order to arrive at a final reading. While these steps did not change throughout the experiment, some of the steps were variable depending on the person performing the process. This experiment was replicated four times over the course of four years. During those experiments 12 different undergraduate and graduate students helped process the large amount of tissue required to measure EL. The step of the EL process that was left to interpretation was the length the leaf blades were to be cut following washing. Students were instructed to cut leaf blades to a length of 1.25--1.5 cm. Over time that size was reduced to 0.5--0.75 cm or increased to over 2.0 cm depending on the student. These inconsistencies in cutting size were considered when EL ratios were not consistent with TQ ratings. We assumed that smaller lengths of tissue exposed more ruptured cells allowing more electrolytes to leak than perhaps the same grass cut to the instructed length. An increase of leakage from smaller cuttings would, therefore, lead to a higher initial EL reading and result in a higher overall EL ratio. The higher ratio could then lead us to believe that the sample is more stressed than others it is being compared to. The opposite could also happen if leaf tissue was cut too long and less leakage occurred resulting in an artificially low EL ratio. Measuring EL is an accurate method for measuring stress in turfgrass, but steps must be taken ensure consistency in the method. To affirm this assumption of differences in cutting lengths resulting in EL differences, a separate study was conducted to compare EL of fine (0.2-

0.3 cm) vs. regular (1.3-1.5 cm) length tissue cuttings. The study included three, unstressed turf species: tall fescue, orchard grass, and KBG. The resulting EL ratios of the fine cut tissue samples for KBG were double that of the regular cut samples and orchard grass and tall fescue EL ratios were nearly triple that of the regular cut samples (Appendix A).

In spite of experimental factors that may have influenced results of this experiment, we were able to see improvements in salinity tolerance from some hybrids bred from tolerant parents. Kentucky bluegrass is historically difficult to breed. Due to apomixis the majority of the offspring are identical to the mother (Albertini et al. 2001, Bushman 2018). When hybridization is confirmed the number of beneficial genes, for traits like salt tolerance, is unknown. This experiment included 21 hybrids and, as expected, a wide range of salinity tolerance was exhibited with the majority of them performing average or between the parental values. The parents that exhibited significantly higher than average TQ were 827, North Star, and Washington, with hybrids (NS × 768)-21 and (557 × 603)-53 also exhibiting TQ significantly above average. Both of these hybrids were statistically better than their tolerant parents, 768 and 603. In terms of EL, 768 and North Star were the parents that exhibited EL ratios significantly below average with hybrids 827 × 768-32, (557 × 603)-51, and (827 × 768)-32 also exhibiting EL ratios significantly below average.

While we did see improvement in hybrids, we generally did not see hybrids with TQ that exceeded their higher parent, with the exception of (NS × 768)-21. For example in the cross (827 x 768), none of the hybrids came close to exhibiting the same TQ as 827, which was selected for its TQ. Border is another example of a parent selected

because of high TQ that had no offspring with comparable TQ. As mentioned in the results, entries with higher TQ under control conditions generally had favorable TQ under salt treated conditions when compared to grasses within their cross. This observation might lead to the question if salinity studies are necessary or if we can simply select a KBG with the best TQ under control conditions and use this to predict performance under saline irrigation conditions. However, upon review of some of the poor performing hybrids, I noticed that some of these hybrids actually did have high TQ under control conditions compared to other siblings in their cross. These entries were: (827 × 768)-31, (B × 557)-43, and (557 × 603)-52. Identifying hybrids with low TQ under salt treated conditions despite having high TQ under control conditions confirms the necessity of salinity experiments as a method to identify salt tolerant turfgrasses, such as the KBG entries herein. These observations also convey the idea that a KBG cultivar with high TQ under non-stressed conditions will not necessarily translate into acceptable TQ in high salinity environments.

Along with identifying poor performing hybrids, this experiment also provided an indication as to which parents were not as impactful as previously thought. One such parent was Border. Border was crossed with 557 and resulted in three hybrids. While Border itself had above average TQ ratings, all of its progeny had poor TQ as well as high EL ratios. Therefore, future breeding programs will de-emphasize Border as a promising parent.

Along with continued emphasis on salt tolerance, field tests are needed to determine the response of the entries under different turf management scenarios as well as the seed yield capabilities. Kentucky bluegrass may respond differently to salinity

stress as it is maintained traditionally; usually with foot traffic and regular mowing. This experiment only evaluated salinity tolerance and was conducted in a greenhouse where temperatures did not exceed 27° C. In realistic situations, grasses irrigated with poor irrigation water will also be managed in temperatures that may exceed 32° C. Would the grasses that performed better under salinity stress in this experiment also adapt well to higher temperatures? My results, compared to Wang (2013), suggest that at least 603 *would* perform better. However, the response of the other entries is largely unknown. Lastly grasses tolerant of saline conditions will need to be economically viable to commercial seed producers because these producers select grasses with a combination of good TQ traits and high seed yield. A grass with good salinity tolerance and TQ traits but low seed yield may not be considered economically viable by a seed producer.

CONCLUSION

These experiments were able to confirm that salinity tolerance can be improved in KBG hybrids through traditional breeding practices. This conclusion is based on performance of hybrids as compared to their parents. Several grasses performed well, however grasses with favorable TQ ratings did not always have favorable EL ratios and vice versa. Because of these differences, better performing grasses from each evaluation method are reported.

The hybrid with the highest TQ at the end of the experiment was (NS × 768)-21, which was an offspring of North Star and 768. North Star had a nearly identical TQ rating as (NS × 768)-21 but TQ of 768 was significantly lower than (NS × 768)-21. (557 × 603)-53 was another hybrid with above average TQ that significantly outperformed parents that were included in this experiment.

The entry with the lowest (most favorable) EL ratio at the end of the experiment was parent 768. This was expected, as 768 has repeatedly exhibited low EL ratios as compared to other KBG varieties. (827 × 768)-32 had the lowest EL ratio of the hybrids tested and was nearly identical to that of 768 and the EL ratio of (827 × 768)-32 was better than its other parent, 827. Hybrid (557 × 603)-51 was another noteworthy hybrid for which EL ratio was significantly below average and significantly lower than its parent, 603.

Given the variations in TQ and EL ratio observed, it is difficult to isolate a single entry that might be deemed the most salt tolerant from this experiment. The only entry that was above average in TQ and below average in EL was parent North Star. Hybrid (557 × 603)-53 was also an entry that could be considered the best performing hybrid

from this experiment. (557 × 603)-53 was significantly above average in TQ and exhibited EL ratios that trended well below the average. These considerations along with the fact that (557 × 603)-53 outperformed parent 603 in TQ and EL is the reason it is selected as the 'best' hybrid from this experiment. Other grasses that performed well will be included in future studies to improve salinity tolerance. Future experiments should also continue to include TQ and EL as a measure of plant health, with perhaps the addition of digital imagery analysis to support visual ratings. Future experiments would also be improved by including fewer entries to help maintain consistency with EL procedures, and larger containers for plants to aid in visual TQ ratings.

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APPENDIX A

Introduction

Electrolyte leakage (EL) has been used extensively as an effective measure of plant stress caused by abiotic factors such as drought (Huang et al., 1997; Fu et al. 2004), cold weather exposure (Ebdon et al., 2002; Webster and Ebdon, 2005), and salinity (Wang 2013; Esmaeili et al., 2015). Methods for measuring EL may vary, but all use a similar series of steps and instrumentation to arrive at the EL ratio. The EL method was used in the experiments described in this thesis to measure stressed caused by salinity on *Poa pratensis*. A step in the process for measuring EL is cutting a leaf tissue sample to size to fit in a sample tube for agitation and autoclaving. Students helping with the project were trained to cut the tissue to specific lengths. Unfortunately, over time, the leaf lengths deviated from the standard, which was to cut tissue 1.25 - 1.5 cm. On occasion, samples were cut smaller (0.5 - 0.75cm) or larger (≥ 2cm) before the student could be corrected. Subsequently, higher than expected variability was observed in some replications of the experiment, which may be attributed to variability in the size of leaf pieces used in the EL method.

Once all data was collected and analyzed I observed grasses with good turf quality (TQ) but poor EL ratios and vice versa. These inconsistencies led us to consider differences in leaf cutting length as a possible explanation. In addition to leaf cutting length we also considered whether their location of the clippings on the leaf blade influenced EL. For example, would EL of cuttings from leaf tips (younger leaf tissue)

differ from those taken from the base of the leaf blade (older leaf tissue)? These two factors were tested as separate experiments. The objective of these experiments was to evaluate how EL might differ with cutting length or leaf blade location. We hypothesize that the finer cut tissue would influence EL by producing a higher EL ratio, and clipping location would have little effect on EL.

Methods

Three grasses were used to evaluate the differences in leaf cutting length (Experiment 1) and location (Experiment 2). The grass species we evaluated were: Kentucky bluegrass (*Poa pratensis*), tall fescue (*Festuca arundinacea*), and orchard grass (*Dactylis glomerata L.*). These grasses were propagated from vegetative clones and established in a 70 grit silica sand growth media. Grasses were maintained in a greenhouse under conditions included 16 hour days, with 21° C day and 18° C night temperatures. Grasses were irrigated every other day to field capacity with green house nutrient solution, and were allowed to establish until enough clippings could be gathered to measure EL, roughly two weeks.

Experiment 1 measured EL from different clipping length. This experiment followed the methods described in Chapter 4 of this thesis with some alterations to improve consistency of leaf cutting length. To measure EL from clipping length, leaf tissue was first cut to the predetermined 'short' and 'regular' lengths. To ensure consistent clipping lengths, visual references were used, such as the width of the scissors (0.3 cm) for the 'short' clippings and half the diameter of the sample tubes (1.5 cm) for

‘regular’ clippings. Clippings for this experiment were taken from the entire length of the leaf.

Experiment 2 measured EL from samples cut from different locations on the leaves using the same grasses as the clipping length experiment. This experiment also followed the methods described in Chapter 4 of this thesis. Collected leaves were divided into thirds: base, middle, and tip. They were then cut to ‘regular’ length (1.25 to 1.5 cm).

Each experiment included three replications from each of the three species of grass for each treatment. Treatments for Experiment 1 were ‘short’ and ‘regular’ clipping lengths. Treatments for Experiment 2 clipping location (base, middle, or tip). In both experiments 0.2 grams of the clippings were weighed out and bathed in a centrifuge tube (VWR, Aurora, Colorado) with 20 ml of deionized water. The clippings were then agitated for 18 to 20 hours.

After agitation, the electrical conductivity (EC) of each sample solution was measured (Orion Star A112 conductivity meter). The first measurement was considered the ‘before’ measurement because it measured the initial leaf cell leakage before the autoclave cycle. Once the ‘before’ measurements were taken, samples were autoclaved to rupture the remaining cells of the plants. The autoclave (Sterivap 669, MMM Group) cycle was run for 15 minutes at 121° C. Samples were then allowed to cool to room temperature, approximately 22° C. The EC of the samples was then measured a second time to quantify the total electrolytes in the cells. This measurement was referred to as the ‘after’ measurement because it was taken after the autoclave cycle. The ‘before’ and ‘after’ EC measurements of each sample were divided and then multiplied by 100: (before/after) x 100, giving us an EL ratio. This process was repeated four times for each

experiment from January to March 2019.

Results from Experiment 1 and 2 were analyzed in R with packages ‘data.table’, ‘ggplot2’, ‘dplyr’, ‘agricolae’, and ‘knitr’.

Results

Electrolyte leakage ratios were only compared within species. All four runs of Experiment 1 resulted in significant differences (Table 1, Figure 1). For Kentucky bluegrass (KBG), EL ratios of ‘short’ cut clippings were roughly double those of ‘regular’ cut clippings. For tall fescue (TF), EL ratios of ‘short’ cut clippings were, on average, quadruple those of ‘regular’ cut clippings. For orchard grass (OG), EL ratios of ‘short’ cut clippings were, on average, triple those of ‘regular’ cut clippings (Table 2).

Table 10. Analysis of variance summary of electrolyte leakage ratios for different leaf cutting sizes for Kentucky bluegrass, tall fescue, and orchard grass.

Species		DF	Sum Sq	Mean Sq	F Value	Pr (>F)
KBG	Cutting Size	1	177.13	177.13	63.457	8.82E-08
	Run	1	5.21	5.21	1.866	0.186
	Residuals	21	58.62	2.79		
TF	Cutting Size	1	1265.7	1265.7	64.302	7.93E-08
	Run	1	105.0	105	5.332	0.0312
	Residuals	21	413.4	19.7		
OG	Cutting Size	1	663.1	663.1	225.28	1.06E-12
	Run	1	52.8	52.8	17.94	0.00037
	Residuals	21	61.8	2.9		

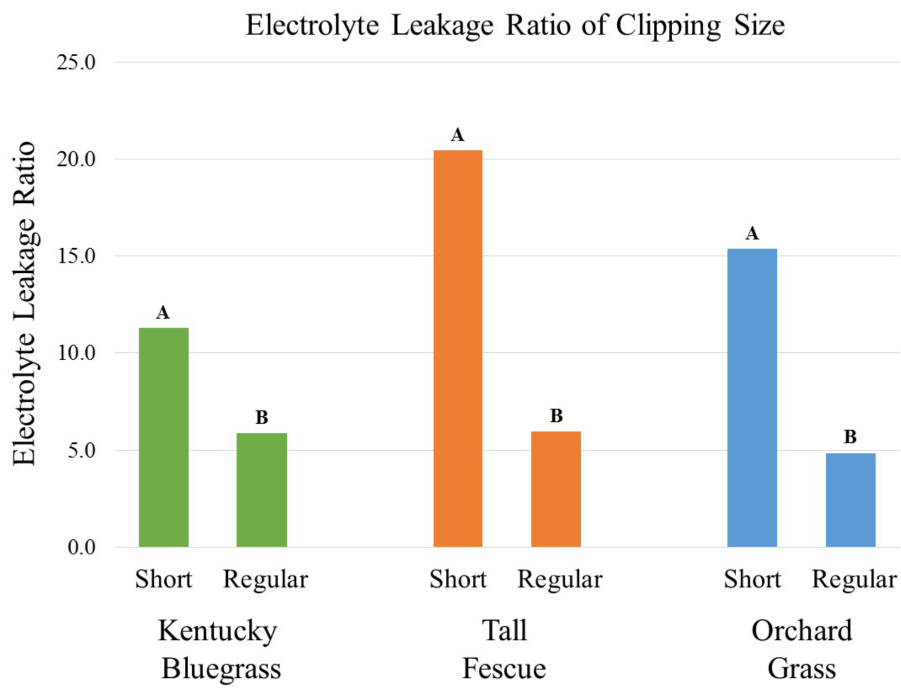

Figure 12. Average electrolyte leakage ratios for ‘short’ and ‘regular’ leaf cutting sizes. Of Kentucky bluegrass, tall fescue, and orchard grass. Significant differences between treatments are indicated by different letters.

Table 11. Average electrolyte leakage ratios for ‘short’ and ‘regular’ leaf cutting sizes of Kentucky bluegrass, tall fescue, and orchard grass across experimental runs.

Treatment	Run 1	Run 2	Run 3	Run 4
KBG Short	8.44	12.39	12.71	11.56
KBG Regular	5.99	5.35	7.04	4.97
TF Short	25.03	18.26	27.19	11.30
TF Regular	6.62	5.99	6.24	4.82
OG Short	18.66	16.15	12.47	14.13
OG Regular	6.59	5.01	3.88	3.88

Electrolyte leakage ratios from different leaf clipping locations, (Experiment 2), did not show significant differences (Table 3, Figure 2).

Table 12. Analysis of variance summary of electrolyte leakage ratios from different leaf cutting locations (base, middle, tip) for Kentucky bluegrass, tall fescue, and orchardgrass.

Species		DF	Sum Sq	Mean Sq	F Value	Pr (>F)
KBG	Treatment	2	3.5	1.75	0.966	0.392
	Run	1	4.69	4.688	2.587	0.118
	Residuals	32	57.98	1.812		
TF	Treatment	2	0.53	0.263	0.087	0.9169
	Run	1	18.97	18.67	6.168	0.0184
	Residuals	32	96.86	3.027		
OG	Treatment	2	1	0.501	0.147	0.864
	Run	1	1.45	1.446	0.426	0.519
	Residuals	30	101.91	3.397		

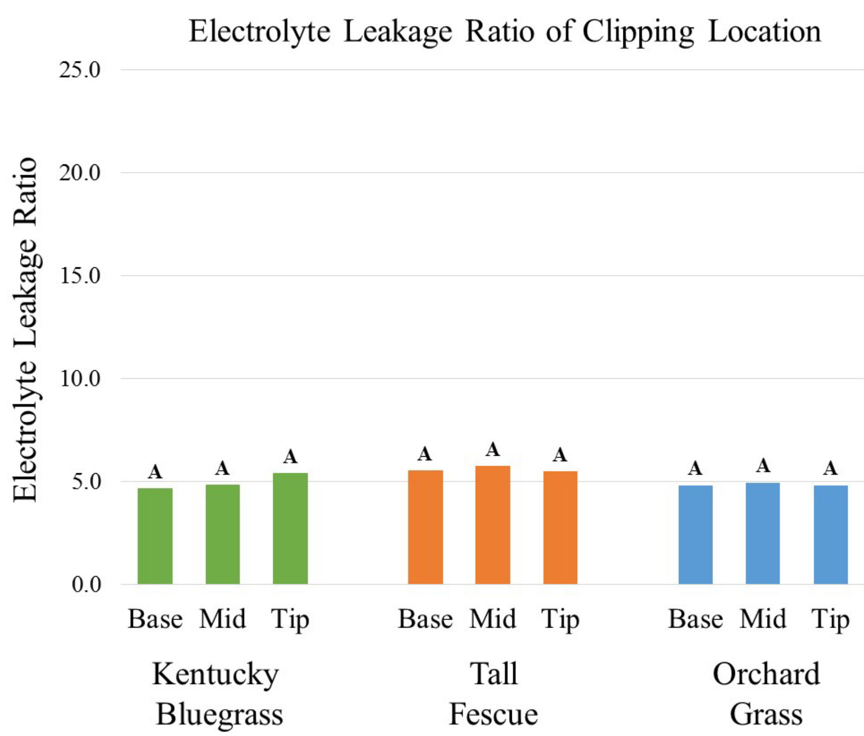

Figure 13. Average electrolyte leakage ratios for leaf cuttings from different locations (base, middle, tip) of Kentucky bluegrass, tall fescue, and orchard grass. Significant differences between treatments are indicated by different letters.

Table 13. Average electrolyte leakage ratios for different leaf cutting locations (base, middle, tip) of Kentucky bluegrass, tall fescue, and orchard grass across experimental runs.

Treatment	Run 1	Run 2	Run 3	Run 4
OG Base	5.76	6.04	2.90	4.61
OG Mid	4.04	5.98	2.98	6.85
OG Tip	5.34	5.72	2.58	5.67
TF Base	5.76	7.60	3.62	5.19
TF Mid	5.38	8.78	4.48	4.40
TF Tip	5.34	8.18	3.69	4.70
KBG Base	6.27	4.96	2.73	4.68
KBG Mid	5.17	5.94	3.04	5.19
KBG Base	5.17	6.76	3.23	6.39

Discussion

The results from these experiments indicate that consistency of leaf cutting length for EL measurements is crucial under unstressed conditions. However, when clippings were taken from different locations under unstressed conditions, no significant differences were noted. The next steps for testing EL methods will be to evaluated these experimental treatments under stressed conditions.

The addition of salinity stress may lead to differences in EL from clippings taken from the base or tip of the leaves that are being sampled, as salts may accumulate in different parts of the leaf. Other steps of the EL method may also be examined to determine if slight modifications will influence EL ratio, such as the duration of agitation prior to the first EL measurement. In this experiment samples were agitated 18-20 hours prior to the first EL measurement. What changes might we expect if samples are agitated

for 18 hours vs 20 hours, or agitated for 16 hours or less? Another step during the procedure, and a step prone to differences among people assisting in the experiment, was washing samples in deionized (DI) water prior to cutting into smaller clipping sizes. This step was designed to remove any salts that may have been left on the leaf surfaces from overhead application of saline solutions, so as not to affect EL ratios. However, if all samples are treated with the same salinity solution and handled similarly, would eliminating the DI water bath affect one entry over another? Logically, it would seem that if all plants were treated the same, a bath in DI water would be unnecessary. However, the grasses all had different leaf textures and canopy densities. Perhaps these physiological differences allow for more water to be stored on the leaf surfaces of some species. If more water remains on the leaf surface, and evaporates, a higher concentration of salts might be expected on some plant leaves as opposed to others, where water may run off the leaves faster. Changes in washing or agitation time may not be necessary for experiments with fewer entries, but improvements gained from modifications of the EL method, such as improving clipping size consistency, are recommended.

Conclusion

The results from Experiment 1 indicate that under non-stressed conditions KBG, TF and OG have higher EL ratios when clipping length is shorter as compared to longer clipping lengths. The results from Experiment 2 indicate that EL ratios from clippings collected from the base, middle or tip of the leaf were not significantly different.