

TURFGRASS PHYSIOLOGY

Interactive effects of waterlogging and salinity on perennial ryegrass and alkaligrass

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Abstract

A lot of salt-affected soils in the world are also affected by compaction and waterlogging due to shallow water tables or decreased infiltration of water due to sodicity. Waterlogging and compaction cause reduced oxygen exchange (hypoxia). Research on the combined impacts of salinity and hypoxia on turfgrass growth is limited. The interactive effects of salinity and oxygen availability on nine perennial ryegrass entries (Lolium perenne L.) and one alkaligrass [Puccinellia tenuiflora (Jacq.) Parl.] cultivar were studied. In a controlled greenhouse, grasses were subjected to 4 treatments: control, salinity, hypoxia, and salinity + hypoxia for 12 weeks. All entries exhibited decreased clipping yield in both salinity and hypoxia + salinity treatments except 'Fults' alkaligrass. With or without hypoxia treatment, 'Fults' alkaligrass was the most salt tolerant grass among all entries. In general, all perennial ryegrass entries had better turf quality in control and hypoxia treatments than in salinity and salinity with hypoxia treatments. All perennial ryegrass entries were more severely affected (quality and yields) under combined hypoxia and salinity treatment compared to salinity or hypoxia only. Plant Na⁺ and Cl⁻ concentrations increased under salinity and salinity + waterlogging treatments. The experimental entries ('10.0824' and '10.0825') were able to maintain better turf quality than other perennial ryegrass cultivars under salinity and the combination of hypoxia + salinity treatments.

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INTRODUCTION 1

Increasing world population and limited water resources have forced many landscape professionals to use non-potable water for landscape irrigation. Using poor quality water can cause salinity problems in many regions. Saline soil and water are major problems that decrease plant growth in many areas of the world. Using saline waters for turfgrass irrigation has become more common in many of these areas due to the shortage of fresh water. Consequently, the demand to identify salinity tolerant turfgrass species and cultivars has increased.

Turfgrass species and cultivars have different degrees of salt tolerance. This study tested the salinity tolerance of new

lines of perennial ryegrass and alkaligrass, both are coolseason grasses. Alkaligrass has been ranked as potentially tolerant (>10 dS m⁻¹ soil salinity value) (Harivandi, 2005). However, the salinity tolerance of perennial ryegrass has been ranked as moderate for commercial cultivars (Tang et al., 2013; Bushman et al., 2020; Friell et al., 2013; Koch & Bonos, 2010).

The effects of saline conditions have been widely studied and reviewed (Marcum & Kopec, 1997; Marcum et al., 1998; Qian et al., 2000; Alshammary et al., 2004; Peng et al., 2004; Pessarakli et al., 2008; Tang et al., 2013). Turfgrass salinity tolerance is complex, and it is affected by different environmental and physiological factors. Plant age,

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temperature, nutrient levels, and humidity are the factors that most influence salinity problems (Maas, 1986). The main reasons for diminished plant growth due to salinity are water deficiency, nutrient imbalance, and ion toxicity (Marschner, 2011).

Many saline soils around the world are also affected by waterlogging due to shallow water tables or decreased infiltration of water in soil due to sodicity causing soil deflocculation. Waterlogging causes hypoxia (oxygen deficiency) and anoxia (absence of oxygen) (Marschner, 2011). Reduced soil oxygen availability under waterlogged conditions decreases turf quality, photosynthetic rate, and chlorophyll and carbohydrate concentration (Jiang & Wang, 2006). Although salinity alone can hinder plant growth and nutrient uptake (Chang et al., 2019, 2020), the combination of salinity and waterlogging has greater effects on plant growth and nutrient uptake than either of them separately (Barrett-Lennard, 2003). In addition, waterlogging can cause the buildup of anaerobic metabolism products such as ethylene, carbon dioxide, and ethanol, which are harmful to plant growth. There are many reports about the combined effects of salinity and hypoxia on crops such as tomato (Solanum lycopersicum), soybean (Glycine max), and wheat (Triticum aestivum) (Alam et al., 2011; Barrett-Lennard, 2003; Rogers et al., 1993; Zheng et al., 2009), but few studies have been done with turfgrass. This study is based on the turf industry's interest, as the results could be helpful for turf breeders to develop cultivars that can maintain acceptable turf quality under saline and low oxygen conditions. The goals of this study are to test the effects of salinity on different perennial ryegrass entries (along with alkaligrass) simultaneously under optimum and low oxygen level. The study's specific objectives are to a) identify perennial ryegrass and alkaligrass lines that can maintain better turf quality under saline conditions, and b) determine the interactive effects of

Core Ideas

- Using poor quality water can cause salinity and sodicity problems
- Turfgrass species and cultivars show differences in salinity and hypoxia tolerance
- All grasses were more severely affected under the salinity + hypoxia treatment
- Selected lines were able to maintain better quality under hypoxia + salinity

salinity and oxygen availability on perennial ryegrass and alkaligrass.

2 | MATERIALS AND METHODS

2.1 | Plant material and growth conditions

The study was conducted at Colorado State University Plant Growth Facility. Nine perennial ryegrass lines and one alkaligrass cultivar were selected (Table 1). Grass seeds were planted in sand, and grown for 30 d before transplanting them as plugs with similar size to deep pots (6.4 cm diameter by 25 cm depth) containing fine sand. Turfgrasses were grown for 60 d before initiation of salinity treatments. Cultivars were distributed randomly in each treatment. The greenhouse temperature was maintained at 23–25 °C days and 18–20 °C nights. Salinity levels were increased daily by 1 dS m⁻¹ in treatment tanks until targeted salinity levels (6, 9, and 12 dS m⁻¹) were reached (control tanks received no salt). Instant ocean salt (Aquarium Systems, Mentor, OH) was used (58% NaCl along with MgCl₂, NaSO₄, CaCl₂ and KCl).

	Clipping yield				
Cultivar	Control	Salinity	Hypoxia	Salinity + Hypoxia	
	mg/cm ²				
Top Hat	5.6 aA ^a	2.0 cC	4.4 bB	1.6 dCD	
Palmer III	4.8 abA	1.6 cD	3.6 bcB	2.0 bC	
Fults	3.9 bB	5.7 aA	3.0 cC	3.0 aC	
Brightstar	5.0 abA	2.0 cB	4.7 bA	1.0 eC	
Paragon	4.7 abA	2.7 bC	3.4 bcB	1.9 bD	
10.0815	4.7 abA	2.0 cC	3.5 bcB	2.0 bC	
10.0824	4.6 abA	3.0 bB	4.4 bA	2.7 aB	
10.0825	4.6 abB	2.5 bcC	7.8 aA	1.7 cdD	
10.0876	5.0 abA	2.0 cC	3.6 bcB	1.9 cdC	
10.0798	4.9 abA	2.0 cC	3.0 cB	0.7 eD	

^aLowercase letters indicate mean separation among different grasses within individual treatments at P = .05. Uppercase letters indicate mean separation among treatment for a given grass at P = .05.

2.2 | Treatments

Four treatments were replicated four times and included:

1. Control: "No salt no hypoxia" plants were submerged in water for 1 h every day for irrigation, and Hoagland nutrient solution (Hoagland & Arnon, 1950) was added to the water.

2. Salinity: plants submerged in water with target salt concentration for 1 h for salt treatments, and Hoagland nutrients were added. Salt was added gradually by 1 dS m⁻¹ per day until four target concentrations (6, 9, and 12 dS m⁻¹) were reached in the treatment solution. For salinity treatment, grasses were first treated with salinity at 6 dS m⁻¹ for a period of 4 wk. Data were collected for treatments and the control. Following data collection, salinity treatment was ramped up to 9 dS m⁻¹, whereas nutrient solution of the control was maintained at <2.0 dS m⁻¹. Salinity was again held at 9 dS m⁻¹ for 4 wk, and data were collected. The cycle was repeated until solution salinity reached 12 dS m⁻¹. All entries were maintained at these individual salinity levels for a period of 4 wk, respectively, for data collection.

3. Hypoxia: plants were kept in enough water to reach the soil surface, then pots drained for 1 h daily and placed back in the water to achieve waterlogging, and nutrients were added to the water. Although soil oxygen concentration was not measured, it is well established that waterlogging (soil was submerged continuously for 23 h per day in this study) causes a hypoxic soil condition because of the low solubility of oxygen in water and the low diffusivity of oxygen in water-filled pores (~10,000-fold slower than through gas-filled soil pores) (Alam et al., 2011; Barrett-Lennard, 2003; Jiang & Wang, 2006).

4. Salinity + hypoxia: plants were kept in saline water at target salt concentration with water level to reach the soil surface, then pots were drained for 1 h daily and placed back in the saline solution to achieve waterlogging, and nutrients were added to the saline waters. Salt concentration was ramped up to 6, 9, to 12 dS m⁻¹. Salinity + hypoxia treatment was held at each of these 4 salt concentrations for 4 wk, and data were collected.

All four treatments were carried out to the termination of the experiment.

2.3 | Measurements

Data collection began 2 weeks after reaching target salinity levels. Grasses were clipped twice per week at 2 cm height. Grass clippings were collected for the last 2 wk and dried at 70 °C for 24 h to determine the dry weight. Turf quality (color, density, and uniformity) was rated visually on a scale of 0 (dead turf) to 9 (optimum quality, with a rating of 6 indicating minimum acceptable quality). Maximum root length was measured at the end of the experiment by measuring the average length of the three longest roots from the crowns to the tips of the roots. Leaf firing percentage was rated visually by estimating the total percentage of bleached leaf area at the end of treatments.

2.4 | Shoot ion concentrations

Ion concentrations analyses were determined according to Gorai et al. (2010). At the end of experiment, dried aboveground plant materials were weighed and ashed for 5 h at 500 °C. Cold ash was dissolved in 3 ml of 1 M HCl and enough deionized water was added to reach 25 ml for the analysis. The concentrations of Na^{+,} K⁺, Ca⁺, and Mg²⁺ were determined by ICP (Inductively Coupled Plasma) at Colorado State University Soil, Water and Plant Testing Laboratory. To determine the concentration of Cl^{-,} shoots were washed with deionized water, and dried at 70 °C for 24 h after which time they were ground by mortar and pestle. A sample of 100 mg was taken and dissolved in 25 ml of 2% acetic acid and filtered. Chloride was analyzed by an ion specific chloride meter (Jenway PC LM3, London, UK).

2.5 | Experimental design and statistical analysis

A split-plot experimental design was used with four replications. The main factor was the interactive effects of salinity and hypoxia, and the four treatments were randomly assigned to the whole plots. Within each whole plot, grass cultivars were randomly assigned as sub-plots. The experimental data were analyzed by analysis of variance (SAS Institute, Cary, NC). Comparisons of salinity × hypoxia treatments were presented, and means were separated by LSD at .95 level of confidence for each turfgrass entry.

3 | **RESULTS AND DISCUSSION**

3.1 | Clipping yield

Clipping yield is one indicator of turf vigor. All cultivars exhibited decreased clipping yield in both the salinity and hypoxia + salinity treatments except 'Fults' (alkaligrass) (Table 1). No differences were found among perennial ryegrass entries for the control treatment. In salinity treatment, perennial ryegrass cultivars showed significant differences compared to the control and to salt tolerant cultivar 'Fults' (alkaligrass). Among perennial ryegrass entries, experimental line '10.0824' had the highest clipping dry weight in 10

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Effects of salinity and hypoxia on turf quality for all perennial ryegrass and alkaligrass entries after one month at the 12 dS m⁻¹ FIGURE 1 salinity level. Varieties with the same lowercase letter in the same treatment are not significantly different (P < .05). There were no significant varietal differences in control and hypoxia treatments

the salinity treatment with or without hypoxia (Table 1). The experimental line '10.0825' had the highest clipping yield (7.8 mg cm^{-2}) among all entries under the hypoxia treatment; however, its clipping yield dropped to 1.7 mg cm^{-2} under salinity + hypoxia treatment. In addition, no differences were noticed between the control and the hypoxia treatment for the cultivar 'Brightstar' and line '10.0824'. However, the dry weight of 'Brightstar' decreased by almost 60% compared to the control in salinity treatment and by 80% under salinity + hypoxia treatment.

Salinity treatment did not reduce clipping yield of 'Fults' alkaligrass with or without hypoxia treatment. It was the most salt tolerant grass among all entries (Table 1). '10.0824', 'Paragon' and '10.0825' were the most salt tolerant entries among perennial ryegrass included. Based on final clipping yield, '10.0824', '10.0825', 'Brightstar', and 'Top Hat' were more tolerant than other entries in the hypoxia treatment. In salinity + hypoxia treatment, 'Fults' and '10.0824' were most tolerant, but all the entries were harmfully affected.

3.2 Turf quality and leaf firing

At the beginning of the experiment, all treatments exhibited desirable turf quality (ranged from 8 to 9 on 0-9 scale, 9 = the best) (data not shown). Turf quality declined over time with salinity treatments. Figure 1 shows the effects of treatment on the turf quality of all cultivars when the highest salinity (12 dS m^{-1}) was reached and held for 4 weeks. In general,

all entries had better turf quality in control and hypoxia treatments than in salinity or salinity with hypoxia treatments. In salinity treatment, '10.0824', '10.0798', '10.0825', '10.0815', '10.0876', and 'Tophat' had better turf quality than 'Palmer III', 'Paragon', and 'Brightstar'. In salinity + hypoxia treatment, 'Tophat', 'Brightstar', '10.0815', '10.0824', '10.0825', '10.0876' and '10.0798' had better turf quality than 'Fults', 'Palmer III', and 'Paragon'. Several perennial ryegrass lines ('10.0824', '10.0825', and '10.0815') were able to maintain acceptable quality under the combination of hypoxia and moderate salinity levels $(6-10 \text{ dS m}^{-1})$ (data not shown).

Leaf firing percentage increased with salinity and salinity +hypoxia treatments (Figure 2). Leaf firing started to appear after salinity reached around 6 dS m^{-1} (data not shown). Leaf firing increased as salinity increased in both the salinity treatment and the salinity + hypoxia treatment and reached the highest percentages when salinity reached 12 dS m⁻¹ in most cultivars (Figure 2). Under 12 dS m^{-1} salinity treatments, 'Palmer III' had the highest leaf firing percentage of 44%, and '10.0798' had the lowest leaf firing percentage of 20% (Figure 2). Cultivar 'Brightstar' had the highest leaf firing percentage of 70 % under salinity + hypoxia treatment, and 'Fults' had the lowest leaf firing percentage of 37%. For all entries, leaf firing was more severe under the salinity + hypoxia treatment than the salinity alone treatment. Severe leaf firing for turfgrass under salinity + hypoxia treatment was reported by Zhang et al. (2013).

Based on data of growth parameters (clipping yield and turf quality), 'Fults' alkaligrass had the best salinity tolerance,



FIGURE 2 Effects of salinity and hypoxia on leaf firing percentage (%) for all perennial ryegrass and alkaligrass entries after one month at the 12 dS m^{-1} salinity level. Varieties with same lowercase letter in the same treatment are not significantly different (P < .05)

followed by '10.0824' and '10.0825'. Cultivars 'Brightstar' and 'Palmer III' had the least salt tolerance.

Although all cultivars had acceptable qualities under the hypoxia treatment, quality decreased for all entries except '10.0815' when compared to the control. Jiang and Wang (2006) reported that reduced soil oxygen availability under hypoxia conditions decreases turf quality, photosynthetic rate, and chlorophyll and carbohydrate concentrations of turfgrass. Another study done in 2003 showed that after 28 d of waterlogging, photosynthesis of all experimental entries of perennial ryegrass was reduced by 30-50% (McFarlane et al., 2003). This experiment demonstrated that the combination of salinity and hypoxia dramatically reduced clipping yield and turf quality, and increased leaf firing of perennial ryegrass and alkaligrass (Figure 1). All grasses were more severely affected under the salinity + hypoxia treatment compared to salinity or hypoxia only. However, 'Fults' and '10.0824' grew the best under salinity + hypoxia treatment. Previously, the combination of salinity and hypoxia has been shown to have greater effects on plant growth than either of them separately in many crops such as wheat, soybean, and legumes (Alam et al., 2011; Rogers et al., 1993; Zheng et al., 2009).

3.3 | Root measurements

Root length was measured at the end of the experiment and significant differences were found among the treatments. All perennial ryegrass cultivars had an increase in root length over control under hypoxia treatments with the roots extending beyond the bottom of the containers (Table 2). 'Paragon' had an average length of 87 cm as the greatest root length in the hypoxia treatment that is a 197% increase compared to the control. On the other hand, 'Fults' alkaligrass had the lowest root length with an average of 27.5 cm in hypoxia treatment. Increased root length under hypoxia treatment was an unexpected result because of the common inhibitor effects of hypoxia on root growth. Many papers found that hypoxia has a negative effect on root growth, and plants under hypoxia generally have shallow root systems. Banach et al. (2012) found that root length of *Plantago lanceolata* L. decreased by more than 60% under hypoxia treatment compared to the control.

Root length results under the hypoxia treatment may not reflect the hypoxia effects; they may be due to the greater water availability in the hypoxia treatment than in control and salinity treatments. In control and salinity treatments, pots were allowed to drain for 23 h per day. In the hypoxia treatment, pots were submerged continuously, except for 1 h daily. Control, salinity and salinity +hypoxia treatments showed no differences in root length. However, a study published in 2013 by Zhang and others supported our results and reported that hypoxia increased root length under hypoxia treatment by 20%, compared to the control. In addition, another study found that some perennial ryegrass cultivars were able to maintain high relative root growth under waterlogging compared to the control (McFarlane et al., 2003). 'Fults' under hypoxia had the same condition as the perennial ryegrass cultivars, but their roots were not longer than the control. Although all

TABLE 2 Root observations and measurements at the termination of the experiment

	Root length					
Cultivar	Control	Salinity	Hypoxia	Salinity + Hypoxia		
	cm					
Brightstar	29 aB ^a	23.8 aB	65 cA	24.3 aB		
Fults	29.5 aA	23.5 aA	27.5 gA	22.8 aA		
Palmer III	29.3 aB	23.8 aB	63.5 cA	24 aB		
Paragon	29.3 aB	24.0 aB	87 aA	24 aB		
Tophat	30.0 aB	25 aB	51.5 deA	23.8 aB		
10.0815	28.8 aB	24 aB	56.3 dA	23.5 aB		
10.0824	29 aB	23.3 aB	62.3 cA	24 aB		
10.0825	29.7 aB	23.3 aB	46.8 fA	23.3 aB		
10.0876	29 aB	23.8 aB	75 bA	23.5 aB		
10.0798	28.8 aB	23.8 aB	45.3 fA	24 aB		

^aLowercase letters indicate mean separation among different grasses within individual treatments at P = .05. Uppercase letters indicate mean separation among treatment for a given grass at P < .05.



FIGURE 3 Effects of salinity and hypoxia on Na concentrations for all perennial ryegrass and alkaligrass entries after one month at the 12 dS m^{-1} salinity level. Varieties with same lowercase letter in the same treatment are not significantly different (P < .05)

perennial ryegrass cultivars had increased root length under hypoxia treatment, significant differences were found among the cultivars. No varietal difference in maximum root length was found among the other treatments (Table 2).

3.4 | Ion concentrations

Sodium concentrations in shoots were increased under salinity and salinity + waterlogging treatments compared to the control in all cultivars (Figure 3). Among all grass entries, 'Paragon' had the lowest Na concentrations under the salinity and salinity+ hypoxia treatments (32.9 and 39.9 mg g⁻¹ d.w., respectively). All entries except '10.0825' and 'Paragon' had higher Na concentrations in shoots under the salinity + hypoxia treatment than salinity treatment alone. In general, the ability of plants to maintain low Na⁺ concentration in shoot cytoplasm is associated with plants salinity tolerance (Blumwald, 2000; Qian et. al, 2000). However, in our studies, no relationship was found between shoot Na⁺ concentration and salinity tolerance. The reason for that could be due to the analysis method that we used which determine the amount of

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FIGURE 4 Effects of salinity and hypoxia on Cl concentration for all perennial ryegrass and alkaligrass entries after one month at the 12 dS m^{-1} salinity level. Varieties with same lowercase letter in the same treatment are not significantly different (P < .05)

Na in the bulk shoots but would not allow us to separate Na present in vacuoles and cytoplasm. Even for salt tolerant plant, active growth processes are sensitive to Na⁺ accumulation in cytoplasm. To maintain low cytoplasmic Na⁺, cells pump toxic ions into the vacuoles via salt sequestration mechanism that is energy dependent. Our results indicated that salinity damage became more severe under waterlogging conditions. The declining ability to tolerate salinity under waterlogging conditions may be associated with the effects of waterlogging on plant energy (ATP) availability. It is well known that waterlogging creates hypoxia that can trigger anaerobic respiration and reduce the production of ATP. Many salinity tolerance mechanisms, including toxic ion exclusion and sequestration into vacuoles may require considerable energy investment and are energy dependent. Many salinity studies suggested that plant Na⁺ increases with salinity (Marcum et al., 1998; Qian et al., 2000; Alshammary et al., 2004). Increased plant Na⁺ concentration under salinity + waterlogging has been indicated in other studies (Barrett-Lennard, 2003).

Shoot Cl⁻ concentration increased by 8–16 times under salinity and salinity + waterlogging treatments compared to non-saline treatments (Figure 4). 'Fults', 'Paragon', 'Top Hat', and '10.0876' had similar Cl⁻concentrations (4–4.6 ppm) under the 12 dS m⁻¹ salinity treatment. Chloride concentrations increased more under salinity + waterlogging treatments in all cultivars except 'Palmer III' and 'Brightstar'.

Increased concentrations of Na⁺ and Cl⁻ in the shoots under waterlogging with salinity together could be due to carbohydrates and energy depletion under hypoxia conditions. Negative effects on plant growth and turf quality can occur with increases in these two elements in the shoots to levels beyond the ability of the cells to transfer these ions into the vacuoles. In addition, waterlogging can cause the buildup of anaerobic metabolism production resulting in ethylene, carbon dioxide, and ethanol which are harmful to plant growth. Therefore, the combined effect of salinity + waterlogging significantly reduced growth and increased the accumulation of these ions.

For all cultivars, K concentrations in shoots were higher under the hypoxia treatment than under the control and salinity treatments (Figure 5). Cultivar 10.0824 had the highest K concentration (328 mg g⁻¹ d.w.) under hypoxia treatment, and the other cultivars showed no significant differences under hypoxia treatment. Entries '10.0825' and 'Paragon' had the highest K concentration (64 and 57 mg g⁻¹ d.w., respectively) under control treatment, while '10.0815' had the lowest concentration (25 mg g⁻¹ d.w.). No differences were found among other cultivars under control treatment. In all cultivars and experimental lines, there was a clear pattern under salinity and waterlogging conditions, that is, a significant increase in Na and CI and a decrease in shoot K. This result was similar to the results reported for several other species (Barrett-Lennard, 1986; Rogers & West, 1993).

In our study, a significant increase in plant Na⁺ concentration was observed in most grasses when grown under combined salinity and waterlogging stress compared to plants grown under salinity only treatment. Lower K⁺/Na⁺ ratios were recorded for most cultivars under the salinity and salinity + hypoxia treatments (Figure 6). The intracellular K⁺/Na⁺ ratio is often used as a key determinant of plant salinity stress/tolerance (Shabala & Cuin, 2007). Salt tolerant plants have high K⁺/Na⁺ ratio because of plasma membrane antiporters' ability to eject Na⁺ from the cytosol while



FIGURE 5 Effects of salinity and hypoxia on K concentrations for all perennial ryegrass and alkaligrass entries after one month at 12 dS m⁻¹ salinity level. Varieties with same lowercase letter in the same treatment are not significantly different (P < .05)



FIGURE 6 Effects of salinity and hypoxia on K/Na ratio for all perennial ryegrass and alkaligrass entries after one month at the 12 dS m⁻¹ salinity level. Varieties with same lowercase letter in the same treatment are not significantly different

holding K⁺ in the cytosol (Shi et al., 2002). Peng et al. (2004) suggest that the K⁺/Na⁺ selectivity of potassium channels and the existence of an apoplastic barrier, the Casparian bands of the endodermis, lead to the lateral gradient of K⁺ and Na⁺ across root tissue, resulting not only in high levels of K⁺ concentration in the shoot but also a large Na⁺ gradient between the root and the shoot. Peng et al. (2004) concluded that alkaligrass resists salt stress through high shoot K⁺ concentration and an endodermis barrier to Na⁺. However, this process

requires energy, and under hypoxia + salinity, roots use anaerobic respiration and produce less ATP that affects this process negatively and decreases this ratio.

4 | CONCLUSION

In summary, grasses showed differences in salinity and hypoxia tolerance. With or without hypoxia treatment, 'Fults' alkaligrass was the most salt tolerant grass among all entries. Among perennial ryegrass entries, '10.0824' and '10.0825' were among the most salt tolerant perennial ryegrass lines. All grasses were more severely affected under the salinity + hypoxia treatment compared to salinity or hypoxia only. However, some perennial ryegrass lines ('10.0824', '10.0825', and '10.0815') were able to maintain better quality than other cultivars under the combination of hypoxia + salinity.

The combined effect of salinity + waterlogging significantly accelerated the increase in shoot Na and Cl accumulation in all entries. Grasses under the combination of waterlogging and salinity were negatively affected; alkaligrass has high salinity tolerance and perennial ryegrass has moderate salinity tolerance, but their tolerance decreased to greater degrees when salinity combined with waterlogging. Further research with different levels of waterlogging is recommended to examine the ability of perennial ryegrass to tolerate hypoxia.

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