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Salt Tolerance of Six Switchgrass Cultivars

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Abstract: *Panicum virgatum* L. (switchgrass) cultivars ('Alamo', 'Cimarron', 'Kanlow', 'NL 94C2-3', 'NSL 2009-1', and 'NSL 2009-2') were evaluated for salt tolerance in two separate greenhouse experiments. In experiment (Expt.) 1, switchgrass seedlings were irrigated with a nutrient solution at an electrical conductivity (EC) of 1.2 dS·m^{-1} (control) or a saline solution (spiked with salts) at an EC of 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10) for four weeks, once a week. Treatment EC 10 reduced the tiller number by 32% to 37% for all switchgrass cultivars except 'Kanlow'. All switchgrass cultivars under EC 10 had a significant reduction of 50% to 63% in dry weight. In Expt. 2, switchgrass was seeded in substrates moistened with either a nutrient solution of EC 1.2 dS·m^{-1} (control) or a saline solution of EC of 5.0, 10.0, or 20.0 dS·m^{-1} (EC 5, EC 10, or EC 20). Treatment EC 5 did not affect the seedling emergence, regardless of cultivar. Compared to the control, EC 10 reduced the seedling emergence of switchgrass 'Alamo', 'Cimarron', and 'NL 94C2-3' by 44%, 33%, and 82%, respectively. All switchgrass cultivars under EC 10 had a 46% to 88% reduction in the seedling emergence index except 'NSL 2009-2'. No switchgrass seedlings emerged under EC 20. In summary, high salinity negatively affected switchgrass seedling emergence and growth. Dendrogram and cluster of six switchgrass cultivars indicated that 'Alamo' was the most tolerant cultivar, while 'NSL 2009-2' was the least tolerant cultivar at both seedling emergence and growth stages. A growth-stage dependent response to salinity was observed for the remaining switchgrass cultivars. 'NSL 2009-1' and 'NL 94C2-3' were more tolerant to salinity than 'Cimarron' and 'Kanlow' at the seedling emergence stage; however, 'Kanlow' and 'Cimarron' were more tolerant to salinity than 'NSL 2009-1' and 'NL 94C2-3' at the seedling growth stage.

Keywords: *Panicum virgatum*; salinity; mineral nutrition; cluster analysis

1. Introduction

Renewable energy is expected to make a significant contribution to meet global energy needs due to diminishing availability of discoverable fossil fuel reserves and the environmental consequences of exhaust gases from fossil fuel. The U.S. Energy Information Administration [1] reported that renewable energy, excluding hydropower, accounted for 28% of the overall growth in electricity generation from 2012 to 2040. Biofuel, one of the most important types of renewable energy, is gaining popularity and the demand for biofuel is increasing. In 2011, a total of 110 billion liters of biofuel were produced worldwide, among which bioethanol accounted for 78.7% [2]. Bioethanol is primarily produced from crops such as corn (*Zea mays*, L.), sugar beet (*Beta vulgaris* var. *altissima* Döll), sweet potato (*Ipomoea batatas*, (L.) Lam.), sugar cane (*Saccharum officinarum*, L.), sorghum (*Sorghum bicolor*, (L.) Moench), and switchgrass (*Panicum virgatum*, L.) [3–5], several of which are food crops. Therefore, the production and use of bioethanol may compete with food crops for arable land in the long term. Ethanol made from ligno-cellulosic feedstocks could play a critical role in promoting energy diversity and reducing carbon dioxide emissions [6]. The market could eventually be worth $20 billion a year, and there is enough feedstock to produce 382 billion liters of cellulosic ethanol in the U.S. [7]. High-yield lignocellulose feedstock is essential for the production of a large quantity of biofuel to sustain an efficient bioenergy processing and production chain.

Switchgrass is a perennial warm season grass that is native to North America from 55° N latitude in Canada southwards into the United States and Mexico. It is primarily used for soil conservation, forage production, and as an ornamental grass in the U.S. It has been identified as a sustainable source of biomass feedstock for energy production with the potential to produce about 380 liters of ethanol per metric ton [8]. Substantial efforts are being made in developing switchgrass for forage production, cellulosic ethanol production, biogas, and direct combustion for thermal energy applications. For instance, 'Alamo' switchgrass is a tetraploid lowland cultivar with coarser foliage and a late maturity date [9]. 'Kanlow' is a tetraploid lowland cultivar that is suited for poorly drained sites or areas subject to periodic flooding [9]. Switchgrass has a broad adaptability, tolerates water and nutrient limitations, and has the ability to produce moderate to high biomass yields on marginal lands [10–12]. Marginal lands refer to lands that have low inherent productivity and have been abandoned or degraded [13]. Soil salinity in some of these lands is too high for most common economically-important crops, and groundwater with high salinity is the major water source. Growing switchgrass on marginal lands would be an alternative way to conserve fresh water, reduce fossil fuel pollution, and secure food safety. Therefore, identifying salt-tolerant switchgrass genotypes and improving the salt tolerance for salt-affected lands is critically important.

Salinity causes reduction in the seed germination [14–16], seedling emergence [17], seedling growth [15,17,18], and yield of switchgrass [19]. It also modifies plants' physiological and biochemical processes [20]. According to Tober et al. [21], switchgrass is moderately sensitive to salt conditions at an electrical conductivity (EC) of 5 to 10 $dS{\cdot}m^{-1}$. Switchgrass is more sensitive to salinity at the seedling emergence stage than at any other stage [21]. However, Ganjegunte et al. [22] reported that 'Alamo' switchgrass grown in soil columns with salt-affected soils collected from an abandoned cotton field in El Paso, Texas, produced appreciable biomass when irrigated with treated wastewater at an EC of 2.6 $dS{\cdot}m^{-1}$ for six years under greenhouse conditions. Additionally, the salt tolerance of switchgrass varies with cultivar [23,24]. To provide more information about such variations, the relative salt tolerance of six switchgrass cultivars was determined in two greenhouse studies by evaluating seedling emergence, plant growth, gas exchange rates, and leaf ion accumulation under different salt conditions.

2. Materials and Methods

2.1. Seedling Growth (Experiment 1, (Expt. 1))

2.1.1. Plant Materials and Treatments

On 18 July, 2013, switchgrass seeds ('Alamo', 'Cimarron', 'Kanlow', 'NL 94C2-3', 'NSL 2009-1', and 'NSL 2009-2') were sown onto the surface of LM-40 high porosity growing mix (Canadian sphagnum peat moss 60%, horticultural perlite 40%, limestone, dolomite, wetting agent, micro- and macronutrients; Lambert Peat Moss Inc., Québec, QC, Canada) in 5.8-L black Poly-tainer™ injection molded containers (22.5 × 19.5 cm) and covered with a thin layer of the growing mix. A reverse osmosis (RO) water-based nutrient solution was used for irrigating plants and to avoid salt accumulation in the root zone before treatments started. The nutrient solution at an electrical conductivity (EC) of 1.2 ± 0.1 $dS{\cdot}m^{-1}$ (mean and standard deviation) was prepared by adding 1 $g{\cdot}L^{-1}$ of 15N-2.2P-12.5K (Peters 15-5-15 Ca-Mg special; Scotts, Marysville, OH, USA) to RO water, with a final pH of 6.4 ± 0.2. When they emerged, seedlings were thinned to five seedlings per pot. All seedlings were grown in a greenhouse in El Paso, TX, USA (lat. 31°41′45″ N, long. 106°16′54″ W, elev. 1139 m). Temperatures in the greenhouse were maintained at 31.4 ± 5.3 °C (mean ± standard deviation) during the day and 25.7 ± 2.7 °C at night. The daily light integral (photosynthetically active radiation) was 16.1 ± 4.1 $mol \cdot m^{-2} \cdot d^{-1}$.

From 7 to 27 August, 2013, seedlings were irrigated with a nutrient solution (control) or saline solutions once a week, for a total of four times. The nutrient solution was prepared as above. The saline solution at 5.0 ± 0.1 $dS \cdot m^{-1}$ (EC 5) was prepared by adding 1.54 $g \cdot L^{-1}$ sodium chloride (NaCl) and 1.47 $g \cdot L^{-1}$ calcium chloride ($CaCl_2$) to the nutrient solution, whereas saline solution at 9.9 ± 0.2 dS·m-1 (EC 10) was prepared by adding 4.37 $g \cdot L^{-1}$ NaCl and 4.16 $g \cdot L^{-1}$ $CaCl_2$ to the nutrient solution. This mixture was used because NaCl is the most common salt in reclaimed water [25] and $CaCl_2$ was used to forestall potential calcium deficiencies [26]. Solutions were prepared in 100-L tanks with a confirmed EC using an EC meter (Model B173; Horiba, Ltd., Kyoto, Japan). The pH of all solutions was adjusted to 6.1 ± 0.4. At each irrigation, plants were irrigated with 1500 mL treatment solution per container, resulting in a leaching fraction of approximately 10% to 20%. Plants were then watered with the nutrient solution two more times before harvest.

The leachate EC was determined following the pour-through method according to Cavins et al. [27] and Wright [28]. In brief, a saucer was placed under the container, which had drained for at least 30 min right after treatment solutions were applied. A total of 100 mL distilled water was poured on the surface of the substrate to obtain the leachate solution, and the EC of the leachate solution was tested for EC using an EC meter. One container per treatment per cultivar was chosen for measurement. Leachate EC readings were averaged across cultivars.

2.1.2. Growth Data

Plant height (cm), from pot rim to the collar of the top leaf, were recorded individually for all plants at the end of the experiment (i.e., 19 September, 2013). The heights of all five plants in the same pot were averaged. The averaged plant height and total number of tillers of all five plants in a pot were used for analysis. At harvest, shoots of all five plants were severed at the substrate surface, and the leaf area was determined using an LI-3100C area meter (LI-COR® Biosciences, Lincoln, NE, USA). Shoot dry weight (DW) was determined after shoots were oven-dried at 65 °C for four days.

2.1.3. Gas Exchange and SPAD Reading

Leaf net photosynthesis (Pn), transpiration (E), and stomatal conductance (gs) of four plants per cultivar per treatment were measured one week before harvest using a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA, USA) with an automatic universal PLC6 narrow leaf cuvette. Fully expanded and healthy leaves were chosen for measurements. The environmental conditions within the cuvette were maintained at leaf temperature = 25 °C, photosynthetic photon flux (PPF) = 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and CO_2 concentration = 375 $\mu mol \cdot mol^{-1}$. Data were recorded when the environmental conditions and gas exchange parameters in the cuvette became stable. These measurements were taken on sunny days between 10:00 and 14:00, and plants were well watered to avoid water stress.

Relative chlorophyll content (Soil-Plant Analysis Development (SPAD) reading) was measured using a handheld SPAD 502 Plus chlorophyll meter (measured as the optical density; Minolta Camera Co., Osaka, Japan) one week before harvest. For each pot, 10 healthy and fully expanded leaves were chosen for measurement.

2.1.4. Mineral Analysis

Five leaf samples per treatment per cultivar were randomly selected for mineral analysis. Dried tissue was ground to pass a 40-mesh screen with a stainless Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). Powdered leaf samples were extracted with 2% acetic acid (Fisher Scientific, Fair Lawn, NJ, USA) to determine Cl^- using the method described in Gavlak et al. [29]. The concentration of Cl^- was determined using a M926 Chloride Analyzer (Cole Parmer Instrument Company, Vernon Hills, IL, USA). Powdered samples were submitted to the Soil, Water, and Forage Testing Laboratory at Texas A&M University (College Station, College Station, TX, USA) for the analyses of the Na^+, Ca^{2+}, and K^+. In brief, powdered samples were digested in nitric acid following the protocol described by Havlin and Soltanpour [30]. Na^+, Ca^{2+}, and K^+ in the digested samples were analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (SPECTRO Analytical Instruments Inc., Mahwah, NJ, USA) and reported on a dry plant basis as described by Isaac and Johnson [31].

2.1.5. Experimental Design and Statistical Analysis

The experiment followed a split-plot design with salinity as the main plot and cultivar as the subplot, with five replications per treatment per cultivar. Two-way analysis of variance (ANOVA) was performed to analyze all data. When a significant difference among treatments occurred for each cultivar, mean separations were conducted using Tukey's Honestly Significant Difference (HSD) multiple comparison at $\alpha = 0.05$.

Relative shoot DW (%) was calculated for each plant in salt treatment as follows: Shoot DW in salt treatment/Shoot DW in control × 100%. Similarly, relative height, relative leaf area, and relative tiller number were calculated. These relative values were used as salt tolerance indices for hierarchical cluster analysis [32]. The dendrogram and clustering of the six switchgrass cultivars were obtained based on the Ward linkage method and squared Euclidian distance on the means of the salt tolerance indices for four multivariate parameters. All statistical analyses were performed using JMP (Version 13.2, SAS Institute Inc., Cary, NC, USA).

2.2. Seedling Emergence (Expt. 2)

On 30 January, 2014, seeds of six switchgrass cultivars ('Alamo', 'Cimarron', 'Kanlow', 'NL 94C2-3', 'NSL 2009-1', and 'NSL 2009-2') were sown at a depth of 0.5 cm in a germination insert (13.5 × 13.5 × 4.5 cm) containing Metro-Mix 360 (SunGro Hort., Bellevue, WA, USA), with 30 seeds per insert and four inserts (replications) per treatment. The potting mix was saturated with a nutrient solution (control) or saline solutions. The nutrient solution was prepared as described in Expt. 1. For the saline solution at EC of 4.8 ± 0.5 $dS \cdot m^{-1}$ (EC 5), NaCl at 1.54 $g \cdot L^{-1}$ and $CaCl_2$ at 1.47 $g \cdot L^{-1}$ were added to the nutrient solution. For the saline solutions at EC of 10.0 ± 0.4 $dS \cdot m^{-1}$ (EC 10) and 19.8 ± 2.9 $dS \cdot m^{-1}$ (EC 20), NaCl at 4.37 and 7.06 $g \cdot L^{-1}$ and $CaCl_2$ at 4.16 and 6.66 $g \cdot L^{-1}$ were added to the nutrient solutions, respectively. As described in Expt. 1, the solutions were prepared in 100-L tanks with a confirmed EC using an EC meter. The pH of all solutions was adjusted to 6.0 ± 0.6. Whenever the substrate surface started to dry, the nutrient or saline solutions were applied through sub-irrigation. This occurred three times during the entire experiment.

Seeds began to emerge 10 days after sowing, and seedling emergence was counted thereafter every day and continued for 12 days. A seedling was considered to have emerged when the hypocotyl hook was visible above the surface. The greenhouse environment was maintained at an average day temperature of 26.0 ± 9.0 °C, night temperature at 12.3 ± 4.4 °C. The relative humidity was 43.2% ± 18.8%.

A split-plot experiment design was employed with salinity as the main plot and cultivar as the subplot, with four replications per treatment per cultivar, 30 seeds per replicate. Emergence percentage (EP) was calculated using the formula: EP (%) = (Number of emerged seedlings)/(Total number of seeds) × 100%. Emergence index (EI) was calculated as: $EI = \sum_{i=1}^{n} \left({EP_i}/{T_i} \right)$; where EPi is the emergence percentage on day i, and Ti is the number of days after sowing [33]. EP and EI data were analyzed as described in Expt. 1.

3. Results and Discussion

3.1. Seedling Growth (Expt. 1)

Plant height, leaf area, tiller number, and shoot dry weight of switchgrass varied with salt treatments and cultivars, but no interactive effects occurred between treatment and cultivar (Table 1). This indicates that all switchgrass cultivars responded similarly to the saline solution applied in this study. All switchgrass cultivars under EC 5 tended to be shorter, with the exception of 'Kanlow'. However, compared to the control, EC 10 significantly decreased plant height by 22% to 35% for all switchgrass cultivars. All switchgrass cultivars had a slight reduction in leaf area under EC 5, but this was still not statistically significant. Treatment EC 10 significantly reduced the leaf area of all switchgrass cultivars by 51% to 65% compared to the control. Treatment EC 5 decreased the tiller number of 'Cimarron' by 25% and tended to inhibit the tiller formation of other switchgrass cultivars (Table 1). Compared to the control, EC 10 reduced the tiller number of all switchgrass cultivars by 32% to 37%, with the exception of 'Kanlow' that had 26% fewer tillers. 'Alamo', 'NL94C2-3', 'NSL 2009-1', and 'NSL 2009-2' switchgrass cultivars under EC 5 had a reduction of 20% to 22% in shoot dry weight, but this was still not statistically different from the control. However, the shoot dry weight of 'Cimarron' and 'Kanlow' switchgrass under EC 5 decreased by 42% and 28%, respectively. All switchgrass cultivars under EC 10 had a significant reduction of 50% to 63% in shoot dry weight. These results are similar to previous reports [34,35] demonstrating that elevated salinity decreases plant growth in terms of plant height, leaf area, and shoot biomass in maize and sorghum. The tiller number of sorghum is also reduced as salinity increased [35].

Salinity also impairs the plant photosynthetic apparatus, especially the photosystem II (PS II) [36]. However, in this study, all switchgrass cultivars had similar leaf net photosynthesis (Pn), transpiration rate (E), and stomatal conductance (gs). Also, leaf Pn, E, and gs were not impacted by salt treatment (data not shown), which was different from a study on maize and sorghum conducted by Niu et al. [35] in a similar fashion. They found that maize and sorghum genotypes under elevated salt treatment had reduced Pn, E, and gs at 35 days after treatment. Salt treatment affected the relative chlorophyll content (SPAD reading). The SPAD readings of switchgrass plants in the control, EC 5, and EC 10 were 28.6, 30.6, and 33.5, respectively. Sun et al. [34] also observed an increased SPAD reading of 'Desert Maize' and 'Tx7078' sorghum irrigated with saline solutions at an EC of 5 and 10 $dS \cdot m^{-1}$. However, maize genotypes had a reduced SPAD reading under salt treatment. The SPAD readings varied with cultivars, with the greatest value of 34 for 'Kanlow' and the smallest value of 26.9 for 'Cimarron'. The SPAD readings ranged from 30.6 to 31.8 for other switchgrass cultivars.

Leaf Na^+ content was different among salt treatments and cultivars, but no interactive effects occurred (Table 2). Compared to the control, leaf Na^+ concentration under EC 5 and EC 10 increased by 53% and 68%, respectively, regardless of cultivar. The highest Na^+ concentration (1.6 $mg \cdot g^{-1}$ DW) was found in 'Alamo' at EC 10. Salt treatment and cultivar interactively affected the leaf Ca^{2+} concentration (Table 2). Leaf Ca^{2+} concentration increased with increasing EC levels in all cultivars except 'Alamo'. Treatment EC 5 significantly increased leaf Ca^{2+} concentration by 19%, 23%, 64%, 35%, and 15%, respectively, for 'Cimarron', 'Kanlow', 'NL 94C2-3', 'NSL 2009-1', and 'NSL 2009-2'. Leaf Ca^{2+} content in 'Cimarron', 'Kanlow', 'NL 94C2-3', 'NSL 2009-1', and 'NSL 2009-2' at EC 10 was 42%, 54%,71%, 57%, and 46%, respectively; higher than that in the control. The leaf Cl^- concentration also differed among salt treatments, but not among cultivars (Table 2). Compared to the control, leaf Cl^- concentration in all cultivars at both EC 5 and EC 10 increased by 22% and 34%, respectively.

Plants can adapt to salt stress through excluding or tolerating Na^+ or Cl^- accumulation in the shoots [20]. In this study, the average leaf Na^+ concentration across all switchgrass cultivars was 0.9 ± 0.1 $mg \cdot g^{-1}$ based on leaf dry weight. This is similar to previous observations on sorghum genotypes [34,35], but is much lower than that in maize genotypes [35]. These results suggest that switchgrass plants, like sorghum, have a better capability of preventing the transport of Na^+ into the shoots to avoid foliar salt damage. The leaf Cl^- concentrations of all switchgrass cultivars in the control, EC 5, and EC 10 were 10.2, 12.4, and 13.8 $mg \cdot g^{-1}$ on leaf dry weight basis, respectively, which are lower than those in maize genotypes [35]. These data suggest that switchgrass can avoid Cl^- accumulation in shoots.

Table 1. Height, leaf area, number of tillers, and dry weight of six switchgrass cultivars irrigated with salt solutions at an electrical conductivity (EC) of 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10) in a greenhouse. A nutrient solution at EC of 1.2 dS·m^{-1} was used as the control. Relative reduction (%) in height, leaf area, number of tillers, and dry weight were calculated as a percent of the control and are presented in parentheses.

Cultivar	Height (cm)			Leaf Area (cm²)			Number of Tillers‡			Dry Weight (g)‡		
	Control	EC 5	EC 10	Control	EC 5	EC 10	Control	EC 5	EC 10	Control	EC 5	EC 10
'Alamo'	51.6a †	49.0 (5)a	40.5 (22)b	3965a	2495 (37)ab	1386 (65)b	44.6a	37.4 (16)ab	29.8 (33)b	43.1a	34.4 (20)a	20.8 (52)b
'Cimarron'	43.0a	37.6 (13)a	28.2 (34)b	3472a	2405 (31)ab	1541 (56)b	50.6a	37.8 (25)b	34.6 (32)b	42.0a	24.6 (42)b	18.9 (55)b
'Kanlow'	54.9a	56.6 (0)a	41.0 (25)b	3453a	2230 (35)ab	1695 (51)b	39.6a	32.8 (17)a	29.2 (26)a	55.2a	39.7 (28)b	24.5 (56)b
'NL 94C2-3'	47.3a	44.2 (7)a	33.2 (30)b	3300a	3026 (8)ab	1259 (62)b	44.8a	46.4 (0)a	30.4 (32)b	52.1a	41.9 (20)a	19.5 (63)b
'NSL 2009-1'	49.6a	42.2 (15)a	32.3 (35)b	3954a	2917 (26)ab	1691 (57)b	58a	44.2 (24)ab	36.8 (37)b	51.0a	40.0 (22)ab	25.4 (50)b
'NSL 2009-2'	53.8a	49.9 (7)a	35.9 (33)b	4664a	3691 (21)a	1754 (62)b	55.8a	48.6 (13)ab	38.0 (32)b	70.5a	54.9 (22)a	26.7 (62)b
Cultivar (V)		*** ‡			***			**			***	
Treatment (T)		***			***			***			***	
V * T		NS			NS			NS			NS	

† For each cultivar, means with the same lowercase letters within a row for the same variable are not significantly different among treatments by Tukey's Honestly Significant Difference (HSD) multiple comparison at $p < 0.05$. ‡ NS, *, **, ***: not significant, significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Adapted from Sun et al. [33]

Potassium plays an important role in the tugor-pressure-driven solute transport in the xylem and the water balance of plants [37]. Na^+ accumulation might cause the reduction of K^+ uptake [38]. In this study, leaf K^+ concentration decreased significantly with an increasing EC in switchgrass 'Alamo' and 'Cimarron' (Table 2). Compared to the control, EC 5 and EC 10 decreased leaf K^+ content by 22% and 30% for 'Alamo', respectively, and by 16% and 30% for 'Cimarron', respectively. Leaf K^+ content in other switchgrass cultivars decreased slightly with increasing EC, and the magnitude of reductions varied with cultivar. These results are different from those records on sorghum genotypes [34], which had an increased leaf K^+ concentration in salt conditions.

Table 2. Leaf ion concentrations of six switchgrass cultivars irrigated with a nutrient solution at an electrical conductivity (EC) of 1.5 dS·m^{-1} (control) or salt solutions at EC of 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10) in a greenhouse.

Cultivar	Treatment	Ion Concentration (mg.g–1 Dry Weight)			
		Na^+	**Ca^{2+}**	**Cl^-**	**K^+**
'Alamo'	Control	1.3 a †	5.0 a	10.9 a	40.0 a
	EC 5	1.3 a	4.5 a	13.5 a	31.1 ab
	EC 10	1.6 a	5.6 a	14.1 a	28.1 b
'Cimarron'	Control	0.9 a	4.1 b	9.1 a	39.5 a
	EC 5	1.2 a	4.9 ab	10.8 a	33.1 b
	EC 10	1.2 a	5.8 a	12.7 a	27.5 c
'Kanlow'	Control	0.4 a	3.6 c	10.5 a	30.3 a
	EC 5	0.7 a	4.4 b	13.1 a	27.7 a
	EC 10	0.5 a	5.5 a	14.0 a	24.3 a
'NL 94C2-3'	Control	0.4 a	3.1 b	11.7 a	36.4 a
	EC 5	0.8 a	5.0 a	12.5 a	28.4 a
	EC 10	0.9 a	5.2 a	15.3 a	33.5 a
'NSL 2009-1'	Control	0.6 a	3.8 b	9.1 a	39.8 a
	EC 5	1.1 a	5.2 ab	11.2 a	36.3 a
	EC 10	0.8 a	6.0 a	13.4 a	31.0 a
'NSL 2009-2'	Control	0.5 b	4.4 b	9.8 a	33.8 a
	EC 5	0.8 ab	5.0 ab	13.3 a	28.4 a
	EC 10	1.1 a	6.4 a	13.2 a	26.8 a
Cultivar (V)		*** ‡	***	NS	***
Treatment (T)		***	*	***	*
V * T		NS	*	NS	NS

† For each cultivar, means with the same lowercase letters within a column are not significantly different among treatments by Tukey's Honestly Significant Difference (HSD) multiple comparison at $p < 0.05$. ‡ NS, *, **, ***: nonsignificant, significant at $p < 0.05$, 0.01, and 0.001, respectively.

3.2. Seedling Emergence (Expt. 2)

No switchgrass seedlings emerged under EC 20. This treatment was excluded from further statistical analysis. Switchgrass seedling emergence percentage and index varied with salt treatment and cultivar, and no interaction occurred between salt treatments and cultivars (Table 3). The seedling emergence percentage of all cultivars under EC 5 was similar to that of the control plants. The seedling emergence index of all cultivars under EC 5 was also similar to that of the control, with the exception of 'NL 94C2-3'. These results indicate that the saline solution at EC of 5 dS·m^{-1} did not inhibit the seedling emergence of the tested switchgrass. Compared to the control, EC 10 significantly reduced the seedling emergence of 'Alamo', 'Cimarron', and 'NL 94C2-3' switchgrass by 44%, 33%, and 82%, respectively. 'Kanlow', 'NSL 2009-1', and 'NSL 2009-2' switchgrass under EC 10 had reductions of 25%, 48%, and 52%, respectively, in seedling emergence percentage. The seedling emergence index of all switchgrass cultivars under EC 10 decreased from 46% to 88%. These results are in line with a previous study on sorghum [34], which decreased its seedling emergence under saline solution conditions.

Table 3. Seedling emergence percentage and index of six switchgrass cultivars sub-irrigated with salt solutions at an electrical conductivity (EC) of 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10) in a greenhouse. A nutrient solution at EC of 1.2 dS·m^{-1} was used as control. Data for saline solution at an EC of 19.8 dS·m^{-1} (EC 20) are not presented as no seedlings emerged. Relative reduction (%) in seedling emergence percentage and index were calculated as a percent of the control and are presented in parentheses.

Cultivar	Seedling Emergence Percentage‡			Seedling Emergence Index		
	Control	EC 5	EC 10	Control	EC 5	EC 10
'Alamo'	41.7a †	35.0 (16) ab	23.4 (44)b	22.9a	18.3 (20) ab	10.3 (55)b
'Cimarron'	57.5ab	63.4 (0)a	38.4 (33)b	40.3a	41.2 (0)a	17.7 (56)b
'Kanlow'	63.4a	61.7 (3)a	47.5 (25)a	40.7a	41.8 (0)a	22.0 (46)b
'NL 94C2-3'	51.7a	46.7 (10)a	9.2 (82)b	34.0a	24.3 (28)b	4.0 (88)c
'NSL 2009-1'	50.8a	34.2 (33)a	26.7 (48)a	31.2a	19.7 (37) ab	12.9 (59)b
'NSL 2009-2'	22.5a	25.9 (0)a	10.8 (52)a	14.3a	16.0 (0)a	5.0 (65)a
Cultivar (V)		*** ‡			***	
Treatment (T)		***			***	
V * T		NS			NS	

† For each cultivar, means with the same lowercase letters within dependent variables are not significantly different among treatments by Tukey's Honestly Significant Difference (HSD) multiple comparison at $p < 0.05$. ‡ NS, *, **, ***: not significant, significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Adapted from Sun et al. [33]

The dendrogram and cluster of six switchgrass cultivars were obtained based on the Ward linkage method and squared Euclidian distance on the means of the salt tolerance indices for four multivariate parameters at seedling growth and emergence stages (Figure 1). 'Alamo' and 'NSL 2009-2' switchgrasses were consistently clustered as the most tolerant and the least tolerant cultivars, respectively, at both seedling growth and emergence stages. However, other tested switchgrass cultivars responded differently from one stage to another. 'NSL 2009-1' and 'NL 94C2-3' were within the same group as 'Alamo' at the seedling emergence stage, but classified into the group of 'NSL 2009-2' at the seedling growth stage. On the other hand, 'Kanlow' and 'Cimarron' stayed in the same group as 'NSL 2009-2' at the seedling emergence stage, but were categorized in the group of 'Alamo' at the seedling growth stage. These results agree with previous reports by Tober et al. [21], Fan et al. [23], and Liu et al. [24], who reported that the salt tolerance of switchgrass varies with cultivar and growth stage. All of these reports suggest that plant physiological adjustment to salinity stress involves trade-offs at different stages, for example, between seedling emergence and seedling growth [36].

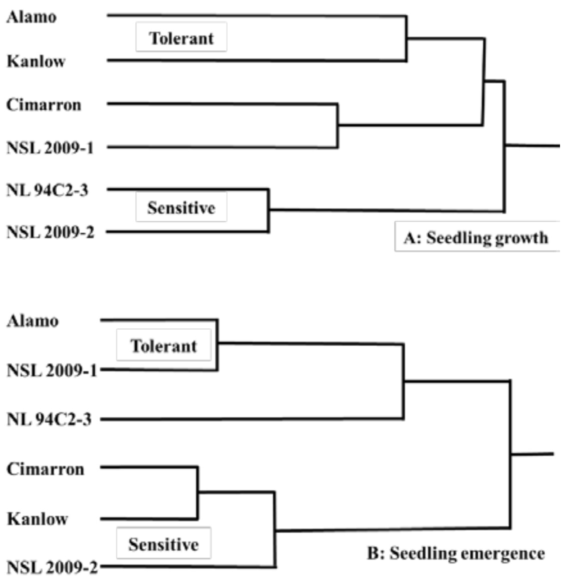

Figure 1. Hierarchical cluster analysis of switchgrass cultivars using multivariate parameters including height, leaf area, number of tillers, and dry weight (**A**), and seedling emergence percentage and index (**B**). The dendrogram is based on Ward linkage using the squared Euclidian distance on the means of multivariate parameters.

4. Conclusions

Salinity negatively affected the switchgrass seedling emergence and growth. The switchgrass cultivar 'Alamo' was the most tolerant cultivar to salinity, while 'NSL 2009-2' was the most susceptible cultivar at both seedling emergence and growth stages. The responses of other tested switchgrass cultivars to salinity are growth-stage dependent. 'NSL 2009-1' and 'NL 94C2-3' were more tolerant to salinity than 'Kanlow' and 'Cimarron' at the seedling emergence stage, but 'Kanlow' and 'Cimarron' were more tolerant to salinity than 'NSL 2009-1' and 'NL 94C2-3' at the seedling growth stage.

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