



The potential of *Alternanthera littoralis* P. Beauv. for saline soil phytoremediation and use as animal feed

Chrislaine Yonara Schoenhals Ritter¹ · Jaqueline da Silva dos Santos¹ · Marcelo Nogueira do Amaral² · Gustavo Muniz Pereira¹ · Charisma Prietto Alles¹ · Priscila Ariane Auler¹ · Milene Lopes dos Santos³ · Márcio Nunes Corrêa³ · Sidnei Deuner¹ · Eugenia Jacira Bolacel Braga¹

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Abstract

Soil salinization is an increasing global concern, demanding sustainable strategies for agricultural recovery and food security. Halophytes such as *Alternanthera littoralis* P. Beauv. represent a promising alternative for phytoremediation and forage production in saline environments. This study evaluated gas exchange, effective quantum yield of PSII (Φ PSII), electron transport rate (ETR), antioxidative enzyme activity, and nutritional composition of *A. littoralis* under 0, 100, 200, and 300 mM NaCl. The species showed high salinity tolerance, with preferential Na⁺ accumulation in shoots (up to 84.51 g kg⁻¹ dry mass at 300 mM NaCl) and maximum accumulation in roots (21.94 g kg⁻¹ at 200 mM), highlighting its potential for Na⁺ phytoextraction. Despite increasing salinity, *A. littoralis* maintained stable Φ PSII and electron transport rate (ETR) values under saline conditions, indicating maintenance of photochemical activity. Leaf SOD activity increased under saline conditions, whereas MDA, H₂O₂, and electrolyte leakage did not differ among treatments, suggesting activation of antioxidative defense response without evidence of severe oxidative damage. Bromatological analyses revealed high protein content, reduced acid detergent fiber, and increased concentrations of essential minerals. These compositional changes were associated with higher in vitro dry matter digestibility under increasing NaCl concentrations, suggesting a potential forage application for the species under saline conditions. Collectively, these findings demonstrate the remarkable salinity tolerance of *A. littoralis*, highlighting its ability to maintain physiological functionality under saline conditions. In addition, the species shows potential as a multifunctional resource for the recovery of salt-affected soils and as a nutritious forage in saline environments.

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✉ Eugenia Jacira Bolacel Braga
jacirabraga@hotmail.com

¹ Departamento de Botânica, Instituto de Biologia,
Universidade Federal de Pelotas, Capão do Leão, RS, Brasil

² Departamento de Biologia, Centro de Ciências Naturais e
Exatas, Universidade Federal de Santa Maria, Santa Maria,
RS, Brasil

³ Núcleo de Pesquisa, Ensino e Extensão em Pecuária
(NUPEEC-Hub), Departamento de Clínica Veterinária,
Instituto Veterinário, Universidade Federal de Pelotas, Capão
do Leão, RS, Brasil

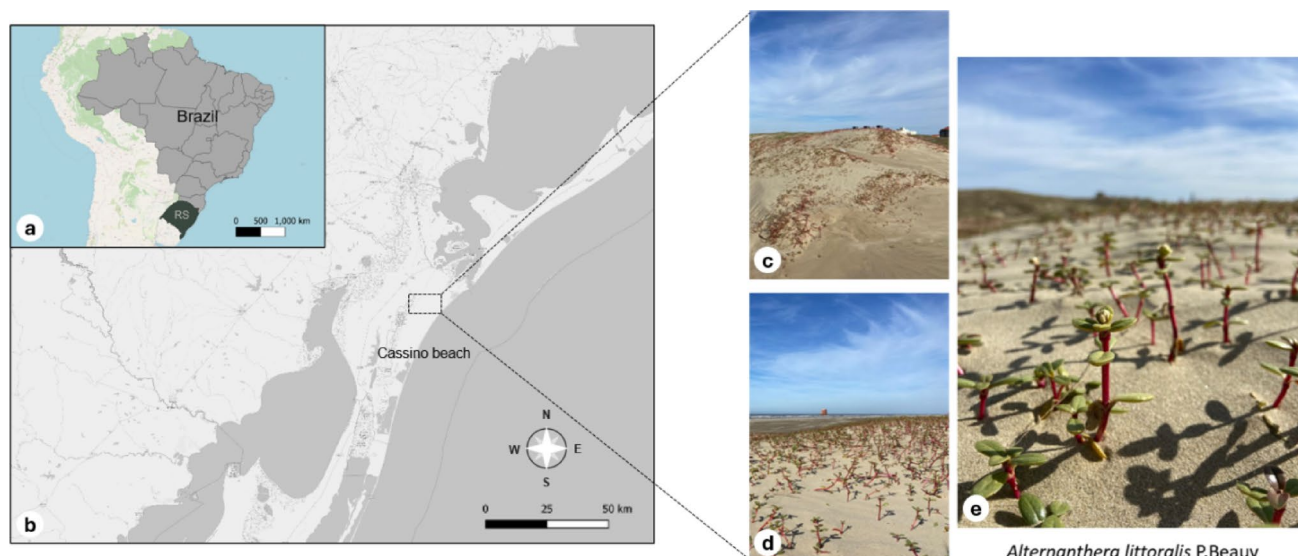


Fig. 1 Location of the study area in the southern region of Brazil. **(a)** Map of Brazil highlighting the state of Rio Grande do Sul (RS). **(b)** Details of the coastal region with the location of Cassino beach (Rio Grande, RS), where the observations were made. **(c–e)** Aspects of the coastal dune environment and occurrence of the species *Alternanthera*

littoralis P. Beauv.: **(c)** general view of the dunes with sparse vegetation, **(d)** occupation of the sandy plain near the coastline, **(e)** detail of the morphology of the plant in its juvenile stage. The mapping was carried out using ArcGIS 10.8

production systems, particularly in regions affected by water scarcity, high temperatures, and land degradation, where seasonal forage shortages are common (Malcolm 1969; Aronson 1989; El Shaer 2010; Slama et al. 2015). Their nutritional composition, often rich in proteins, phenolic compounds, lipids, and essential minerals such as K^+ , Ca^{2+} , and Mg^{2+} , further increases their potential as supplementary feed resources (Ksouri et al. 2008; Benhammou et al. 2009; Mishra et al. 2015). Nevertheless, factors such as elevated salt content and the presence of antinutritional compounds still represent important limitations that must be considered to optimize their use in animal nutrition (Khan et al. 2009).

Within this context, species belonging to the Amaranthaceae family, such as *Alternanthera littoralis* P. Beauv., have attracted increasing attention due to their remarkable tolerance to adverse environmental conditions, including high salinity, low soil fertility, and extreme temperatures (Zhigila et al. 2015; Bhargava et al. 2020). Naturally distributed along the sandy coastal regions of eastern Brazil, this herbaceous species has been recognized not only for its ecological adaptability, but also for its rich composition of bioactive compounds. Its antioxidant potential is associated with the presence of natural enzymatic antioxidants and secondary metabolites, including phenols, flavonoids, anthocyanins, and tannins, which contribute to the neutralization of reactive oxygen species and protection against environmental stress (Fahn and Cutler 1992; Cordazzo et al. 2006; Yu et al. 2008).

Given these attributes, *A. littoralis*, a native Brazilian species, emerges as a promising candidate for dual-purpose use: **(I)** phytoremediation of saline agricultural soils, contributing

to the removal and accumulation of excess salts, and **(II)** use as a forage resource to supplement animal feed in salt-affected regions. However, to date, no studies have investigated the species from this perspective, and its actual tolerance to salinity, as well as its potential for these applications, remains unclear. Thus, this work represents the first effort to investigate the physiological responses of *A. littoralis* to saline environments, aiming to elucidate its salt tolerance mechanisms and evaluate its potential for dual-purpose applications, including phytoremediation and forage use, thereby contributing to the sustainable valorization of Brazilian native flora.

Materials and methods

Plant material and growth conditions

Alternanthera littoralis P. Beauv. plants were collected at Cassino beach, located in the Southern region of the Rio Grande do Sul State ($32^{\circ}10'51''$ S; $52^{\circ}09'31''$ W; 7 m altitude) in Brazil (Fig. 1) and Registered in SISGEN under number ABDB2A3.

Salinity treatments

To investigate plant responses to salinity, seedlings were carefully washed and transferred to 2 L plastic pots in a floating hydroponic system. Plants were maintained in a floating hydroponic system containing half-strength Hoagland and Arnon nutrient solution (pH 5.8) for 15 days for

acclimation (Hoagland and Arnon 1950). After the acclimation period, NaCl was added directly to the nutrient solution in the hydroponic system, establishing four salinity treatments: 0 mM (control), 100 mM (moderate salinity; EC: 5–7 dS m⁻¹), 200 mM (moderate salinity; EC: 8–10 dS m⁻¹), and 300 mM (severe salinity; EC: >12 dS m⁻¹) (Akpınar 2023). Plants remained under saline treatments for 21 days, and nutrient solutions were renewed weekly. Each treatment consisted of four biological replicates, with two plants per replicate. After the experimental period, plant material was collected and stored at –80 °C for subsequent physiological and biochemical analyses.

For bromatological and nutritional analyses, plants were exposed to three salinity levels (0, 200, and 300 mM NaCl) under the same hydroponic conditions. After 21 days in saline conditions, samples were collected for bromatological and in vitro digestibility analyses, which were conducted at the Center for Research, Teaching, and Extension in Livestock (NUPEEC-HUB/UFPEL).

Ion quantification (Na⁺ and K⁺)

The quantification of Na⁺ and K⁺ was performed on the shoot part (leaves + stem) and roots of plants across all proposed treatments. The plant material was dried in an oven at 50 °C until a constant mass was achieved, ground using a ball mill, and digested with nitric acid (HNO₃) and perchloric acid (HClO₄) in a 2:1 ratio for ion extraction, accordingly methodology described by Tedesco (1995).

Gas exchange and PSII efficiency

Stomatal conductance (gs; mol H₂O m⁻² s⁻¹), transpiration rate (E; mmol H₂O m⁻² s⁻¹), effective quantum yield of PSII (ΦPSII), and electron transport rate (ETR; μmol e⁻ m⁻² s⁻¹) were measured using a LI-600 porometer/fluorometer (LI-COR Biosciences, Lincoln, NE, USA). Measurements were performed in the morning (approximately 9:00 am) on fully expanded intermediate leaves, on the adaxial surface, under ambient light conditions and without prior dark adaptation, with photosynthetically active radiation ranging from approximately 800 to 1200 μmol m⁻² s⁻¹, ambient CO₂ concentration of approximately 400 ppm, and air temperature between 25 and 30 °C.

Photosynthetic pigments

The photosynthetic pigments were measured according to the methodology described by Arnon (1949). Approximately 100 mg of *A. littoralis* leaves were ground in liquid nitrogen, followed by the addition of 4 mL of acetone, and centrifuged at 12,000 g for 5 min. Subsequently, using

a spectrophotometer, absorbance readings were taken at wavelengths of 663 nm (chlorophyll a), 645 nm (chlorophyll b), and 470 nm for carotenoids.

Antioxidative enzymes

To prepare the enzyme extract, 200 mg of leaf tissue was ground in liquid nitrogen and homogenized in 2.0 mL of extraction buffer (100 mM K⁺ phosphate, pH 7.0, 0.1 mM EDTA, and 10 mM ascorbic acid). The homogenate was centrifuged at 13,000 g for 15 min at 4 °C, and the supernatant was used to determine the activities of the enzymes superoxide dismutase (SOD) (EC 1.15.1.1), catalase (CAT) (EC 1.11.1.6), and ascorbate peroxidase (APX) (EC 1.11.1.1).

SOD activity was evaluated based on the enzyme's ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) (Giannopolitis and Ries 1977) in a reaction medium containing 100 mM K⁺ phosphate (pH 7.8), 14 mM methionine, 0.1 mM EDTA, 75 μM NBT, and 2 μM riboflavin. Absorbance readings were taken at 560 nm. One unit of SOD was defined as the amount of enzyme that inhibits 50% of the NBT photoreduction under the assay conditions.

Total protein concentration was determined using the Bradford assay to calculate the specific activity of superoxide dismutase. Coomassie Brilliant Blue G-250 was used as the dye. Aliquots of 50 μL of the extract were mixed with 2.5 mL of the reagent, and absorbance was measured at 595 nm after 5 min. Quantification was performed using a standard curve prepared with bovine serum albumin (BSA) (Bradford 1976).

Catalase activity was determined according to the methodology described by Azevedo et al. (1998), based on the oxidation of H₂O₂ (molar extinction coefficient 39.4 mM cm⁻¹). The reaction medium consisted of 100 mM K⁺ phosphate buffer (pH 7.0), 12.5 mM H₂O₂, water, and the enzyme extract. The activity was monitored by the decrease in absorbance at 240 nm for 2 min and expressed as μmol H₂O₂ min⁻¹ mg⁻¹ protein.

APX activity was determined using the method proposed by Nakano and Asada (1981), by assessing the rate of ascorbate oxidation at 290 nm (extinction coefficient 2.8 mM cm⁻¹). The reaction mixture, incubated at 28 °C, consisted of 100 mM K⁺ phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H₂O₂, water, and the enzyme extract. The activity was monitored by the decrease in absorbance at 290 nm for 2 min and expressed as μmol ascorbate min⁻¹ mg⁻¹ protein.

Oxidative stress indicators

Lipid peroxidation was assessed by quantifying malondialdehyde (MDA) following the protocol outlined by Cakmak

and Horst (1991). Frozen samples (0.200 g) were ground in 2 mL of 0.1% trichloroacetic acid and centrifuged at 12,000 g for 15 min at 4 °C. The supernatant was collected, and 1.5 mL of thiobarbituric acid solution (0.5% TBA in 10% TCA) was added. The samples were then incubated in a water bath at 90 °C for 20 min. Subsequently, the samples were centrifuged again at 12,000 g for 4 min, and absorbance readings were taken at 535 nm and 600 nm using a spectrophotometer.

The same extract used for lipid peroxidation was utilized for the quantification of hydrogen peroxide. The reaction medium consisted of 10 mM K⁺ phosphate buffer (pH 7.0) and 1M K⁺ iodide (KI). An aliquot of the extract, ranging from 0.100 to 0.500 µL, was mixed with 1.0 mL of 1M K⁺ iodide and 10 mM K⁺ phosphate buffer to reach a final volume of 2 mL. After gentle shaking, the samples were subjected to spectrophotometric reading at 390 nm (Velikova et al. 2000).

Electrolyte leakage from the cells was quantified following the methodology outlined by Vasquez-Tello et al. (1990) and Pimentel (2002), with modifications. In this procedure, three leaf discs of 1 cm² were washed and incubated in 20 mL of deionized water for 24 h at room temperature. Subsequently, the leaf discs were autoclaved for 20 min, and the conductivity of the resulting solutions was measured using a portable conductivimeter.

Bromatological analysis

The fresh samples were weighed to determine the fresh mass and then pre-dried in a forced air incubator at 55 °C for 72 h and ground in a stationary Wiley Miller mill with a 1 mm screen. To definitively analyze the dry mass content, the ground samples were dried at 105 °C for 8 h (Easley et al. 1965).

To estimate the organic and mineral matter content, the samples were weighed and placed in a muffle furnace for 2 h at 600 °C. Upon reaching room temperature, their mass was measured again. The ethereal extract analysis was carried out in a Soxhlet extractor, using petroleum ether as a solvent (AOAC 1995).

The total protein content was calculated from the nitrogen content determined using the modified Kjeldahl method (AOAC 1995). The modifications included using a 4% (w/v) boric acid solution as the receiver for free ammonia during distillation, a 0.2% (w/v) bromocresol green solution with 0.1% methyl red as an indicator, and a standard sulfuric acid solution. The titration solution was used as described by Kozloski et al. (2003). The method described by Van Soest and Robertson (1985) was used to analyze neutral detergent fiber and acid detergent fiber, determined according to Licitra et al. (1996).

In vitro digestibility

Rumen fluid collection

Ruminal fluid was collected from two cannulated Texel × Corriedale sheep, approximately three years old. In each round, 1.2 L of ruminal fluid was obtained through the ruminal fistula, filtered to remove solid particles, and maintained under continuous CO₂ flushing to preserve anaerobic conditions.

Preparation of fermentation vessels

The in vitro incubator at the Nupeec Nutrition Laboratory consisted of four fermentation vessels (2 L capacity each). Three vessels were used for the treatments (0, 200, and 300 mM NaCl), each containing six bags with 2.5 g of sample (15 g total per vessel). One additional vessel containing only ruminal fluid and buffer solution was used as a control.

Two buffer solutions and one reducing solution were prepared to maintain the pH between 6.7 and 6.9 (Martins et al. 2019). The incubation medium consisted of 80% buffer solution and 20% ruminal fluid. Each gram of sample received 100 mL of buffered ruminal fluid; therefore, each vessel received 300 mL of ruminal fluid and 1.2 L of buffer solution (Martins et al. 2019). All vessels were continuously supplied with CO₂.

The incubator was maintained at 39.0±0.5 °C with constant rotation at 2 rpm. Before incubation, all solutions and ruminal fluid were preheated to 39 °C to avoid thermal shock to the ruminal microbiota.

Two bags from each treatment were removed after 6, 24 and 48 h of incubation for dry matter digestibility analysis. The bags were dried at 105 °C and weighed, and digestibility was calculated from the difference between initial and final dry weights (Martins et al. 2019). At each sampling time, pH and temperature were recorded, and ruminal fluid samples were microscopically evaluated to verify microbiota viability (Zilio et al. 2008).

Mineral composition of *A. littoralis* P. Beauv

Mineral composition analysis was performed at 3rlab Analytical Laboratory (Chapecó, SC, Brazil) using inductively coupled plasma optical emission spectrometry (ICP-OES).

Samples were subjected to acid digestion using nitric acid (HNO₃) and hydrogen peroxide (H₂O₂), and the resulting extracts were diluted to appropriate volumes prior to analysis. The solutions were then introduced into the ICP-OES system for determination of macro and micronutrient concentrations, with element quantification based on calibration curves prepared from certified standard solutions.

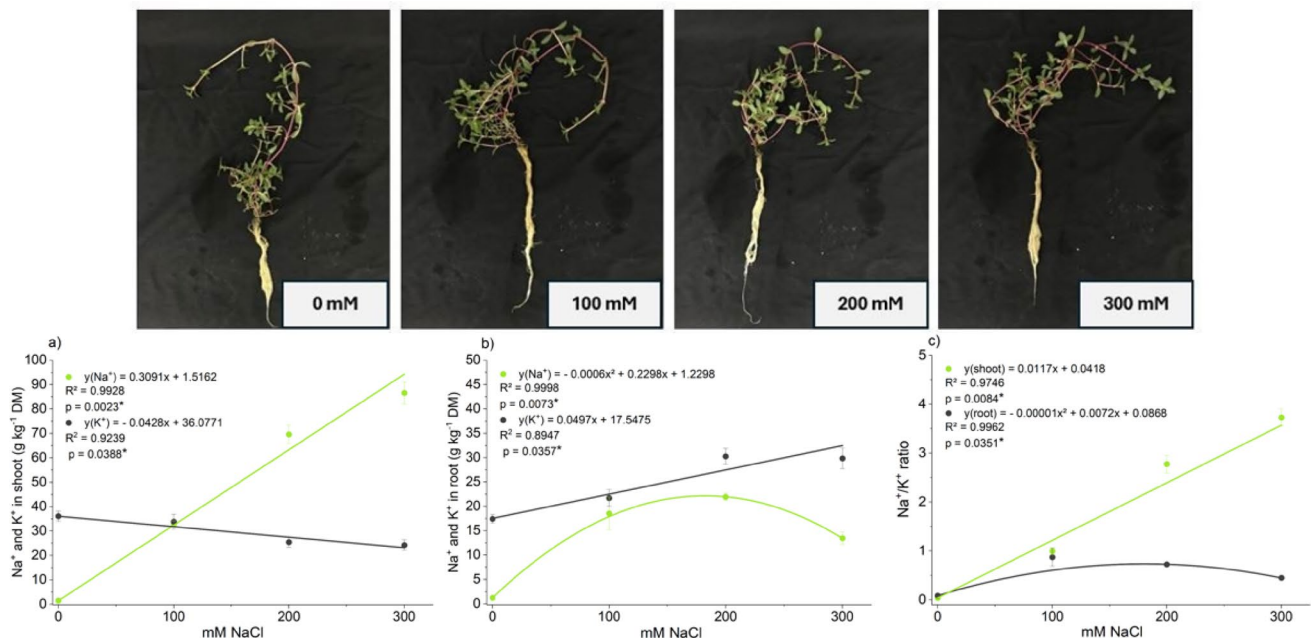


Fig. 2 Sodium and potassium content in shoot (a), root (b), and sodium and potassium ratios in shoot and root (c), in *A. littoralis* P. Beauv. plants, exposed to 0, 100, 200, and 300 mM NaCl for 21 days under a hydroponic cultivation system. Green lines represent Na⁺ content, while gray lines indicate K⁺ content. Data are presented as

means $\pm$ standard error (SE), based on four biological replicates ($n=4$). Regression equations, coefficients of determination (R^2), and p-values are shown in the respective panels. Asterisks (*) indicate statistically significant regression models ($p \leq 0.05$), whereas models without an asterisk were not significant ($p > 0.05$)

Fig. 3 Stomatal conductance (gs) (a), transpiration rate (E) (b), effective quantum yield of PSII (Φ PSII) (c), and electron transport rate (ETR) (d) in *A. littoralis* P. Beauv. plants exposed to 0, 100, 200, and 300 mM NaCl for 21 days under a hydroponic cultivation system. Data are presented as means $\pm$ standard error (SE), based on four biological replicates ($n=4$). Regression equations, coefficients of determination (R^2), and p-values are shown in the respective panels. Asterisks (*) indicate statistically significant regression models ($p \leq 0.05$), whereas models without an asterisk were not significant ($p > 0.05$)

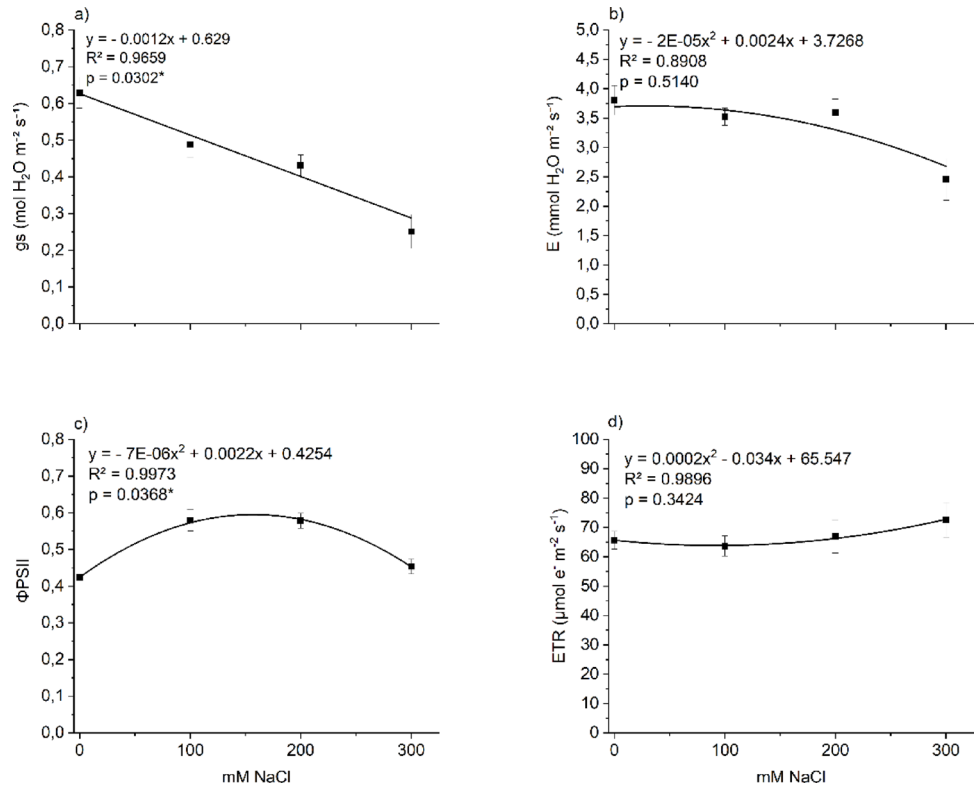
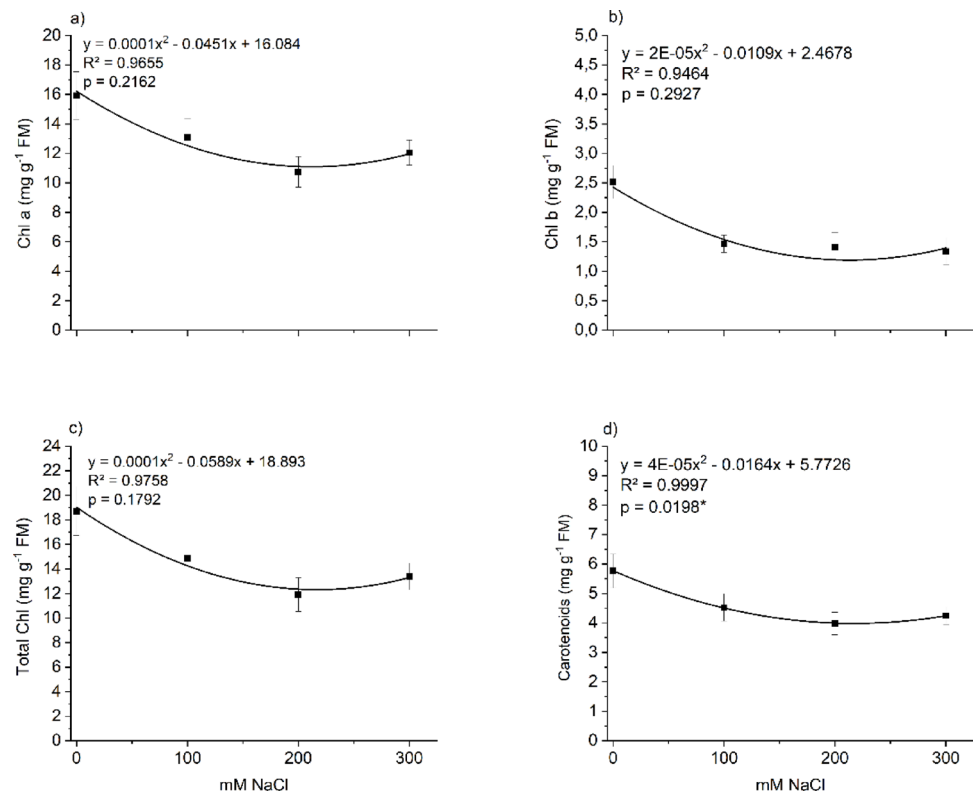


Fig. 4 Concentration of photosynthetic pigments of chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), and carotenoids (d) in *A. littoralis* P. Beauv. plants exposed to 0, 100, 200, and 300 mM NaCl for 21 days under a hydroponic cultivation system. Data are presented as means $\pm$ standard error (SE), based on four biological replicates ($n=4$). Regression equations, coefficients of determination (R^2), and p-values are shown in the respective panels. Asterisks (*) indicate statistically significant regression models ($p \leq 0.05$), whereas models without an asterisk were not significant ($p > 0.05$)



Quality control procedures included the use of reagent blanks and reference materials to ensure analytical accuracy and minimize contamination. All analyses followed the methodologies described in the *Compêndio Brasileiro de Alimentação Animal* (2017).

Dry matter content was determined gravimetrically by oven-drying ground samples at 103 ± 2 °C until constant weight. Approximately 2 g of sample were weighed into pre-dried containers, dried in a ventilated oven, and subsequently cooled in a desiccator before final weighing. Dry matter content was calculated from the difference between fresh and dry weights. The analysis was performed according to the methodologies outlined in the *Compêndio Brasileiro de Alimentação Animal* (2017) and the guidelines provided in the *Animal Production and Health Manual* by the FAO (2011).

Statistical analysis

All variables were tested for normality using the Shapiro-Wilk test. Subsequently, an analysis of variance (ANOVA) was performed, adopting a significance level of $p \leq 0.05$. For bromatological variables, when significant differences were observed, mean comparisons were conducted using Tukey's test at a 5% probability level, and the results were presented in tables. For physiological and biochemical variables, regression analyses were performed considering NaCl concentration as a quantitative factor. Regression

models were considered significant when $p \leq 0.05$. A summary of all regression models, coefficients of determination (R^2), p-values, and significance levels is provided in Table S1 (Supplementary Material).

A principal component analysis (PCA) was performed using log-transformed mean values of physiological, biochemical, and ionic variables measured in leaves and roots, including gas exchange, PSII efficiency, photosynthetic pigments, oxidative stress markers, antioxidative enzyme activities, and Na⁺ and K⁺ concentrations, to identify patterns of association among treatments. PCA results were represented by a biplot based on the first and second principal components. All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA).

Results

Sodium and potassium content

Sodium content in the shoots of *A. littoralis* increased linearly with salinity, reaching $84.51 \text{ g kg}^{-1} \text{ DM}$ at 300 mM NaCl (Fig. 2a), compared to $1.51 \text{ g kg}^{-1} \text{ DM}$ in control plants (0 mM NaCl). In roots, Na⁺ content peaked at 200 mM NaCl ($21.94 \text{ g kg}^{-1} \text{ DM}$; Fig. 2b) and decreased at the highest salinity level.

In contrast, potassium (K⁺) content in shoots decreased progressively with increasing salinity, with the lowest

Fig. 5 Concentration of malondialdehyde (MDA) in leaves (a) and roots (b), and hydrogen peroxide (H_2O_2) in leaves (c) and roots (d) in *A. littoralis* P. Beauv. plants exposed to 0, 100, 200, and 300 mM NaCl for 21 days under a hydroponic cultivation system. Data are presented as means $\pm$ standard error (SE), based on four biological replicates ($n=4$). Regression equations, coefficients of determination (R^2), and p-values are shown in the respective panels. Asterisks (*) indicate statistically significant regression models ($p \leq 0.05$), whereas models without an asterisk were not significant ($p > 0.05$)

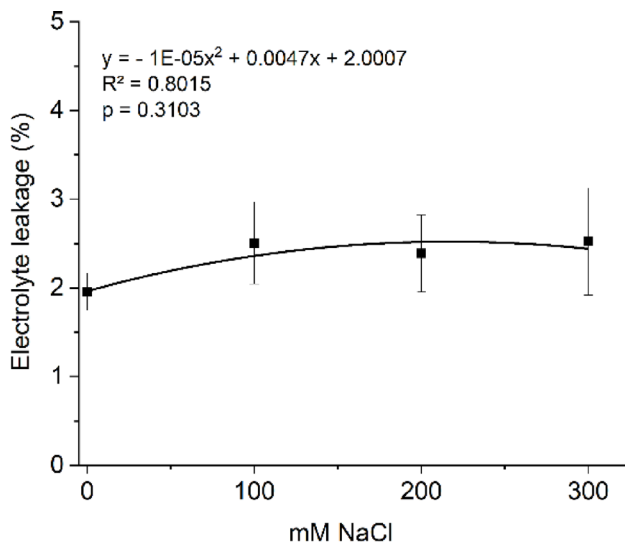
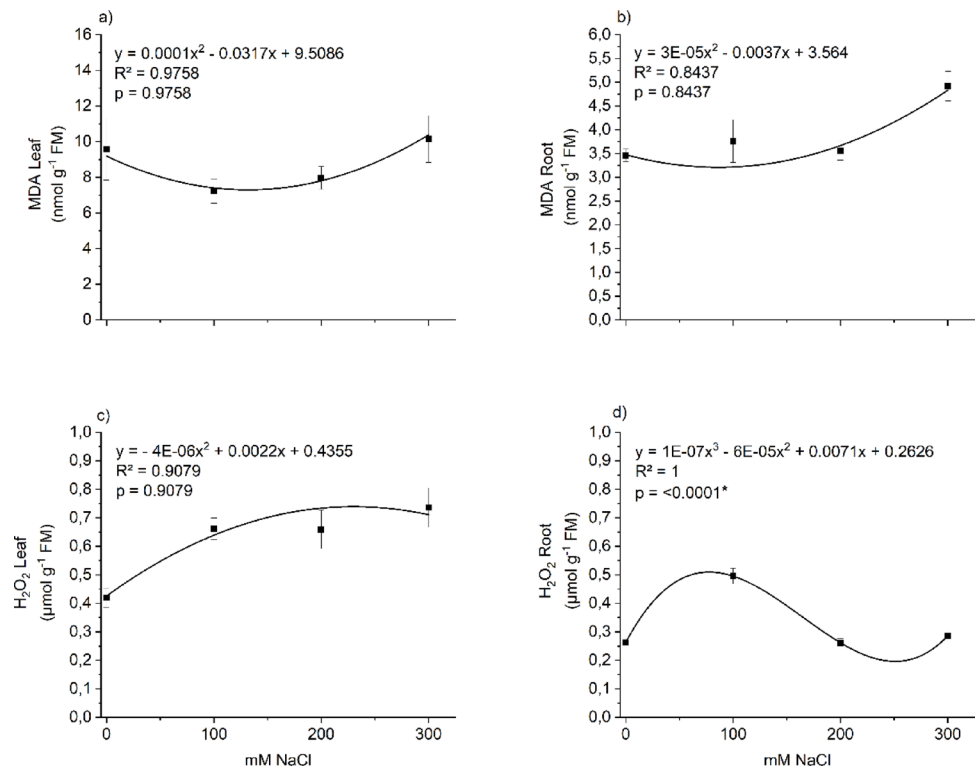


Fig. 6 Electrolyte leakage in *A. littoralis* P. Beauv. plants exposed to 0, 100, 200, and 300 mM NaCl for 21 days under a hydroponic cultivation system. Data are presented as means $\pm$ standard error (SE) based on four biological replicates ($n=4$). Regression equations, coefficients of determination (R^2), and p-values are shown in the panel. Asterisks (*) indicate statistically significant regression models ($p \leq 0.05$), whereas models without an asterisk were not significant ($p > 0.05$)

values recorded at 300 mM NaCl (Fig. 2a). Root K^+ content, however, was higher at 200 and 300 mM NaCl than in control plants (Fig. 2b). Consequently, the Na^+/K^+ ratio

differed markedly between tissues. In shoots, Na^+ exceeded those of K^+ at 200 and 300 mM NaCl, resulting in ratios above 1. In roots, the Na^+/K^+ ratio remained comparatively lower across treatments.

Gas exchange and PSII efficiency

Stomatal conductance (g_s) declined significantly with increasing salinity (Fig. 3a), from approximately $0.63 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ under control conditions to values $\sim 60\%$ lower at 300 mM NaCl. By contrast, transpiration rate (E) (Fig. 3b) and electron transport rate (ETR) (Fig. 3d) did not show significant differences among treatments. The effective quantum yield of PSII (ΦPSII) (Fig. 3c) showed a significant quadratic response to increasing NaCl concentrations, with maximum values observed under intermediate salinity levels.

Photosynthetic pigments

Chlorophyll a (Chl a) (Fig. 4a), chlorophyll b (Chl b) (Fig. 4b), and total chlorophyll (Fig. 4c) did not differ significantly among salinity treatments. In contrast, carotenoid concentration (Fig. 4d) declined significantly, with the lowest values observed at 300 mM NaCl, approximately 27% lower than the control.

Fig. 7 Activity of the enzyme superoxide dismutase (SOD) in leaves (a) and roots (b), catalase (CAT) in leaves (c) and roots (d), and ascorbate peroxidase (APX) in leaves (e) and roots (f) in *A. littoralis* P. Beauv. plants exposed to 0, 100, 200 and 300 mM NaCl for 21 days under a hydroponic cultivation system. Data are presented as means $\pm$ standard error (SE), based on four biological replicates ($n=4$). Regression equations, coefficients of determination (R^2), and p-values are shown in the respective panels. Asterisks (*) indicate statistically significant regression models ($p \leq 0.05$), whereas models without an asterisk were not significant ($p > 0.05$)

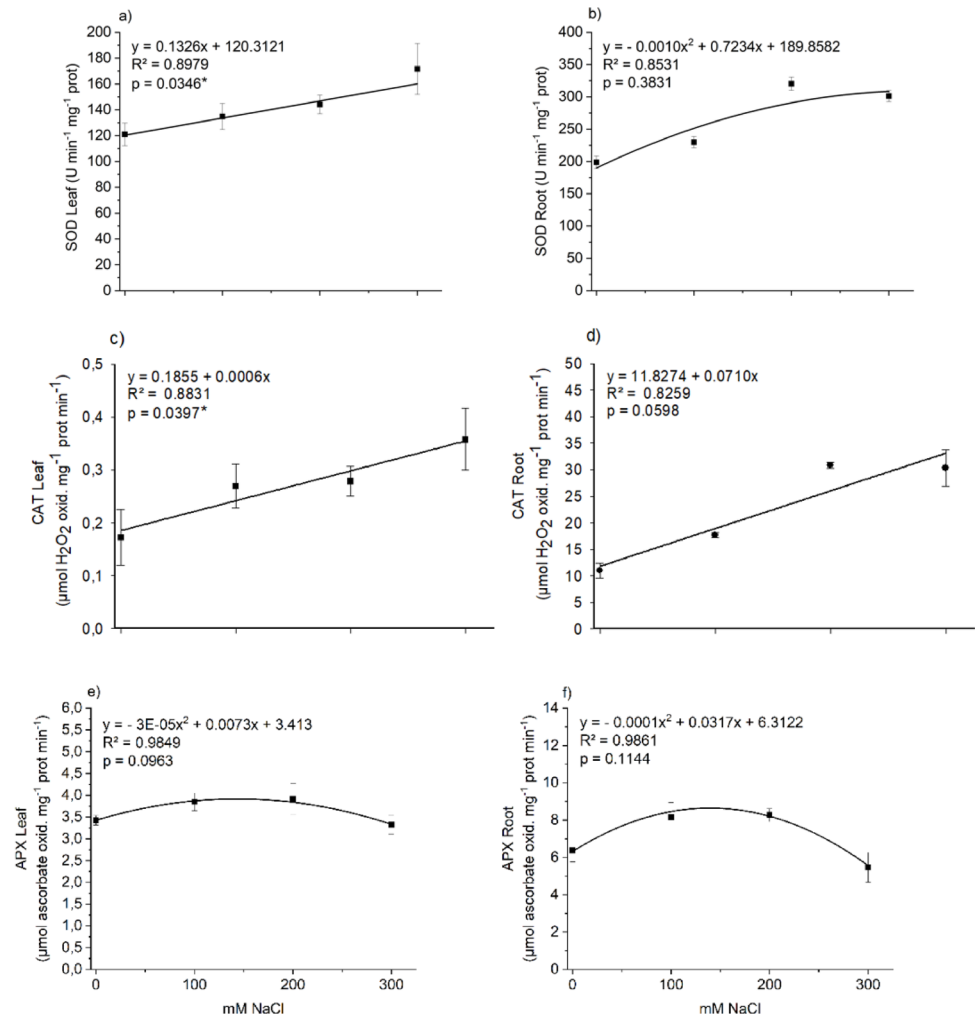


Fig. 8 Principal component analysis (PCA) of the variables analyzed with *A. littoralis* P. Beauv. plants exposed to 0, 100, 200, and 300 mM NaCl for 21 days under a hydroponic cultivation system

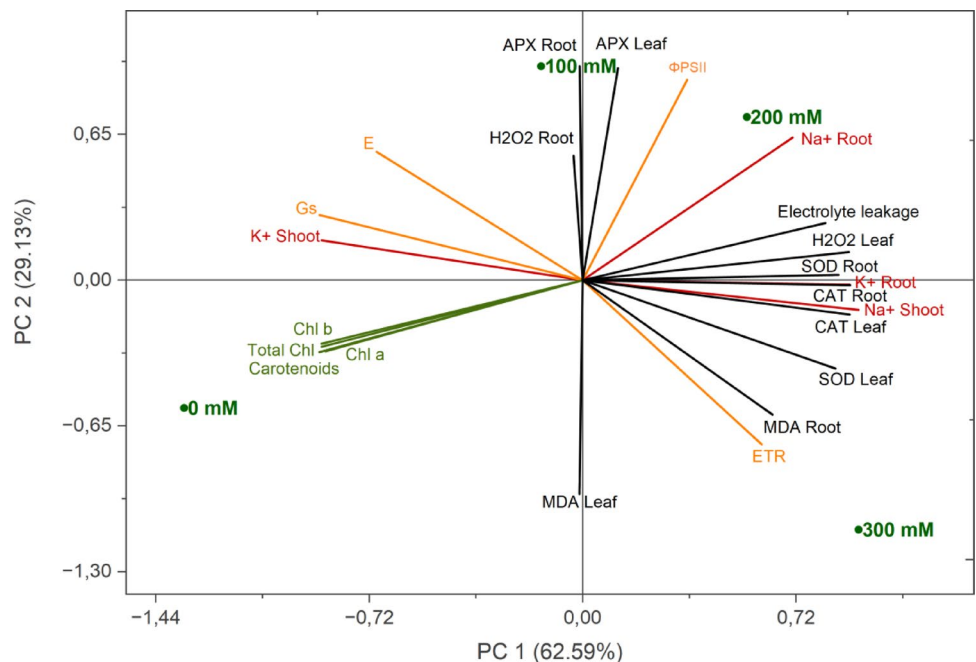


Table 1 Variables contribution to PCA in *A. littoralis* P. Beauv. plants exposed to 0, 100, 200, and 300 mM NaCl for 21 days under a hydroponic cultivation system

PC1	Variable	PC2	Variable
0.260	Na ⁺ Shoot	0.382	APX Root
0.252	CAT Root	0.379	APX Leaf
0.251	K ⁺ Root	0.358	ΦPSII
0.251	CAT Leaf	0.255	Na ⁺ Root
0.250	H ₂ O ₂ Leaf	0.229	E

Oxidative stress indicators

Among the oxidative stress indicators evaluated, only H₂O₂ content in roots showed significant differences across salinity treatments (Fig. 5). In contrast, malondialdehyde (MDA) levels in both shoot and root, as well as electrolyte leakage in shoot, remained statistically unchanged under saline conditions (Figs. 5 and 6).

Enzymatic antioxidant system

The antioxidant responses of *A. littoralis* to salinity were more pronounced in shoot tissues than in roots. Leaf SOD activity showed a significant positive linear response to increasing NaCl concentrations, with maximum activity observed at 300 mM NaCl (Fig. 7a). A similar trend was observed for CAT activity in shoots, indicating enhanced antioxidant capacity under elevated salinity (Fig. 7c). In contrast, APX activity in shoots and the activities of SOD, CAT, and APX in roots did not show significant differences among treatments.

Principal component analysis (PCA)

Figure 8 shows the principal component analysis (PCA) integrating physiological, biochemical, photosynthetic, and ionic variables of *Alternanthera littoralis* under different NaCl concentrations. The first two principal components explained 91.72% of the total data variability, with PC1 accounting for 62.59% and PC2 for 29.13%, indicating clear separation among salinity treatments. The variables that contributed most to this separation are detailed in Table 1.

PC1 clearly separated control plants (0 mM NaCl) from salt-treated plants. The control treatment was positioned

Table 3 In vitro digestibility of *A. littoralis* P. Beauv. plants exposed to 0, 200, and 300 mM NaCl and digestion times (6, 24, and 48 h)

Treatment (mM NaCl)	6 h	24 h	48 h
0 mM	32.37	54.31	52.74
200 mM	42.75	69.95	67.87
300 mM	53.35	70.31	79.65

The data represents the percentage of maintenance, the percentage of digested material, and digestibility in dry matter (DM). Values correspond to two biological samples per treatment/hour ($n=2$) and are presented for descriptive purposes only. Due to the lack of replication, no statistical analysis was carried out

Table 4 Mineral composition and moisture content of *A. littoralis* P. Beauv. plants exposed to 0, 100, 200, and 300 mM NaCl for 21 days under a hydroponic cultivation system

Mineral	0 mM NaCl	200 mM NaCl	300 mM NaCl
Calcium (%DM)	1.43	0.75	0.97
Phosphorus (%DM)	0.73	0.77	0.90
Magnesium (%DM)	1.06	0.82	0.97
K ⁺ (%DM)	6.67	5.51	5.74
Na ⁺ (%DM)	0.52	2.33	2.72
Sulfur (%DM)	0.82	0.69	0.78
Chloride (%DM)	0.96	2.76	3.97
Aluminum (ppm)	88.04	74.95	62.33
Boron (ppm)	61.77	46.09	49.23
Copper (ppm)	5.16	6.22	6.08
Iron (ppm)	96.15	75.48	62.19
Manganese (ppm)	25.60	47.75	41.38
Zinc (ppm)	48.90	72.96	74.40
Humidity (%)	88.69	86.21	85.09
Dry matter (%)	11.31	13.79	14.91

The values represent the percentage of dry matter (% DM) for macronutrients and parts per million of dry matter (ppm) for micronutrients. The parameters evaluated include calcium, phosphorus, magnesium, potassium, sodium, sulfur, chloride, aluminum, boron, copper, iron, manganese, and zinc, in addition to moisture (%) and dry matter (%). Values correspond to a single biological sample per treatment ($n=1$) and are provided for descriptive purposes only. Due to the absence of replication, statistical analysis was not performed

on the negative side of PC1 and was strongly associated with photosynthetic pigment-related variables, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids. In contrast, saline treatments were distributed on the positive side of PC1, indicating distinct clustering patterns under salinity. Treatments at 100 and 200 mM NaCl were positioned closer to each other, suggesting similar response

Table 2 Chemical Composition of *A. littoralis* P. Beauv. plants exposed to 0, 100, 200, and 300 mM NaCl for 21 days under a hydroponic cultivation system

Treatment (mM NaCl)	Dry matter (%)	protein content (%)	Ethereal extract (%)	Neutral detergent fiber (%)	Acid detergent fiber (%)
0 mM	10.06±0.81 ^c	21.35±0.21 ^a	0.99±0.38 ^a	27.84±0.61 ^a	21.51±1.98 ^a
200 mM	12.93±0.46 ^b	19.15±1.34 ^a	1.90±0.52 ^a	26.91±1.07 ^a	17.63±0.47 ^b
300 mM	15.20±0.89 ^a	20.40±0.28 ^a	2.27±0.62 ^a	25.39±1.15 ^a	16.81±0.53 ^b

Values represent the mean±standard deviation. Different letters in the same column indicate significant differences between treatments ($p<0.05$)

patterns under moderate salinity. Among these treatments, the 100 mM treatment was positively associated with APX activity in both leaves and roots, as well as root H₂O₂ content. The 200 mM NaCl treatment was more strongly associated with root Na⁺ accumulation and ΦPSII.

In contrast, the 300 mM treatment is positioned in the lower positive quadrant of the PCA, opposite to the control treatment and spatially separated from the 100 and 200 mM treatments along PC2. This treatment was more strongly associated with electron transport rate (ETR), leaf SOD activity, CAT activity, and MDA accumulation.

Bromatological composition

Dry matter content increased significantly with increasing NaCl concentration, with clear differences observed among treatments. The highest dry matter values were observed at 300 mM NaCl, where dry matter content was approximately 50% higher compared to control plants (Table 2).

In contrast, the protein content, ethereal extract, and neutral detergent fiber did not exhibit significant differences among the salt treatments. On the other hand, acid detergent fiber (ADF) content decreased significantly with increasing salinity, particularly at 200 mM and 300 mM NaCl, showing reductions of 21% and 22%, respectively, compared to the control.

In vitro digestibility test

The in vitro dry matter (DM) digestibility of *A. littoralis* varied according to NaCl concentration and incubation time (Table 3). Although no statistical analysis was performed due to limited replication, the data descriptively suggested a tendency toward higher digestibility under saline conditions and longer incubation periods. Plants exposed to 200 and 300 mM NaCl showed numerically higher digestibility values compared to the control treatment across the evaluated incubation times.

Mineral composition

Mineral composition data obtained from descriptive analyses ($n=1$) indicated differences in nutrient concentrations under saline conditions. Higher values of phosphorus (+32%), copper (+17%), manganese (+61%), zinc (+52%), Na⁺ (+423%), and chloride (+313%) were observed under elevated salinity levels (Table 4). In contrast, lower values of calcium (-32%), magnesium (-8%), sulfur (-4%), K⁺ (-13%), aluminum (-29%), boron (-20%), and iron (-35%) were observed under saline conditions.

Discussion

Physiological and biochemical mechanisms underlying salt tolerance

Soil salinization is a major challenge for sustainable agriculture, requiring strategies that combine stress tolerance and resource-use efficiency (Gupta et al. 2019). In this context, *Alternanthera littoralis* emerges as a highly salt-tolerant halophyte capable of maintaining physiological functionality under moderate to high salinity.

At 300 mM NaCl, Na⁺ concentration in shoots reached 84.51 g kg⁻¹ DM, while root levels peaked at 21.94 g kg⁻¹ DM at 200 mM, resulting in a high Na⁺/K⁺ ratio (>1) and indicating preferential Na⁺ accumulation in aerial tissues. This suggests efficient ionic compartmentalization via vacuolar and apoplastic sequestration, a mechanism also reported in *Atriplex nummularia* and *Salicornia europaea* (Wu et al. 2019; Mazhari et al. 2022; Akter et al. 2023). From a phytoremediation perspective, this strong Na⁺ translocation may contribute to soil desalination by transferring ions to harvestable biomass (Qadir et al. 2007), which could subsequently be removed or processed for other uses depending on its composition (Manousaki and Kalogerakis 2011).

The concurrent increase in root K⁺ under high NaCl, despite reduced K⁺ levels in shoots, suggests preferential K⁺ retention in root tissues as a mechanism to preserve ionic homeostasis under salinity. Maintaining K⁺ in roots is essential for enzymatic activity, osmotic regulation, membrane stability, and stress signaling, particularly under conditions of elevated Na⁺ accumulation. This response is likely mediated by transport systems such as HAK, HKT1, and Na⁺/H⁺ antiporters (SOS1 and NHX1), which play central roles in ion selectivity and compartmentalization in halophytes (Zhu 2003; Munns and Tester 2008).

The marked reduction in stomatal conductance under saline conditions suggests partial stomatal closure as a mechanism to regulate water loss under salt stress. However, transpiration rates remained relatively stable, without statistical difference, indicating that water fluxes were not severely restricted, possibly due to osmotic adjustment promoted by Na⁺ accumulation in shoot tissues. According to Monteith (1995), partial stomatal closure may occur in response to increased transpirational demand without necessarily causing proportional reductions in transpiration. Although VPD was not directly measured in the present study, the maintenance of transpiration despite reduced stomatal conductance may suggest a response similar to the feedback mechanism described by Monteith (1995) and Matzner and Comstock (2001).

Unlike previous studies showing that salinity impairs PSII efficiency, promotes photoinhibition, and alters

pigment composition in non-halophytic species (Bongi and Loreto 1989; Mishra et al. 1991; Masojídek and Hall 1992; Belkhdja et al. 1994; Stefanov et al. 2023), several halophytic species such as *Suaeda salsa* have demonstrated stable photosynthetic performance and PSII functionality under high salinity levels (ranging from 0 to 400 mM NaCl) (Lu et al. 2003). In agreement with these findings, *A. littoralis* exhibited high photochemical stability under saline conditions. Even under elevated NaCl concentrations (200–300 mM), the species maintained Φ PSII and ETR values close to or higher than those observed in control plants. In addition, chlorophyll a, chlorophyll b, and total chlorophyll contents were not significantly affected by salinity, suggesting maintenance of the structural integrity of the photosynthetic apparatus. Similar responses have also been reported in halophytic species such as *Artemisia anethifolia* and *Beta vulgaris* (Lu et al. 2003; Jamil et al. 2007). Although carotenoid levels decreased under saline conditions, this reduction was not associated with photochemical impairment as evidenced by the maintenance of Φ PSII, ETR, and chlorophyll contents or increased oxidative damage. These findings suggest that the remaining carotenoid pool, together with other protective mechanisms, was sufficient to sustain photochemical stability under salt stress. The absence of severe toxic effects on PSII photochemistry despite elevated Na^+ and Cl^- accumulation may be associated with efficient ionic compartmentalization through vacuolar sequestration of salt ions (Krause and Weis 1991; Peng et al. 2016). This mechanism likely contributed to the maintenance of photosynthetic performance and physiological stability of *A. littoralis* under saline conditions.

Salinity is commonly associated with excessive production of reactive oxygen species (ROS) (Mittler 2002). However, in *A. littoralis*, most oxidative stress indicators remained relatively stable under saline conditions. Although H_2O_2 content in roots showed significant variation in response to salinity, no marked increases in MDA or electrolyte leakage were observed, suggesting the absence of severe oxidative damage. The preservation of membrane integrity and PSII functionality under elevated NaCl concentrations suggests the maintenance of effective protective mechanisms against oxidative stress. In this context, the progressive increase in superoxide dismutase (SOD) activity in shoots, particularly at 300 mM NaCl, together with enhanced CAT activity, likely contributed to oxidative stability under saline conditions (Bose et al. 2014). These responses may have contributed to the maintenance of membrane integrity, photosynthetic performance, and cellular stability in *A. littoralis* under elevated NaCl concentrations.

In contrast, APX activity in shoots and the activities of SOD, CAT, and APX in roots did not show significant

changes under salinity, suggesting that baseline antioxidant activity in these tissues was sufficient to maintain cellular homeostasis. Similar antioxidant responses have been reported in halophytic species such as *Suaeda salsa* and *Atriplex nummularia* (Gupta et al. 2019; Wu et al. 2019; Ullah et al. 2021). The PCA analysis reinforced the integrated nature of the physiological responses of *A. littoralis* to salinity. Control plants were clearly separated from salt-treated plants, while plants exposed to 300 mM NaCl were associated with Na^+ accumulation, increased SOD activity, and maintenance of ETR. Overall, the PCA supports the high physiological plasticity and salt tolerance of *A. littoralis*, highlighting its potential for application in saline agriculture and phytoremediation approaches (Ali et al. 2013; Sarwar et al. 2017).

Nutritional value and forage potential of *A. littoralis*

A. littoralis combined marked salt tolerance with a favorable bromatological profile under saline conditions, maintaining elevated crude protein (CP), ethereal extract (EE), and neutral detergent fiber (NDF) contents. While halophytic forages generally contain between 8 and 20% CP (Benjamin et al. 1992; El Shaer 2010), *A. littoralis* reached values close to 21%, reinforcing its potential as a nutritional resource for saline environments. In this context, future determination of the non-protein nitrogen (NPN) fraction may contribute to a more accurate evaluation of its nutritional quality and protein utilization efficiency in ruminant diets.

An important response observed under high salinity was the reduction in acid detergent fiber (ADF) at 300 mM NaCl, accompanied by increased in vitro dry matter digestibility. Since ADF is negatively correlated with forage digestibility (Goering and Van Soest 1970; Jung et al. 1997; Moore and Jung 2001), this reduction may indicate salinity-induced modifications in cell wall composition or lignification patterns that favored biomass digestibility. Similar responses have been reported in other halophytic species cultivated under saline conditions (Ameixa et al. 2016).

The elevated Na^+ accumulation observed under severe salinity deserves careful consideration regarding the use of *A. littoralis* in animal feeding systems. The maximum Na^+ concentration detected in the biomass reached 84.51 g kg^{-1} dry matter (DM), while plant DM content was approximately 14.9%, consistent with values commonly reported for halophytes cultivated under saline conditions. Considering a voluntary dry matter intake (DMI) equivalent to 2.3% of body weight (BW) for adult grazing cattle (Galyean et al. 2016), exclusive consumption of the plant under this extreme scenario could result in Na^+ intake levels substantially exceeding the nutritional requirements recommended

for beef and dairy cattle (NASEM 2021). For example, estimated Na^+ intake could reach approximately 924 g day^{-1} for non-lactating beef cows (475 kg BW) and $1,215 \text{ g day}^{-1}$ for high-producing dairy cows (625 kg BW) if *A. littoralis* were provided as the sole forage source.

This issue becomes particularly relevant considering that Na^+ represents approximately 39.34% of the molecular weight of NaCl, meaning that the Na^+ concentration observed in the present study corresponds to nearly 214.8 g kg^{-1} NaCl equivalent. Under the simulated intake conditions, estimated NaCl consumption would exceed the threshold associated with acute salt toxicity in cattle, reported at approximately $2.2 \text{ g NaCl kg}^{-1}$ BW (Thompson 2024). Excessive salt intake, particularly when associated with restricted water availability or poor-quality drinking water, may result in severe clinical manifestations, including gastrointestinal disorders, neurological impairment, muscular tremors, convulsions, and, in extreme cases, death. Moreover, although NASEM (2021) does not establish a maximum tolerable limit specifically for Na^+ , recommended dietary concentrations for cattle range from 0.06 to 0.10% of DM, values substantially lower than those estimated under the exclusive consumption scenario evaluated here.

Nevertheless, it is important to emphasize that the highest Na^+ concentrations reported in this study were obtained under controlled experimental conditions with severe salinity (300 mM NaCl), which likely exceed salinity levels commonly found under practical agricultural conditions. In field environments, lower soil salinity would probably reduce Na^+ accumulation in plant tissues, meaning that the present estimates should be interpreted primarily as a worst-case scenario for risk assessment rather than as representative of conventional forage production systems.

Therefore, although the exclusive use of *A. littoralis* as forage under extreme salinity conditions may present risks associated with excessive Na^+ intake, its use as a complementary forage resource or dietary supplement may still be feasible when properly managed. In this context, bromatological and mineral characterization become essential tools for establishing safe inclusion rates in animal diets, allowing the productive and ecological potential of the species to be explored without compromising feed intake, water balance, or animal health. This graphical summary is based on a heatmap constructed from key physiological, biochemical, and nutritional data. Variations in responses can be observed through the color gradient presented. The values represent the meaning of each treatment relative to the control, with red colors indicating responses above the control and blue colors indicating responses below the control. Physiological and biochemical values were log₂-transformed for better visualization of the differences.

Source: Created by the author in BioRender.com.

Conclusion

Alternanthera littoralis exhibited high tolerance to saline conditions through coordinated physiological and biochemical adjustments that enabled the maintenance of photosynthetic functionality and oxidative stability under elevated NaCl concentrations. The species showed pronounced Na^+ accumulation, particularly in aerial tissues, without severe impairment of photochemical performance or cellular integrity, highlighting its ability to maintain physiological stability under salt stress. In addition, the preservation of important nutritional traits and the descriptively higher in vitro digestibility observed under salinity suggest that *A. littoralis* may represent a promising halophytic resource for saline agriculture. Together, these findings highlight the ecological and agronomic relevance of the species and support its potential use in phytoremediation approaches and as an alternative forage resource for salt-affected environments.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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