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## **Biostimulant use in pangolão grass *Digitaria pentzii* subjected to saline stress<sup>1</sup>**

### **Utilização de bioestimulante no capim-pangolão *Digitaria pentzii* submetido a estresse salino**

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#### **HIGHLIGHTS:**

*Pangolão grass is moderately salinity tolerant.*

*Biostimulant did not favor growth and production of pangolão grass in any salinity.*

*Accumulation of sodium in leaves reduced phytomass production of pangolão grass.*

**ABSTRACT:** Salinity caused by excess salts in soil solutions is one of the most limiting environmental stresses in agriculture worldwide. In this scenario, among strategies that favor the expression of the genetic potential of plants, the use of biostimulants stands out. The objective of this study was to evaluate the influence of a seaweed-based biostimulant on growth, forage production, gas exchange, and accumulation of sodium and potassium ions in pangolão grass (*Digitaria pentzii*) under saline stress. The experiment was conducted from March to July 2019 using a randomized block design, in a 2 × 3 factorial scheme with two concentrations of biostimulant (0 and 8 mL L<sup>-1</sup>) and three electrical conductivities of irrigation water (0.03, 2 and 4 dS m<sup>-1</sup>), with four replicates. The accumulation of 50 μmol g<sup>-1</sup> of sodium in leaves corresponded to a reduction of 0.3 g of dry matter in the leaf blade production per plot. The biostimulant did not influence the structural characteristics, phytomass accumulation, or stomatal conductance of the pangolão grass, regardless of salinity. At the level of 4 dS m<sup>-1</sup> in irrigation water, the ionic stress toxicity due to the accumulation of salts in the aerial part of pangolão grass was more severe. This is the first evidence of the “moderate” salinity tolerance of pangolão grass in semi-arid regions.

**Key words:** abiotic stress, phytomass accumulation, salinity, seaweed extract, sodium content

**RESUMO:** A salinidade causada pelo excesso de sais na solução do solo é um dos estresses ambientais mais limitantes na agricultura no mundo. Nesse cenário, dentre as estratégias para favorecer a expressão do potencial genético das plantas, destaca-se o uso de bioestimulantes. Objetivou-se avaliar a influência de um bioestimulante à base de extrato de algas marinhas no crescimento, produção de forragem, trocas gasosas e acúmulo de íons sódio e potássio em capim-pangolão *Digitaria pentzii*, submetido a condições de estresse salino. O experimento foi conduzido de março a julho de 2019, em delineamento de blocos casualizados, em esquema fatorial 2 × 3, com duas concentrações do bioestimulante (0 e 8 mL L<sup>-1</sup>) e três condutividades elétricas da água de irrigação (0,03; 2 e 4 dS m<sup>-1</sup>), com quatro repetições. O acúmulo de 50 μmol g<sup>-1</sup> de sódio nas folhas correspondeu a uma redução de 0,3 g de matéria seca na produção de lâminas foliares por vaso. O bioestimulante não influenciou as características estruturais do capim-pangolão, acúmulo de fitomassa e condutância estomática, independentemente da salinidade. No nível de 4 dS m<sup>-1</sup> na água de irrigação, a toxicidade do estresse iônico devido ao acúmulo de sais na parte aérea do capim-pangolão é mais severa. Esta é a primeira evidência da tolerância “moderada” à salinidade do capim-pangolão em regiões semiáridas.

**Palavras-chave:** estresse abiótico, acúmulo de fitomassa, salinidade, extrato de algas marinhas, teor de sódio

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## INTRODUCTION

Currently, there are more than 950 million hectares subjected to the process of soil salinization worldwide, mainly in arid and semi-arid regions (Zörb et al., 2019). The salinization process is associated with the predominance of evaporation over rainfall and soil formation factors, which in turn directly impact water sources intended for irrigation (Hassan et al., 2021).

Excess salts cause a reduction in plant growth (Saidimoradi et al., 2019), affecting metabolism through osmotic stress and ionic toxicity (Kaya et al., 2020). However, the concentration of salts that determines this reduction varies with plant species, and this fact is related to the tolerance of each species to salinity (Kataria & Verma, 2018).

Pangolão grass (*Digitaria pentzii*) has been used in animal feed in the Brazilian semi-arid region (Bezerra et al., 2020) because of its adaptation to the edaphoclimatic conditions of the region, high qualitative potential of forage production (Coelho et al., 2021; Menor et al., 2022), and high acceptability. However, there are no studies on the tolerance of pangolão grass to saline stress, which is one of the main challenges in its management under low quality irrigation water.

Several studies (Dourado Neto et al., 2014) have explored the use of biostimulants as a strategy to express the genetic potential of plants. These compounds can favor the growth and production of forage in the presence of salts in irrigation water (Simões et al., 2022), providing vigor to the immune system and reactivating physiological processes in the different life phases of the plant (Deinlein et al., 2014).

There are still few studies on strategies capable of favoring the productive potential of pangolão grass, such as the use of biostimulants in the absence of salts or in increasing levels of salinity. In view of the above, the objective was to evaluate the influence of the biostimulants on growth, forage production, gas exchange, and accumulation of sodium and potassium ions in the leaves and roots of pangolão grass subjected to saline stress conditions.

## MATERIAL AND METHODS

The study was conducted from March to July 2019 using pots kept under field conditions at the Universidade Federal Rural de Pernambuco (UFRPE) in the municipality of Serra Talhada, PE, Brazil (07° 57' 01" S, 38° 17' 53" W, at an altitude of 429 m).

According to Koppen, the climate regime of the region is BSw, with the rainy season occurring during the summer, beginning in November and ending in April. The long-term average annual rainfall is 632.2 mm, with an annual long-term average air temperature of 26 °C and a relative air humidity of 60% (Dubreuil et al., 2018; Leite et al., 2021). Meteorological data were collected during the experiment, with data available on the website of the National Institute of Meteorology (INMET) (<http://www.inmet.gov.br>, post A350, latitude -7.95°, longitude -38.30°, and altitude 499 m). During the experimental period, there was an accumulation of 87 mm of

rain concentrated in the first 20 days after a standardization cut. The average air temperature fluctuated between 25 and 30 °C.

Soil was collected from the 0-20 cm layer of an inceptisol in the experimental area, then homogenized and passed through a sieve with a 2.0 mm mesh, and subsequently packed in plastic pots of dimensions 24 and 17 cm (largest and smallest internal diameter, respectively) and 23 cm (height), with perforations in the bottom and with a layer of 2.0 cm of coarse gravel and 2.0 cm of fine gravel to facilitate drainage of irrigation water. Approximately 7 kg of soil was added to each pot.

A soil sample was analyzed by the Laboratory of Soil Fertility of the Instituto Agrônomo de Pernambuco (IPA), and was characterized by the following chemical attributes: pH (H<sub>2</sub>O) 7.20; Ca<sup>2+</sup> 5.30 cmol dm<sup>-3</sup>; Mg<sup>2+</sup> 1.10 cmol dm<sup>-3</sup>; K<sup>+</sup> 0.45 cmol dm<sup>-3</sup>; CTC 8.14 cmol dm<sup>-3</sup>; base saturation 84.89%; organic matter 1.38% and phosphorus 40 mg dm<sup>-1</sup>.

The experiment was set up in randomized blocks, in a 2 × 3 factorial scheme of two concentrations (0 and 8 mL L<sup>-1</sup>) of a commercial biostimulant (ACADIAN<sup>®</sup>), and three electrical conductivities of irrigation water (0.03, 2, and 4 dS m<sup>-1</sup>), with four replicates; each experimental plot consisted of a pot. According to Ayers & Westcot (1987), the water used in the control treatment (0.03 dS m<sup>-1</sup>) is classified as C1, with no use restrictions; water of electrical conductivity (ECw) of 2 dS m<sup>-1</sup> is classified as C2, moderate salinity and ECw of 4 dS m<sup>-1</sup> is classified as C3, severe salinity.

To obtain the salinity levels, sodium chloride salts (NaCl) corresponding to 1.168 and 2.337 g L<sup>-1</sup> were added to the public water supply (0.03 dS m<sup>-1</sup>), to obtain levels 2 and 4 dS m<sup>-1</sup>, respectively. In the control, water from the public water supply (0.03 dS m<sup>-1</sup>) without the addition of NaCl salts was used.

The 8 mL dosage of ACADIAN<sup>®</sup> biostimulant, a commercial product based on seaweed extract (*Ascophyllum nodosum* (L)), following the manufacturer's recommendations was diluted with the aid of a graduated pipette using water from the public supply, in a beaker corresponding to a volume of 1.0 L. The solution was stirred for one hour, aiming for a uniform distribution when spraying the product on the pangolão grass (*D. pentzii*). No biostimulant was applied to the control.

The spacing adopted was 0.40 m between pots in the same row and between rows. Two pangolão grass seedlings were planted per pot (March 11, 2019). During the first 28 days after planting (DAP), to avoid water stress, the seedlings were kept in conditions close to saturation, aiming at the full establishment of the seedlings.

At 28 DAP (April 8, 2019), the standardization cut was performed at 10 cm from the soil surface of all pangolão grass plants, using pruning shears and a millimeter ruler. At 29 DAP, treatments of biostimulant and saline stress were initiated. A biostimulant (83 mL per pot) was applied by foliar spray every seven days. The cultures received a fixed irrigation depth, considering the maintenance of soil moisture at close to field capacity, with 0.5 L per pot being applied at intervals of one day throughout the experimental period.

At 47 days after harvesting the first cycle (117 DAP), growth analyses were performed: stem length (distance from the plant base to the ligule of the last expanded leaf), stem diameter

(measured at 1.0 cm from the neck), number of live tillers (obtained by counting in a marked clump), number of live leaves (fully expanded and presenting more than 50% of the leaf area without being compromised by senescence), expanded number of leaves (counting of leaves with ligules), and number of expanding leaves (growing leaves with inconspicuous ligules).

Leaf area was determined in a non-destructive way, based on the linear dimensions of length (distance between the ligule and the leaf apex) and width (measured in the middle region of the oldest fully expanded leaf) of the leaf blade, according to Eq. 1 (Bezerra et al., 2020):

$$LA = L \times W^{1.007} \quad (1)$$

where:

- LA - leaf area;
- L - leaf blade length value; and,
- W - leaf blade width.

Measurements were made using a millimeter tape and digital caliper.

In addition to these above variables, stomatal conductance ( $g_s$ ) was measured in the middle part of the third fully expanded leaf of each plant for all treatments at 78 DAP using a Porometer System (from HOJA, SC-1 InSak SAS).

At the end of the second growth cycle (70–117 DAP), the plants were cut close to the ground, separated, fractionated, and their morphological components (leaf blade, stem plus sheath, roots, and dead material) were weighed on a semi-analytical balance. The soil was removed from the pot, crushed, and passed through a sieve (4 mm mesh) to retain the roots, which were then washed in running water.

Soon after, the plant material was placed in an air circulation oven at 65 °C until it reached a constant mass (Detmann et al., 2012). Subsequently, it was removed from the oven and weighed again to measure the dry mass of the leaf blade, stem plus sheath, dead material (all plant material with more than 70% chlorosis was considered dead), root dry mass, and shoot total dry mass.

To determine the percentage of morphological components in dry matter, Eq. 2 was used:

$$PC = \frac{DMC}{TDM} \times 100 \quad (2)$$

where:

- PC - percentage of the component in relation to the plant;
- DMC - dry mass of the component; and,
- TDM - the total dry mass.

Thus, the percentage of leaf blades (PLB), percentage of stem plus sheath (PSS), and percentage of dead material (PDM) were determined.

At the end of the experiment (118 DAP), electrical conductivity analyses of the soil saturation extract were performed according to the soil analysis methods manual of EMBRAPA (Teixeira et al., 2017). Soil samples (150 g) were collected, passed through a 2 mm sieve, and distilled water

was added until they had a shiny or mirror-like appearance. After 12 hours, the soil solution was extracted using a vacuum pump, and the electrical conductivity was measured using a conductivity meter.

Sodium and potassium contents were determined using flame photometry (Viégas et al., 2001). Leaf and root samples were dried in an oven at 65 °C for up to 72 hours. Subsequently, they were ground using a bench mill. The extraction was performed using 100 mg of ground material added to 10 mL of distilled water in a water bath at 100 °C for one hour. The extract obtained was filtered and then analyzed in a flame photometer (Micronal, model B462) calibrated with standard solutions of NaCl and KCl. The contents ( $\mu\text{mol g}^{-1}$ ) of Na and K were expressed based on the dry mass of the leaves and roots.

The results of these evaluations were initially subjected to the Shapiro-Wilk normality test and Bartlett's homoscedasticity test. Given the assumptions of normality and homoscedasticity were satisfied, an analysis of variance (ANOVA) was performed using the F-test ( $p \leq 0.05$ ). If F was significant, the treatment means were compared using Tukey's test ( $p \leq 0.05$ ). Regression analysis was performed between the leaf sodium content ( $\text{Na}^+$ ) and dry matter (DM) of the leaf blade (LB) of the pangolão grass. The statistical program R version 3.5.3 was used for all the analyses.

## RESULTS AND DISCUSSION

The differing electrical conductivities of the irrigation water led to significant differences in electrical conductivities of the saturation extracts of the soil solutions between the salinity levels studied. As expected, the application of biostimulant did not influence the electrical conductivities of the soil solutions. Salinity levels of 0.03, 2, and 4  $\text{dS m}^{-1}$  resulted in electrical conductivities of 0.5, 2.32 and 4.22  $\text{dS m}^{-1}$ , respectively, indicating that there was an accumulation of salts in the soil equivalent to the salinity of the treatments (Table 1).

Soil salinization increases when the amount of salt accumulated by irrigation water is greater than the amount removed by leaching (Rodrigues et al., 2018; Jesus & Borges, 2020). During the experiment there was an accumulation of 87 mm of rainfall and the pots remained exposed, simulating real field conditions; however, this amount of rain was not sufficient to dilute the salts, favoring accumulation in the soil.

The interaction between salinity and biostimulant had an effect on the  $\text{Na}^+$  content of the leaves (Table 2). Only live tiller number (LTN) and leaf area (LA) were affected by salinity. Furthermore, the isolated effects of electrical conductivity of

**Table 1.** Electrical conductivity of saturation extracts (ECse) of soil solutions as a function of electrical conductivity of irrigation water (ECw)

ECw	ECse <sup>1</sup>
(dS m <sup>-1</sup> )	
0.03	0.50 ± 0.06 c
2	2.32 ± 1.14 b
4	4.22 ± 0.95 a
p-value	0.0012

<sup>1</sup> Means and standard deviations of the electrical conductivity of the saturation extract as a function of salinity level; Means followed by the same letters do not differ according to Tukey's test ( $p > 0.05$ )

**Table 2.** Analysis of variance of salinity levels (S) and biostimulant (B) and their effects on structural characteristics, plant production, stomatal conductance ( $g_s$ ) and  $Na^+$  and  $K^+$  content in the leaves and roots of pangolão grass

Effect	Structural features							
	SL	SD	LTN	LLN	LEN	ELN	LA	
S	ns	ns	*	ns	ns	ns	***	
B	ns	ns	ns	ns	ns	ns	ns	
S × B	ns	ns	ns	ns	ns	ns	ns	
CV (%)	50	24	26	35	34	46	21	
	Plant production							
	LB	SPS	DM	TDM	R	PLB	PSS	PDM
S	*	ns	ns	ns	ns	*	ns	ns
B	ns	ns	ns	ns	ns	ns	ns	ns
S × B	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	23	21	32	25	61	6	51	55
	Stomatal conductance				$Na^+$ and $K^+$ content			
			NaL	NaR	KL	KR		
S			*	**	**	ns	ns	
B			ns	***	ns	ns	ns	
S × B			ns	*	ns	ns	ns	
CV (%)			25	47	43	13	41	

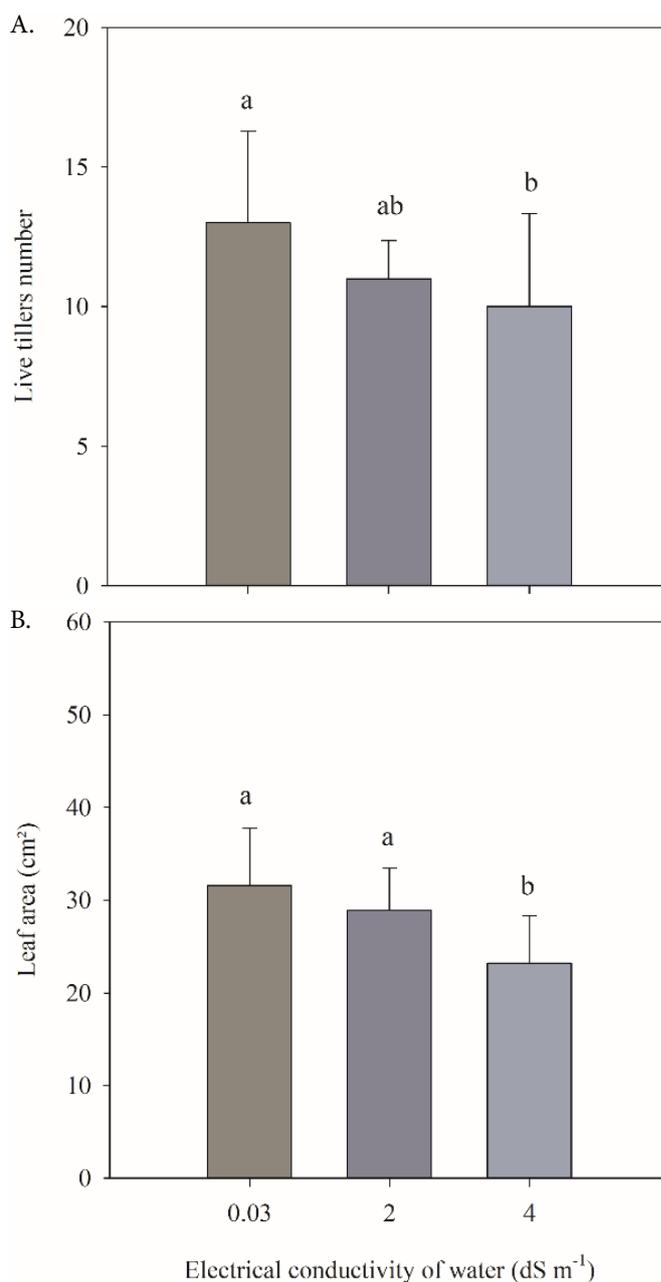
SL - Stem length; SD - Stem diameter; LTN - Live tillers number; LLN - Live leaves number; LEN - Leaves expanded number; ELN - Expanding leaves number; LA - Leaf area; LB - Leaf blade; SPS - Stem plus sheath; DM - Dead material; TDM - Total dry matter; R - Root; PLB - percentage of leaf blade; PSS - percentage of stem plus sheath; PDM - percentage of dead material; NaL - Sodium in the leaf; NaR - Sodium in the root; KL - Potassium in the leaf; KR - Potassium in the root. \*, \*\*, \*\*\*, ns - Significant at  $p \leq 0.05$ , at  $p \leq 0.01$ , at  $p < 0.001$ , and not significant, respectively; CV - Coefficient of variation

irrigation water on the leaf blade, percentage total dry matter of components, stomatal conductance, and  $Na^+$  content of leaves and roots were verified. The biostimulant did not influence the structural characteristics, plant production, or stomatal conductance of the pangolão grass (Table 2).

These results contradict the hypothesis that application of the biostimulant would increase tolerance to saline stress under semi-arid conditions. In the current literature (Cunha et al., 2016; Simões et al., 2022) there are divergent records regarding the efficiency of biostimulants, particularly when associated with stressful conditions. According to Cunha et al. (2016), the use of biostimulants can have beneficial effects on plant development. However, the efficiency of this product can be affected by soil and climate conditions, plant species, management, and dosage.

The live tillers number (LTN) reduced from 13 at 0.03 dS  $m^{-1}$  to 10 at 4 dS  $m^{-1}$ , however there was no difference between these treatments and moderate salinity (2 dS  $m^{-1}$ ) (Figure 1A). The ability to emit and maintain new tillers is directly related to the availability of nutrients in the soil (Simões et al., 2022). From this perspective, the lower LTN in severe salinity conditions is due to excess NaCl in the soil solution. According to Kumari et al. (2017), an increase in the concentration of these ions in the soil affects the ability of the roots to absorb the nutrients necessary for the healthy growth of plants.

The lowest leaf area (23.2  $cm^2$ ) was observed under severe salinity, compared to leaf areas of 31.6 and 28.9  $cm^2$  observed respectively in low and moderate salinities (ECws of 0.03 and 2 dS  $m^{-1}$  respectively) (Figure 1B). The effects of salinization on plants can be due to the limitation of water absorption, ion toxicity, and interference of salts in physiological processes (Gheyi et al., 2016). The absorption of water by plants is only possible when the imbibition forces of the roots are greater



Vertical bars indicate standard deviation from the mean. Means followed by different letters differ from each other ( $p \leq 0.05$ ), according to Tukey's test

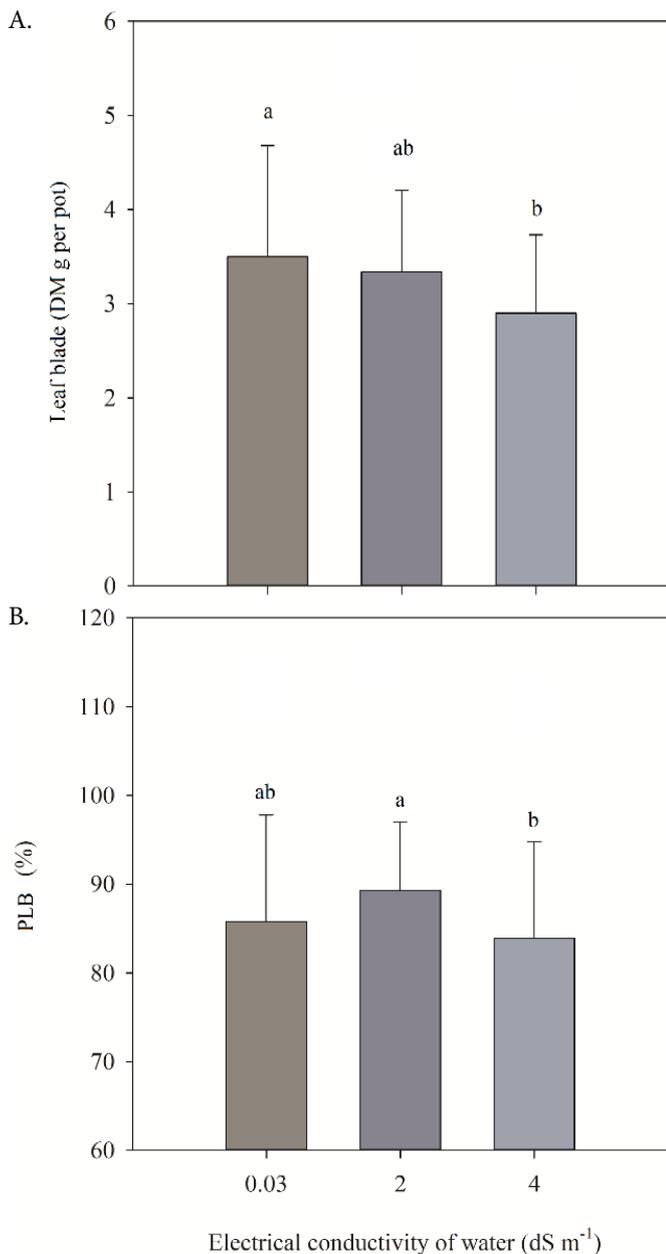
**Figure 1.** Live tillers number (A) and leaf area (B) of pangolão grass as a function of different electrical conductivities of the irrigation water

than the soil retention forces. Excess salts in the soil solution increase retention forces through the osmotic effect; therefore, even under water availability, plants cannot absorb water and this is referred to as a physiological drought (Gheyi et al., 2016; Kataria & Verma, 2018).

The decrease in LA with severe salinity (Figure 1B) is considered the first line of defense of plants against osmotic stress, as it reduces the transpiration surface and, consequently, the loss of water by transpiration. Subsequently, due to the continuation of excess salts in the soil-plant system, the pangolão grass suffered from ionic stress that corresponded to the accumulation of  $Na^+$  ions in plant tissues. Under these conditions, changes in cell wall properties and a reduction in photosynthesis occur, leading to a reduction in the total leaf area (Kataria & Verma, 2018).

The results showed that for 4 dS m<sup>-1</sup> ECw in irrigation water, the forage yield of pangolão grass was significantly compromised. Leaf blade dry matter production decreased from 3.5 g per plot at 0.03 dS m<sup>-1</sup> to 2.9 g per plot with severe salinity (Figure 2A). Furthermore, the results indicated that there was a difference in the percentage of leaf blades in total dry matter between levels 2 and 4 dS m<sup>-1</sup>, with severe salinity reducing the percentage of leaf blades in relation to the other morphological components (2 dS m<sup>-1</sup> = 89% vs. 4 dS m<sup>-1</sup> = 83%) (Figure 2B).

The difference in the ECse of the soil solution between the salinity levels of 0.03 and 2 dS m<sup>-1</sup> (Table 2) was not sufficient to reduce the forage production of pangolão grass. This finding is unprecedented evidence of the “moderate” salinity tolerance of pangolão grass in semi-arid conditions. On the other hand,



Vertical bars indicate standard deviation from the mean. Means followed by different letters differ from each other ( $p \leq 0.05$ ) by Tukey's test; DM - Dry matter

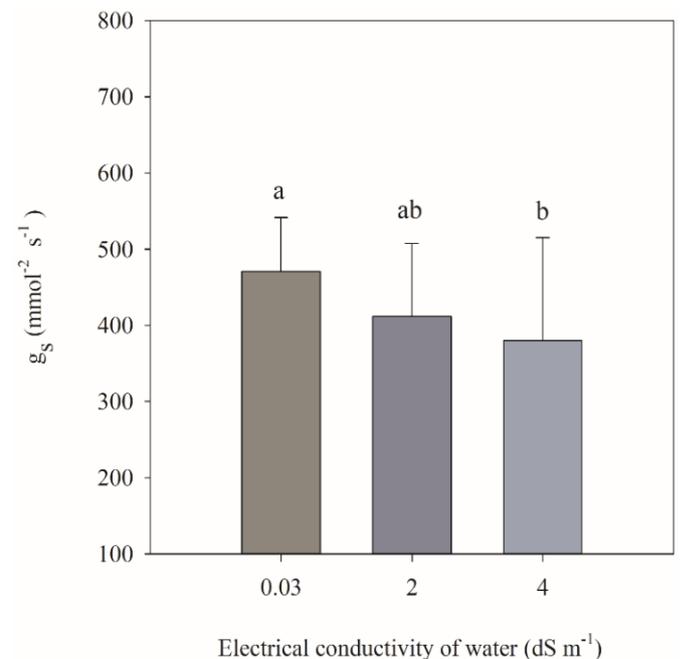
**Figure 2.** Leaf blade dry matter production (A) and percentage of leaf blade (PLB) in dry matter (B) of pangolão grass as a function of different electrical conductivities of irrigation water

the accumulation of salts at the level of 4 dS m<sup>-1</sup> reduced the production of leaf blades by 21% in comparison to the absence of salinity (0.03 dS m<sup>-1</sup>) and reduced the relationship of this morphological component with the production of total dry matter by 6% compared to the level of 2 dS m<sup>-1</sup>. According to Emerenciano Neto et al. (2019), the nutritive value of the stem is lower than that of the leaf; therefore, a lower leaf mass in relation to that of stems indicates a decrease in forage quality. These results suggest that under conditions of severe salinity, pangolão grass compromises the quantity and quality of forage produced.

Under high salinity at an ECw of 4 dS m<sup>-1</sup>, plants act against osmotic and ionic stresses. Initially, with the increase of salts in the soil, osmotic stress decreases water absorption and, consequently, cellular expansion. Over time, Na<sup>+</sup> and Cl<sup>-</sup> ions are absorbed and accumulate in plant tissues, which leads to a reduction in activities essential for metabolism, such as photosynthesis, resulting in a significant decrease in crop growth and productivity (Gheyi et al., 2016; Kataria & Verma, 2018).

Stomatal conductance was not affected by salinity in the reading taken at 7:00 a.m. However, under full sun conditions in the reading at 12:00, salinity effects on the stomatal conductance of pangolão grass were observed (Figure 3). The stomatal conductance responses to salinity were similar to the results verified for LTN and leaf blade production, suggesting that the reduction of gas exchange in severe salinity has a negative impact on plant yield.

In this study, the absence of salinity effects on  $g_s$  during the 7:00 a.m. assessment was justified by mild radiation and temperature conditions at that time. On the other hand, when subjected to equivalent increases in radiation and temperature in the 12 hours assessment, plants in severe salinity considerably reduced stomatal conductance.



Vertical bars indicate standard deviation from the mean. Means followed by different letters differ from each other ( $p \leq 0.05$ ), according to Tukey's test

**Figure 3.** Stomatal conductance ( $g_s$ ) at 101 days after planting of pangolão grass as a function of different electrical conductivities of irrigation water

A decrease in stomatal conductance under saline stress conditions has been frequently reported in the literature (Sadaqat Shah et al., 2020; Khedr et al., 2021). The effects of osmotic stress caused by salinity on water absorption from the soil induce plants to reduce stomatal conductance to reduce water loss through leaves. However, stomatal closure reduces the internal concentration of CO<sub>2</sub> in plants and decreases the activity of several enzymes, including RuBisCo (Kataria & Verma, 2018). In this way, oxidative stress begins, reducing the net photosynthetic rate and leading to growth inhibition, and even plant death.

There was no isolated effect or interaction between salinity and biostimulant on potassium content in pangolão grass leaves and roots. However, there was a significant interaction between these factors (salinity and biostimulant) on the sodium content of the leaves (Table 1). The results indicated that the interaction between the highest concentration of biostimulant (8 mL L<sup>-1</sup>) and severe salinity caused the highest accumulation of Na<sup>+</sup> in the leaves (Table 3). In this treatment, there was an increase of 221.6 and 168.4% in the Na<sup>+</sup> content in the leaves compared to the levels of 0.03 and 2 dS m<sup>-1</sup>, respectively, and 87.9% higher in the absence of biostimulant.

The use of seaweed extracts can stimulate root growth and, consequently, increase nutrient uptake by plants (Sible et al., 2021). This characteristic can favor the yield of plants when subjected to the availability of essential elements for their growth. However, when exposed to excess Na<sup>+</sup> ions in the soil and under the effects of biostimulants on root growth, greater soil exploration by the roots also favors the absorption of saline ions.

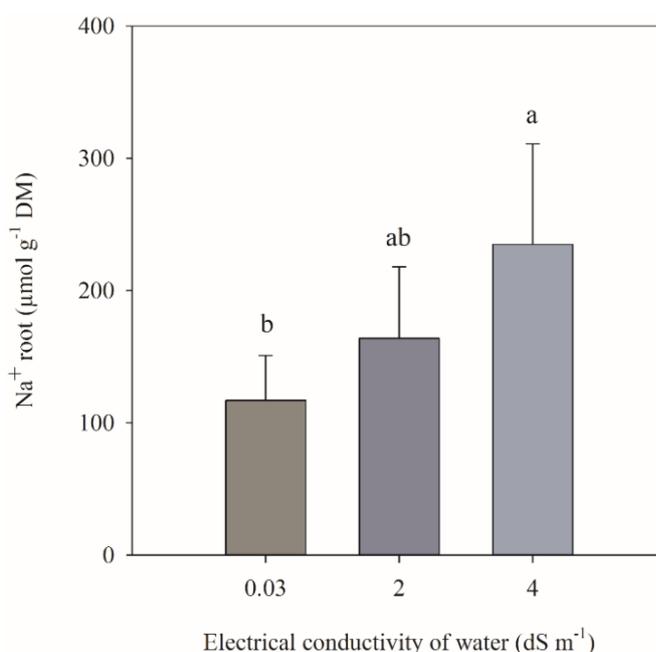
As shown in Table 1, there were no significant effects of the factors salinity and biostimulant on the accumulation of potassium in the leaves and roots of pangolão grass. In salinity studies, potassium is a much-explored nutrient because of its multiple roles in maintaining cell turgor, membrane potential, and enzymatic activities (Khedr et al., 2021). In addition, several studies (Kumar et al., 2018; Khedr et al., 2021) indicated negative correlations between Na<sup>+</sup> and K<sup>+</sup> levels, with a reduction in K<sup>+</sup> levels in plant tissues after the application of salts having Na<sup>+</sup> in their composition. These authors attributed these results to the antagonism between Na<sup>+</sup> and K<sup>+</sup> due to competition for absorption sites in the plasmalemma and a possible increase in the efflux of K<sup>+</sup> from the roots due to disturbances in membrane integrity. In this sense, despite the negative impact on EC<sub>w</sub> plant yield of 4 dS m<sup>-1</sup>, the positive balance of K<sup>+</sup> in plant tissues was not affected.

There was no significant interaction between the studied factors in terms of Na<sup>+</sup> concentration in the roots (Table 2). There was a salinity effect alone (Figure 4), with significant

**Table 3.** Sodium (Na<sup>+</sup>, μmol g<sup>-1</sup> DM) content in pangolão grass leaves as a function of different electrical conductivities of irrigation water and concentration of biostimulant

Biostimulant concentration (mL L <sup>-1</sup> )	Electrical conductivity of water (dS m <sup>-1</sup> )		
	0.03	2	4
0	77.73 bA	127.63 abA	182.34 aB
8	106.52 bA	127.63 bA	342.61 aA

Means followed by the same capital letter in the column and lowercase letter in the row do not differ statistically from each other by Tukey's test at p ≤ 0.05; DM – Dry matter



Vertical bars indicate standard deviation from the mean. Means followed by different letters differ from each other (p ≤ 0.05) by Tukey's test; DM – Dry matter

**Figure 4.** Sodium content (Na<sup>+</sup>) in roots of pangolão grass as a function of different electrical conductivities of irrigation water

differences between EC<sub>w</sub> of 4 and 0.03 dS m<sup>-1</sup> (235 and 117 μmol g<sup>-1</sup> dry matter, respectively). These results were in agreement with those observed for the leaves, regardless of biostimulant; the severe salinity promoted the highest concentrations of Na<sup>+</sup> in the pangolão grass leaves and roots.

There is considerable variation in the tolerance of plants to salinity, and one of the main mechanisms associated with this tolerance is the ability of plants to reduce the translocation of Na<sup>+</sup> to the shoot. Some studies have shown that salinity tolerance is directly related to a reduction in N<sup>+</sup> concentration at toxic levels in the shoot (Gheyi et al., 2016; Kataria & Verma, 2018). Thus, the translocation of Na<sup>+</sup> from the roots to the shoot in the EC<sub>w</sub> of 4 dS m<sup>-1</sup> and, consequently, the excess of potentially toxic ions in the photosynthetic tissues, is another explanation for the results observed in the structural characteristics, accumulation of phytomass, and gaseous exchange.

A relatively low coefficient of determination (R<sup>2</sup> = 0.46) was found for the linear regression analysis (Eq. 3, p ≤ 0.01) of the effect of Na<sup>+</sup> content in leaves on leaf blade production. These results suggest that, in addition to the effects of ionic stress due to the toxicity of ion accumulation in the shoot, other factors related to osmotic stress, such as inhibition of water and nutrient absorption, disruption of membranes, impairment of the ability to detoxify reactive oxygen species, decreased photosynthetic activity, and decreased stomatal opening (Kataria & Verma, 2018) are involved in the production of leaf blades in pangolão grass.

$$LB = 3.60 - 0.006 \times Na^+ \quad (3)$$

The Na<sup>+</sup> highest concentrations in the leaves were reflected in the reduction in pangolão grass forage yield. The accumulation of 50 μmol g<sup>-1</sup> DM of Na<sup>+</sup> in the leaves corresponded to a reduction of 0.3 g of DM per pot in the production of leaf blade.

## CONCLUSIONS

1. The seaweed extract used as a biostimulant did not influence the structural characteristics, phytomass accumulation, or stomatal conductance of pangolão grass, regardless of the presence of salts in the irrigation water.

2. With 4 dS m<sup>-1</sup> of electrical conductivity in irrigation water, pangolão grass suffered from osmotic stress, and even more intensely from the toxic effects of ionic stress due to the accumulation of salts in the aerial part.

3. This is the first evidence of the “moderate” salinity tolerance of pangolão grass in semi-arid regions.

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