PAB
Pesquisa Agropecuária Brasileira

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Plant Phisiology/ Original Article

Na$^+$ exclusion and proline accumulation in *Urochloa* spp. cultivars

Abstract – The objective of this work was to evaluate the effect of salinity on Na$^+$ and K$^+$ distribution and on proline content in *Urochloa brizantha* and *Urochloa decumbens* grass cultivars. Plants were grown in nutrient solution and subjected to salt stress for nine days. Shoot and root lengths and proline, total chlorophyll, K$^+$, and Na$^+$ contents were determined. Salt stress reduced the shoot length of cultivar Marandu but not of Xaraés and Basilisk. Total chlorophyll content was not affected by salinity in *U. brizantha* cultivars, but increased in cultivar Basilisk of *U. decumbens*. Salt-induced proline accumulation was observed in all cultivars, with a higher increase in Marandu. The K$^+$ content in leaf blades increased in the Marandu and Basilisk cultivars under salinity conditions. 'Basilisk' showed Na$^+$ exclusion in shoot tissues, whereas 'Marandu' and 'Piatã', followed by 'Xaraés', exhibited a higher Na$^+$ in leaf blades. The pattern of ion distribution among cultivars was attributed mainly to differences in the ability to control the ion transport from root to shoot, since the rates of ion uptake were similarly affected by salinity. Tolerance to salt stress in grasses of the *Urochloa* genus is associated to Na$^+$ exclusion and not to proline accumulation in shoot tissues.

Index terms: *Urochloa brizantha*, *Urochloa decumbens*, salt stress.

Exclusão de Na$^+$ e acúmulo de prolina em cultivares de *Urochloa* spp.

Resumo – O objetivo deste trabalho foi avaliar o efeito da salinidade na distribuição de K$^+$ e Na$^+$ e no conteúdo de prolina em cultivares dos capins de *Urochloa brizantha* e *Urochloa decumbens*. As plantas foram cultivadas em solução nutritiva e expostas a estresse salino durante nove dias. Foram determinados os comprimentos da parte aérea e das raízes e os conteúdos de prolina, clorofila total, K$^+$ e Na$^+$. O estresse salino reduziu o comprimento da parte aérea da cultivar Marandu, mas não de Xaraés e Basilisk. O conteúdo total de clorofila não foi afetado nas cultivares de *U. brizantha*, mas aumentou na cultivar Basilisk de *U. decumbens*. Observou-se acúmulo de prolina em todas as cultivares sob estresse salino, com maior aumento em Marandu. O conteúdo de K$^+$ nos limbos foliares aumentou nas cultivares Marandu e na Basilisk, em condições de estresse. 'Basilisk' apresentou exclusão de Na$^+$ nos tecidos aéreos, enquanto 'Marandu' e 'Piatã', seguidas por 'Xaraés', apresentaram maior acúmulo de Na$^+$ nos limbos foliares. O padrão de distribuição de íons entre as cultivares foi atribuído, principalmente, a diferenças na habilidade para controlar seu transporte da raiz aos tecidos da parte aérea, já que as taxas de absorção foram similarmente afetadas pela salinidade. A tolerância à salinidade em gramíneas do gênero *Urochloa* está associada à exclusão de Na$^+$ e não ao acúmulo de prolina na parte aérea da planta.

Termos para indexação: *Urochloa brizantha*, *Urochloa decumbens*, estresse salino.

Cicero Lucas da Silva(1),
Rita de Cássia Alves de Britto Ferreira(1)
and Juan Carlos Alvarez-Pizarro(1 ✉)

(1) Universidade Federal do Cariri, Centro de Ciências Agrárias e da Biodiversidade, Rua Ícaro de Sousa Moreira, nº 125, Barro Branco, CEP 63130-025 Crato, CE, Brazil.
E-mail: cicero_lucas@outlook.com, engagro.ritaferreira@gmail.com, juan.alvarez@ufca.edu.br

✉ Corresponding author

Received
September 22, 2022

Accepted
November 22, 2022

How to cite
SILVA, C.L. da; FERREIRA, R. de C.A. de B.; ALVAREZ-PIZARRO, J.C. Na$^+$ exclusion and proline accumulation in *Urochloa* spp. cultivars. **Pesquisa Agropecuária Brasileira**, v.58, e03131, 2023. DOI: https://doi.org/10.1590/S1678-3921.pab2023.v58.03131.

Introduction

Soil salinity is a major abiotic constraint affecting plant growth and productivity. Under such conditions, plants are exposed to osmotic and ionic stresses that lead to metabolic alterations, nutritional unbalance and oxidative damages (Parihar et al., 2015). A primary mechanism of salt stress tolerance, which is displayed in several halophytes and tolerant glycophytes, consists of root-mediated control on differential partition of K^+ and Na^+ ions among the plant organs after its uptake from growth medium (Munns & Tester, 2008). A low Na^+ content and high K^+/Na^+ ratio in leaves are the physiological manifestations of an efficient root exclusion in salt-stress tolerant plants (Tao et al., 2021).

From a physiological point of view, the exclusion of Na^+ results from the contribution of selective uptake at the level of root surface, and from the selective transport of K^+ over Na^+ from roots to shoot (Yang et al., 2018). The efficient partition of Na^+ and K^+ reduces the salt-induced oxidative damages on biological molecules and membrane integrity, therefore ensuring an adequate operation of the photosynthetic metabolism and plant growth (Koyro et al., 2013; Kim et al., 2016).

Plants that exclude toxic ions must maintain the water status of their aerial tissues by selective K^+ uptake and produce organic solutes such as proline (Munns & Tester, 2008). Thus, the high proline accumulation in the shoot is a remarkable trait of salt-stress tolerant plants, and it was described in wheat (Ashraf et al., 2012) and oat (Liu et al., 2020). However, the presence of a salt-avoiding mechanism has been reported to reduce the dependence by this osmolyte, characterizing cultivars with better performance in saline soils. For instance, in wheat cultivars with low indices of stress sensitivity, the Na^+ and proline concentration in upper leaves were low (Poustini et al., 2007), which suggests that the salt tolerance depends primarily on the control of Na^+ and Cl^- homeostasis in plant tissues. In contrast, a less proline accumulation was also observed in salt-tolerant *Panicum hemitomom* populations, without showing differences for their ability to control leaf Na^+/K^+ ratio, in comparison with less salt-tolerant populations (Hester et al., 2001).

Urochloa brizantha (A. Rich.) R.D. Webster [Syn. *Brachiria brizantha* (Hochst. ex A.Rich.) Stapf] and *Urochloa decumbens* (Stapf) R.D. Webster (Syn. *Brachiria decumbens* Stapf) are tropical grasses with high nutritional value and good biomass production. These species of *Urochloa* genus exhibit morphophysiological adaptations to grow in both dry and harm environments, which have allowed its cultivation in semiarid regions (Véras et al., 2020). Soil salinization is a quite recurrent phenomenon in such environments, and some species of *Urochloa* grasses have low tolerance to soil salinity (Alvarez-Pizarro et al., 2020; Amorim et al., 2015). In some *U. brizantha* cultivars, the proline accumulation in blades was not enough to minimize the deleterious effects of salinity, and the Na^+ exclusion was postulated as a key mechanism to tolerate the salt stress in this crop (Alvarez-Pizarro et al., 2020). Therefore, it is necessary to understand the role of Na^+ exclusion by roots and the proline-mediated protection when young *Urochloa* seedlings are exposed to salt stress.

The objective of this work was to evaluate the effect of salinity on Na^+ and K^+ distribution and the proline content in *U. brizantha* and *U. decumbens* grass cultivars.

Materials and Methods

Seeds of Marandu, Piatã, and Xaraés – cultivars from *U. brizantha* –, and Basilisk – cultivar from *U. decumbens*, were superficially disinfected with 3% NaClO for 5 min and, then, thoroughly rinsed with tap water. Seeds were sown in vermiculite moistened with 0.5 mmol L^{-1} $CaCl_2$ solution and irrigated daily with distilled water for 10 days. Seedlings with uniform size were transferred to trays containing 10 L Hoagland nutrient solution with 1/3 ionic strength and grown for one week. Then, the plants were transferred to pots (two plants per pot) containing 2.3 L of nutrient solution without NaCl (control treatment) and with NaCl at 75 mmol L^{-1} (salt stress treatment) for nine days. The salinity of each nutrient solution was gradually increased with the addition of NaCl, using the necessary amount to elevate the salt level by 37.5 mmol L^{-1} at 12-hour intervals until reaching the desired level. In the sixth day after the beginning of the saline treatment, the solutions were renewed to avoid nutritional deficiency. The growth solutions were subjected to pH between 5.5 and 6.0, and the K^+ level was adjusted daily to 0.75 mmol L^{-1}. Plants were grown in a naturally lit greenhouse subjected to conditions of day/night air temperature at 28.5±1.6°C/ 24.3±1.0°C and relative humidity at 58.5±6.8%/

67.5±7.0%, respectively. At the experiment end, the plants were collected; their shoot and root lengths were measured, and their leaf blades, leaf sheath, and roots were weighted and stored at -20°C for further analysis.

For K^+ and Na^+ determination, fresh tissues (ca 200 mg) of leaf blades, leaf sheaths, and distal segments from roots were homogenized with 7.0 mL of deionized water, with the aid of a mortar and pestle for 10 min, and the extraction proceeded in an orbital shaker for 1 hour. Then, the extracts were centrifuged at 3.000 *g* for 10 min. The K^+ and Na^+ contents in the water supernatants were determined by flame photometry (FC-280, CELM, Barueri, SP, Brazil) according to Malavolta et al. (1989).

From fresh mass (FM) and ion content data, the transport net rate (TR, in %) and uptake net rate (UR, in mmol kg^{-1} FM) for K^+ and Na^+ were calculated using the following equations:

$$TR_{K,Na} = \frac{\text{total amount of ion in shoot}}{\text{total amount of ion in the whole plant}} \times 100$$

$$UR_{K,Na} = \frac{\text{total amount of ion in the whole plant}}{\text{root fresh mass}}$$

Chlorophyll was extracted with pure ethanol from 200 mg of fully-expanded leaf blades. The extraction was performed in the dark for 24 hours. The homogenate was centrifuged at 3.000 *g* for 10 min, and the absorbance of the supernatant was measured with a spectrophotometer at 645 nm and 663 nm. The total chlorophyll (Chl) content was estimated using the following equation (Hiscox & Israelstam, 1979):

$$Chl = 20.2\ A_{645} + 8.02\ A_{663}$$

For proline determination in leaf blades, fresh tissues (ca 200 mg) were homogenized for one hour with 7.0 mL of 80% ethanol and, then, centrifuged at 3.000 *g* for 10 min. The diluted supernatant was mixed with a similar volume of acid ninhydrin (1.25 g ninhydrin, 20 mL 6 mmol L^{-1} phosphoric acid, 30 mL glacial acetic acid), incubated at 95ºC for 45 min, cooled in an ice bath and, then, the chromophore was separated with two volumes of toluene for the reading of its absorbance at A_{520} (Bates et al., 1973). The free proline content (mg g^{-1} FM) was estimated based on the standard curve developed with L-proline.

The experimental design was completely randomized with 2x4 factorial arrangement (two salinity levels, without or with NaCl at 75 mmol L^{-1}, and four *Urochloa* cultivars). Each treatment consisted of five replicates composed of two plants each. Data were subjected to a two-way analysis of variance, and the means were compared by Tukey's test at 5% probability. The Pearson's correlation coefficient (r) between different sets of fresh mass and physiological parameters were worked out using the statistical software Jamovi version 2.3 (The Jamovi Project, Sydney, Australia).

Results and Discussion

The shoot length varied between cultivars under salt stress (Figure 1 A). Thus, the absence of salt-stress effects on shoot length in 'Basilisk' and 'Xaraés', and the reductions observed in 'Marandu' (43.8%) and 'Piatã' (30.8%), in comparison with the control treatment, suggest a higher tolerance in the former than in the latter cultivars. The negative effect on shoot length of some *Urochloa* cultivars, after nine days of the exposure to salt, may be due to the gradual Na^+ accumulation. Similar responses between tolerant and sensitive cultivars were observed in rice (Singh et al., 2018), and in wheat and barley (Zeeshan et al., 2020). The root length did not vary with the exposure to salt, in comparison with the control treatment (Figure 1 B), which indicates its lower sensitivity than that of the shoot length.

The Chl content was not affected by salinity in the cultivars of *U. brizantha*, whereas it significantly increased in *U. decumbens* 'Basilisk' (Figure 1 C), which was significantly higher than the content observed *U. brizantha* 'Marandu'. The chlorophyll content is an indicative parameter of tolerance level to salt stress; highly-tolerant wheat (Saddiq et al., 2021) and rice (Singh et al., 2018) genotypes showed lower reduction of chlorophyll pigments than more sensitive ones. As salt stress adversely affects the chlorophyll pigments, it causes the decrease of the net photosynthetic rate, maximum quantum, and photon yield of photosystem II (Farooq et al., 2015; Parihar et al., 2015). The higher chlorophyll content in 'Basilisk' may be attributed to an induced synthesis under salt stress, which did not occur in the other cultivars. In accordance with these results, the total chlorophyll levels tend to increase in

DOI: 10.1590/S1678-3921.pab2023.v58.03131

Aeluropus littoralis during salt stress, which points out to the better use of leaf resources in light-harvesting processes (Hashemipetroudi et al., 2022). Thus, the increased total chlorophyll in 'Basilisk' under stress may improve the light energy capture under saline conditions. The chlorophyll content in salt-tolerant plants has been also correlated with an improved K^+/Na^+ ratio and an efficient protection by compatible solutes (Rady & Mohamed, 2018).

Proline content increased in all salt-treated cultivars, and it was higher in Marandu than in the others (Figure 1 D). For instance, the proline level in leaf blades of 'Marandu' was 1.80-fold higher than those in 'Basilisk'. Proline acts as a tissue osmoprotector during salt stress, and its accumulation in sheath cell sap and xylem confers a high tolerance to salinity in oat genotypes (Liu et al., 2020). Moreover, the genetic improvement through overexpressing *PuP5CS*, which is involved in the proline biosynthesis, enhances the plant stress tolerance and maintains the growth and chlorophyll content in *Panicum virgatum* under salinity conditions (Guan et al., 2018). Nonetheless, in the current study, the shoot length and chlorophyll contents in 'Marandu' were affected more under salinity conditions (Figure 1 A and C). Similar results have been observed in wheat cultivars (Poustini et al., 2007) and *Panicum virgatum* (Kim et al., 2016), showing that proline accumulation in plant tissues is not related with the protection of

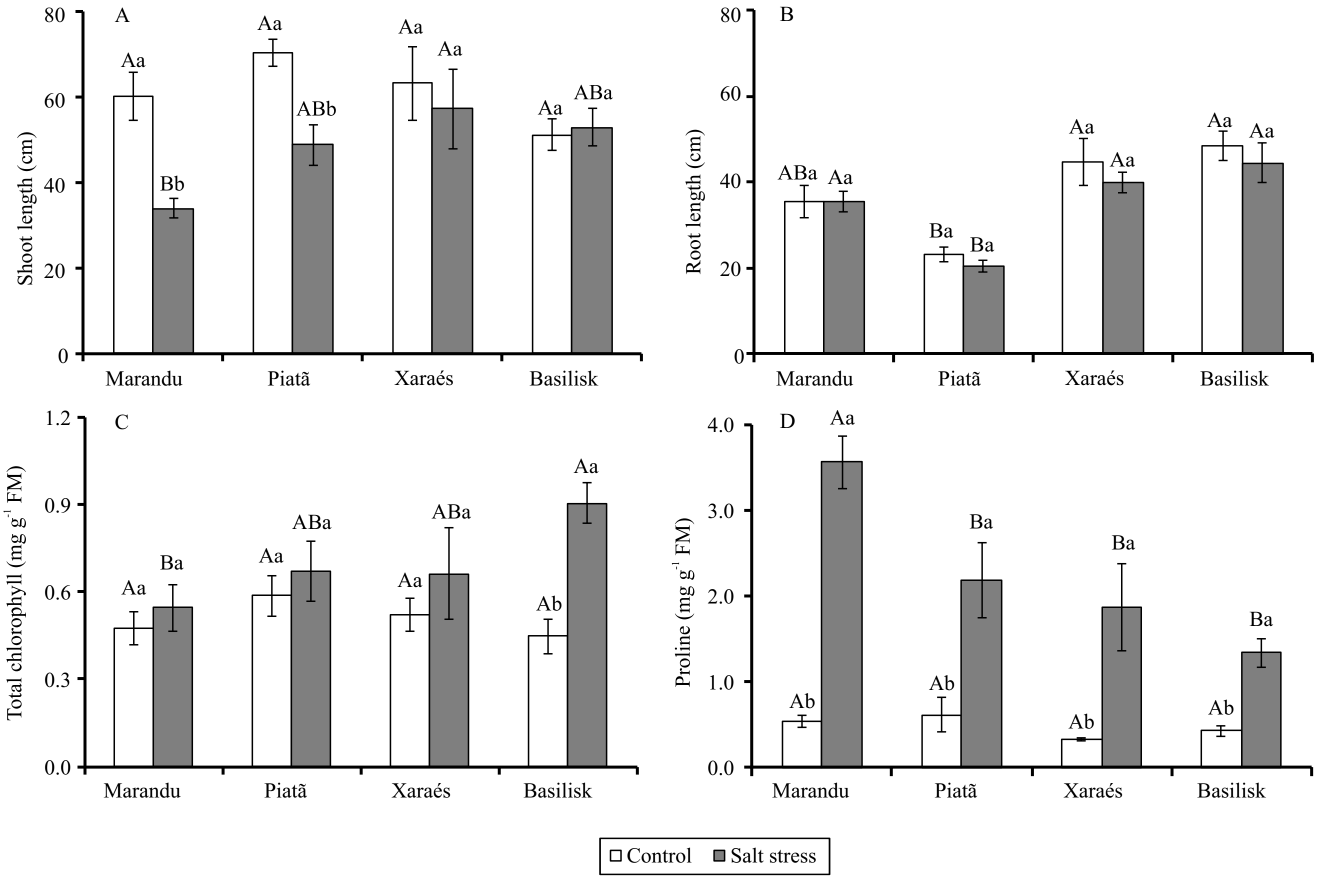

Figure 1. Length of shoot (A) and roots (B), and chlorophyll (C) and proline (D) contents in leaf blades of young seedlings of *Urochloa decumbens* Basilisk and *U. brizantha* Marandu, Piatã, and Xaraés cultivars grown in nutrient solution, without (control) or with NaCl at 75 mmol L^{-1} (salt stress). FM: fresh mass. Means followed by different uppercase letters (comparing cultivars) and lowercase letters (comparing salt levels) differ by Tukey's test, at 5% probability. The values represent mean±SE from five replicates (n = 5).

chlorophyll pigments and salt tolerance. Therefore, the high proline increase in *Urochloa* grasses may be interpreted as a negative symptom of salt stress.

Under salinity, K^+ content increased in leaf blades of 'Marandu' and 'Basilisk' (Figure 2 A), and in roots of 'Xaraés' (Figure 2 G). However, reductions of the K^+ content in leaf sheaths were observed (Figure 2 D), and it could be involved in the K^+ distribution toward the leaf blades, attenuating the salt-induced K^+ deficiency and contributing to improve the ionic relations of these tissues. Similar results were observed in leaves of durum wheat, in which the displacement of K^+ from the parenchyma cells of leaf sheath was caused by Na^+ accumulation (James et al., 2006).

Under saline conditions, the Basilisk (cultivar from *U. decumbens*) showed a higher capacity to retain Na^+ in its roots in comparison with the cultivars from *U. brizantha*, effectively reducing the Na^+ accumulation in its aerial tissues. In comparison with *U. decumbens* 'Basilisk', the accumulated Na^+ in roots of the *U. brizantha* cultivars Marandu, Piatã, and Xaraés was 51.0, 44.5, and 30.8% lower (Figure 2 H), whereas in blades it was 141.3, 124.4, and 88.5% higher (Figure 2 B) and, in sheaths, it was 43.2, 19.4, and 40.6% higher (Figure 2 E), respectively. Only *U. decumbens* 'Basilisk' displays an Na^+ exclusion mechanism from aerial tissues, which may have contributed to improve the growth of plants under mild salinity conditions

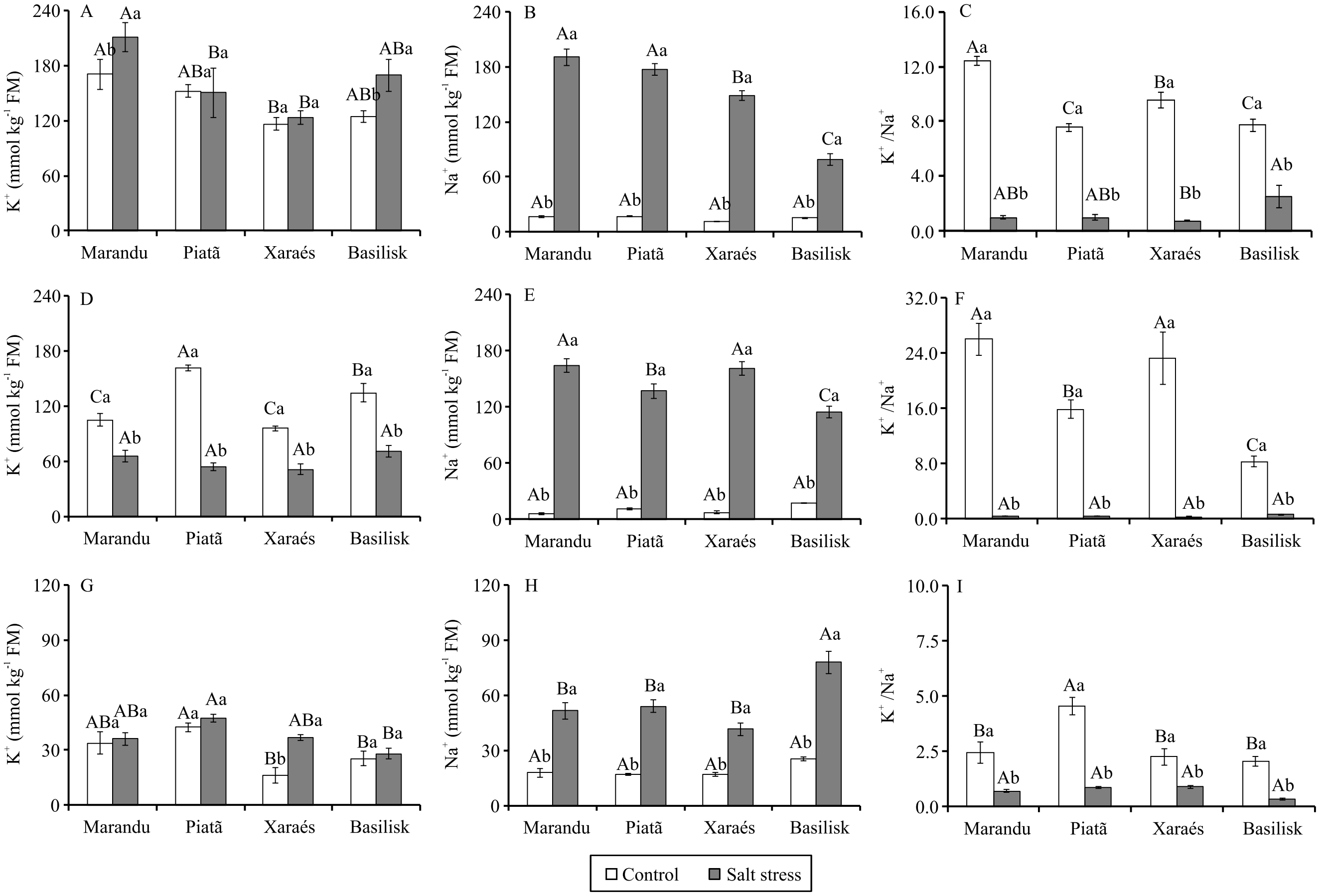

Figure 2. Content of K^+ (A, D, and G), Na^+ (B, E, and H), and K^+ /Na^+ ratio (C, F, and I) in leaf blades, leaf sheaths, and roots of young seedlings of *Urochloa decumbens* Basilisk and *U. brizantha* Marandu, Piatã, and Xaraés cultivars grown in nutrient solution without (control) or with NaCl at 75 mmol L^{-1} (salt stress). FM: fresh mass. Means followed by different uppercase (comparing cultivars) and lowercase letters (comparing salt levels) differ by Tukey's test, at 5% probability. The values represent mean±SE from five replicates (n = 5).

(Figure 1 A). Among the *U. brizantha* cultivars, Xaraés showed a lower Na^+ accumulation in its leaf blades and a higher shoot length than Marandu and Piatã, which would be attributed to the involvement of the leaf sheaths as a plausible Na^+ exclusion point; however, a positive effect on K^+/Na^+ ratio of leaf blades was not observed (Figure 2 C). In grasses such as durum wheat, the base of the leaves and roots represent crucial points for the Na^+ exclusion from the blades, leading to a high root/sheath to blade ratio of Na^+ concentration (James et al., 2006). Thus, the sensitivity to salt stress of *U. brizantha* young seedlings could be attributed to the lower efficiency of Na^+ exclusion mechanisms operating in roots as well as in sheaths.

The uptake net rate (UR) for K^+ was not affected by NaCl at 75 mmol L^{-1}. However, all cultivars, except for Marandu, had a reduced TR for K^+, and it was higher in Basilisk than in Piatã and Xaraés (Table 1). The high TR for K^+ from root to shoot can be promoted by a high Na^+ retention in the root system (Munns & Tester, 2008). *U. decumbens* 'Basilisk' had TR for Na^+ 20.5% lower than that of Marandu and Xaraés cultivars of *U. brizantha* (Table 1). Likely, Na^+ removal operates with high efficiency in 'Basilisk' by improving the K^+/Na^+ ratio in leaf blades (Figure 2 C) and chlorophyll content (Figure 1 C), in relation to some cultivars of *U. brizantha*. However, the UR for Na^+ at root surface did not differ between the cultivars, suggesting that the rates of Na^+ unidirectional influx and efflux, across the plasma membrane of root cells, would be similar under tested conditions. This assumption entails that differences in abundance, selective properties, and regulation of plasma membrane cation transporters did not occur between species of *Urochloa* genus. The main role of Na^+ loading in xylem vessels for the reduced transfer to the shoot has been observed in wild rice species (Shahzad et al., 2022) and barley (Wu et al., 2019).

The correlation analysis showed a negative correlation of the total chlorophyll content (r=-0.60, p=0.01) and root length (r=-0.62, p=0.003) with Na^+ level in leaf blades, and a positive correlation of those parameters (r=0.46, p=0.04, and r=0.51, p=0.02, respectively) with K^+ transport rate to shoot (Table 2). The ability to preferentially exclude Na^+ and to control the long-distance K^+ transport through a more efficient xylem loading has been recognized as a relevant characteristic for salt tolerance in grass species (Wu et al., 2018; Ebrahim et al*.,* 2019). In the present study, 'Basilisk' exhibited these tolerance responses (Figure 2 B, Table 1), which determined its higher performance under saline conditions than that of other *Urochloa* cultivars. Unexpectedly, a negative correlation between the total chlorophyll content and the K^+/Na^+ ratio in leaf blades (r=-0.48, p=0.03) was observed, and it may be attributed to the narrow differences for K^+ content in leaf blades of *Urochloa* cultivars (Figure 2 A). High K^+/Na^+ ratio in aerial tissues is a useful selection parameter for salt stress-tolerant genotypes (Tao et al., 2021). The correlation between proline in leaf blades and the analyzed traits was inverse but not significant, which indicates that there is a weak relation among the salt stress tolerance and proline accumulation in *Urochloa* grass leaves. Similarly, no correlation among salt tolerance and the proline accumulation was observed in wheat cultivars (Poustini et al., 2007).

Table 1. Net uptake and transport of K^+ and Na^+ in young seedlings of *Urochloa decumbens* Basilisk and *U. brizantha* Marandu, Piatã, and Xaraés cultivars grown in nutrient solution, without (control) or with NaCl at 75 mmol L^{-1} (salt stress)(1).

Cultivar	K^+ net uptake (mmol g^{-1} FM)		K^+ transport (%)		Na^+ net uptake (mmol g^{-1} FM)		Na^+ transport (%)	
	Control	Salt stress	Control	Salt stress	Control	Salt stress	Control	Salt stress
Marandu	152.7±6.6Ba	155.6±14.2ABa	78.3±3.3Ba	76.5±1.7ABa	30.8±2.7Ab	212.2±9.0Aa	42.7±3.3Bb	75.8±1.3Aa
Piatã	234.3±10.3ABa	152.8±15.2Aa	84.2±3.0ABa	68.2±1.9Bb	54.7±16.0Ab	194.7±19.1Aa	61.8±6.2Ab	71.8±1.5ABa
Xaraés	138.7±11.3Ba	117.1±8.4Ba	88.6±2.3Aa	68.0±1.9Bb	29.2±2.0Ab	193.2±21.0Aa	41.8±2.0Bb	76.9±4.0Aa
Basilisk	294.8±42.1Aa	174.0±29.7Aa	92.0±0.8Aa	82.9±2.0Ab	67.9±5.2Ab	202.7±18.0Aa	62.1±1.3Aa	60.7±3.8Ba

(1)Means followed by equal letters, uppercases in the columns and lowercases in the lines, do not differ by Tukey's test, at 5% probability. FM: fresh mass. The values represent mean±SE from five replicates (n = 5).

Table 2. Correlation of chlorophyll and shoot and root lengths with different parameters of *Urochloa decumbens* Basilisk and *U. brizantha* Marandu, Piatã, and Xaraés cultivars grown under saline conditions$^{(1)}$.

Variable	Chlorophyll content	Shoot length	Root length
Leaf Na$^+$	-0.60*	-0.42	-0.62*
Culm Na$^+$	-0.32	-0.11	-0.14
Root Na$^+$	0.35	-0.00	0.21
Leaf K$^+$	-0.20	0.03	-0.01
Leaf K$^+$/Na$^+$	-0.48	0.17	-0.40
Leaf proline	-0.36	-0.28	-0.18
Na$^+$ UR	-0.05	0.04	-0.11
Na$^+$ TR	-0.30	-0.07	-0.33
K$^+$ UR	-0.00	0.12	0.05
K$^+$ TR	0.46*	0.08	0.51*

*Significant at 5% probability. UR: uptake net rate. TR: transport net rate.

Conclusions

1. The tolerance to salt stress in *Urochloa* grass cultivars seedlings is, at least in part, linked to the Na$^+$ exclusion from shoot by the root system.
2. The weak regulation of Na$^+$ transport in *U. brizantha* 'Marandu' explains its high sensitivity to mild salt stress.
3. Proline accumulation in leaf blades is not an indicative parameter of tolerance to salt stress in *Urochloa* seedlings.

Acknowledgments

To Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for financial support.

References

ALVAREZ-PIZARRO, J.C.; SOUSA, K.C. de; SILVA, J. de S.; SILVA, A.A. da; GOMES-FILHO, E. Osmolyte accumulation in leaves and Na$^+$ exclusion by roots in two salt-treated forage grasses. **Grassland Science**, v.66, p.231-237, 2020. DOI: https://doi.org/10.1111/grs.12274.

AMORIM, Y.F.; AMORIM, R.S.; MARQUES, P.R.R.; AMARAL, C.L.F.; SANTANA, T.M. Tolerância de cultivares de capim *Brachiaria brizantha* à salinidade. **Agropecuária Científica no Semiárido**, v.11, p.31-37, 2015.

ASHRAF, M.A.; ASHRAF, M.; SHAHBAZ, M. Growth stage-based modulation in antioxidant defense system and proline accumulation in two hexaploid wheat (*Triticum aestivum* L.) cultivars differing in salinity tolerance. **Flora**, v.207, p.388-397, 2012. DOI: https://doi.org/10.1016/j.flora.2012.03.004.

BATES, L.S.; WALDREN, R.P.; TEARE, I.D. Rapid determination of free proline for water-stress studies. **Plant and Soil**, v.39, p.205-207, 1973. DOI: https://doi.org/10.1007/BF00018060.

EBRAHIM, F.; ARZANI, A.; RAHIMMALEK, M.; SUN, D.; PENG, J. Salinity tolerance of wild barley *Hordeum vulgare* ssp. *spontaneum*. **Plant Breeding**, v.139, p.304-316, 2019. DOI: https://doi.org/10.1111/pbr.12770.

FAROOQ, M.; HUSSAIN, M.; WAKEEL, A.; SIDDIQUE, K.H.M. Salt stress in maize: effects, resistance mechanisms, and management. A review. **Agronomy for Sustainable Development**, v.35, p.461-481, 2015. DOI: https://doi.org/10.1007/s13593-015-0287-0.

GUAN, C.; HUANG, Y.-H.; CUI, X.; LIU, S.-J.; ZHOU, Y.-Z; ZHANG, Y.-W. Overexpression of gene encoding the key enzymes involved in proline-biosynthesis (*PuP5CS*) to improve salt tolerance in switchgrass (*Panicum virgatum* L.). **Plant Cell Reports**, v.37, p.1187-1199, 2018. DOI: https://doi.org/10.1007/s00299-018-2304-7.

HASHEMIPETROUDI, S.H.; AHMADIAN, G.; FATEMI, F.; NEMATZADEH, G.; YAMCHI, A.; KUHLMANN, M. Ion content, antioxidant enzyme activity and transcriptional response under salt stress and recovery condition in the halophyte grass *Aeluropus littoralis*. **BMC Research Notes**, v.15, art.201, 2022. DOI: https://doi.org/10.1186/s13104-022-06090-4.

HESTER, M.W.; MEDELSSOHN, I.A.; MCKEE, K.L. Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physiological constraints. **Environmental and Experimental Botany**, v.46, p.277-297, 2001. DOI: https://doi.org/10.1016/S0098-8472(01)00100-9.

HISCOX, J.D.; ISRAELSTAM, G.F. A method for the extraction of chlorophyll from leaf tissue without maceration. **Canadian Journal of Botany**, v.57, p.1332-1334, 1979. DOI: https://doi.org/10.1139/b79-163.

JAMES, R.A.; DAVENPORT, R.J.; MUNNS, R. Physiological characterization of two genes for Na$^+$ exclusion in durum wheat, *Nax1* and *Nax2*. **Plant Physiology**, v.142, p.1537-1547, 2006. DOI: https://doi.org/10.1104/pp.106.086538.

KIM, J.; LIU, Y.; ZHANG, X.; ZHAO, B.; CHILDS, K.L. Analysis of salt-induced physiological and proline changes in 46 switchgrass (*Panicum virgatum*) lines indicates multiple response modes. **Plant Physiology and Biochemistry**, v.105, p.203-212, 2016. DOI: https://doi.org/10.1016/j.plaphy.2016.04.020.

KOYRO, H.-W.; HUSSAIN, T.; HUCHZERMEYER, B.; KHAN, M.A. Photosynthetic and growth responses of a perennial halophytic grass *Panicum turgidum* to increasing NaCl concentrations. **Environmental and Experimental Botany**, v.91, p.22-29, 2013. DOI: https://doi.org/10.1016/j.envexpbot.2013.02.007.

LIU, L.; HAN, G.; NAGAOKA, T.; SANEOKA, H. A comparative study of the growth and physiological parameters of two oat (*Avena sativa* L.) lines under salinity stress. **Soil Science and Plant Nutrition**, v.66, p.847-853, 2020. DOI: https://doi.org/10.1080/00380768.2020.1820756.

MALAVOLTA, E.; VITTI, G.C.; OLIVEIRA, S.A. de. **Avaliação do estado nutricional das plantas**: princípios e aplicações. Piracicaba: Associação Brasileira para Pesquisa da Potassa e do Fosfato, 1989. 201p.

MUNNS, R.; TESTER, M. Mechanisms of salinity tolerance. **Annual Review of Plant Biology**, v.59, p.651-681, 2008. DOI: https://doi.org/10.1146/annurev.arplant.59.032607.092911.

PARIHAR, P.; SINGH, S.; SINGH, R.; SINGH, V.P.; PRASAD, S.M. Effect of salinity stress on plants and its tolerance strategies: a review. **Environmental Science and Pollution Research**, v.22, p.4056-4075, 2015. DOI: https://doi.org/10.1007/s11356-014-3739-1.

POUSTINI, K.; SIOSEMARDEH, A.; RANJBAR, M. Proline accumulation as a response to salt stress in 30 wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. **Genetic Resources and Crop Evolution**, v.54, p.925-934, 2007. DOI: https://doi.org/10.1007/s10722-006-9165-6.

RADY, M.M.; MOHAMED, G.F. Improving salt tolerance in *Triticum aestivum* (L.) plants irrigated with saline water by exogenously applied proline or potassium. **Advances in Plants and Agricultural Research**, v.8, p.193-199, 2018. DOI: https://doi.org/10.15406/apar.2018.08.00312.

SADDIQ, M.S.; IQBAL, S.; HAFEEZ, M.B.; IBRAHIM, A.M.H.; RAZA, A.; FATIMA, E.M.; BALOCH, H.; JAHANZAIB; WOODROW, P.; CIARMIELLO, L.F. Effect of salinity stress on physiological changes in winter and spring wheat. **Agronomy**, v.11, art.1193, 2021. DOI: https://doi.org/10.3390/agronomy11061193.

SHAHZAD, B.; YUN, P.; SHABALA, L.; ZHOU, M.; SELLAMUTHU, G.; VENKATARAMAN, G.; CHEN, Z.-H.; SHABALA, S. Unravelling the physiological basis of salinity stress tolerance in cultivated and wild rice species. **Functional Plant Biology**, v.49, p.351-364, 2022. DOI: https://doi.org/10.1071/FP21336.

SINGH, V.; SINGH, A.P.; BHADORIA, J.; GIRI, J.; SINGH, J.; VINEETH, T.V.; SHARMA, P.C. Differential expression of salt-responsive genes to salinity stress in salt-tolerant and salt-sensitive rice (*Oryza sativa* L.) at seedling state. **Protoplasma**, v.255, p.1667-1681, 2018. DOI: https://doi.org/10.1007/s00709-018-1257-6.

TAO, R.; DING, J.; LI, C.; ZHU, X.; GUO, W.; ZHU, M. Evaluating and screening of agro-physiological indices for salinity stress tolerance in wheat at the seedling stage. **Frontiers in Plant Science**, v.12, art.646175, 2021. DOI: https://doi.org/10.3389/fpls.2021.646175.

VÉRAS, E.L.L.; DIFANTE, G.S.; GURGEL, A.L.C.; COSTA, C.M.; EMERENCIANO NETO, J.V.; RODRIGUES, J.G.; COSTA, A.B.G.; PEREIRA, M.G.; ÍTAVO, L.C.V. Tillering capacity of *Brachiaria* cultivars in the Brazilian Semi-arid Region during the dry season. **Tropical Animal Science Journal**, v.43, p.133-140, 2020. DOI: https://doi.org/10.5398/tasj.2020.43.2.133.

WU, H.; SHABALA, L.; ZHOU, M.; SU, N.; WU, Q.; UL-HAQ, T.; ZHU, J.; MANCUSO, S.; AZZARELLO, E.; SHABALA, S. Root vacuolar Na^+ sequestration but not exclusion from uptake correlates with barley salt tolerance. **Plant Journal**, v.100, p.55-67, 2019. DOI: https://doi.org/10.1111/tpj.14424.

WU, H.; ZHANG, X.; GIRALDO, J.P.; SHABALA, S. It is not all about sodium: revealing tissue specificity and signalling roles of potassium in plant responses to salt stress. **Plant Soil**, v.431, p.1-17, 2018. DOI: https://doi.org/10.1007/s11104-018-3770-y.

YANG, Z.; ZHENG, H.; WEI, X.; SONG, J.; WANG, B.; SUI, N. Transcriptome analysis of sweet sorghum inbred lines differing in salt tolerance provides novel insights into salt exclusion by roots. **Plant Soil**, v.430, p.423-439, 2018. DOI: https://doi.org/10.1007/s11104-018-3736-0.

ZEESHAN, M.; LU, M.; SEHAR, S.; HOLFORD, P.; WU, F. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. **Agronomy**, v.10, art.127, 2020. DOI: https://doi.org/10.3390/agronomy10010127.