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Does brassinosteroid mitigate salt stress in sorghum?

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Abstract

The objective of the present study was to assess the mitigating effect of brassinosteroid regulator plant on damages caused by salt stress in sorghum. The experiment was carried out in pots containing 5 kg of substrate, on benches in a greenhouse covered with transparent plastic film and with a 50% shade screen on the sides. A completely randomized design with four replications was used, in a 2×5 factorial arrangement consisted of application and non-application of brassinosteroids at concentration of 0.1 mg L^{-1} applied at the 30, 37, and 45 days after emergence (DAE) and five salinity levels of the irrigation water (electrical conductivities of 0, 2, 4, 8, and 16 dS m^{-1}). The plants were irrigated with water from the public supplying system up to 45 DAE and, then, with brackish water up to 60 (DAE), when they were evaluated. Our results indicate that Brassinosteroids mitigated damages caused by salt stress by increasing the Ca to Na ratio. Sorghum is moderately resistant to salt stress by presenting a sodium extrusion mechanism activated by calcium. Further studies with longer exposure time to salt stress are recommended for a more thorough understanding and recommendation of the use of brackish water in sorghum crops.

Keywords: Elicitation; Physiology of Production**;** Grains; Plant growth regulator; *Sorghum bicolor* L.

Abbreviations: DAE_days after emergence; dS_decisiemens; pH_hydrogen potential; P_phosphorus; K_Potassium; H_Hydrogen, Al_aluminium; Ca_ calcium; Mg_magnesium; Zn_zinc; Na_sodium; Kc_coefficient of culture; ETc_evapotranspiration of the crop; Reference ETo_Evapotranspiration; Cea_Electrical conductivity of water; NaCl_sodium chloride; SPAD_chlorophyll index; NL_number of leaves, PH_height of plants; E_perspiration; SWR_ stem mass ratio; TB_total biomass; RWR_root mass ratio; S/R_aerial part/root system ratio and LWR_leaf mass ratio.

Introduction

Sorghum is the fifth most grown cereal in the world, after wheat, maize, rice, and barley. This plant has an ecophysiological importance due to its high-water use efficiency, which makes it tolerant to droughts. The high genetic diversity of sorghum has allowed the selection of many cultivars for different functions, from grain consumption to dye production, making this crop to be used as an option for biomass production for several purposes (Perlein et al. 2021).

Sorghum is a staple food in parts of Africa and Asia, different from other countries where it is traditionally used as animal feed. Sorghum grains present compounds with beneficial characteristics to human health, such as absence of gluten, making it attractive for use in foods that benefit human health (Akin et al., 2021).

The economic importance of sorghum in Brazil is connected to the production of raw materials for animal feed production. In the Central-West region, 464,500 hectares of grain sorghum were grown in the 2020-2021 crop season, with a mean yield of 2,472 kg ha^{-1} (Ibge, 2022). Goias is the largest sorghum producing state in Brazil, with 377,900 hectares, representing more than 40% of the national production.

The species *S. bicolor* L. is characterized by its high biomass yield; it is a C_4 plant that presents high adaptability and photosynthetic capacity, which reflect on its energy potential (Prasad et al., 2021). Abiotic stresses, such as water, thermal, light, and salt stresses, are limiting factors for sorghum yield; however, the species has mechanisms of tolerance to these adverse conditions, from changes in leaf and root architectures to stomatal and osmotic regulations (Mwamahonje et al., 2021; Abreha et al., 2022), which enable the plant to support stresses and present a reasonable grain yield (Rajarajan et al., 2021).

Sorghum is grown as a second crop in Brazil in a period of high risk due to conditions of rainfall irregularity and prolonged drought (Batista et al., 2019). Therefore, water deficit and thermal stress commonly occur, as well as significant losses in crop production (Souza et al., 2020). Under these restrictions, sorghum plants develop mechanisms of tolerance through strategies that can ensure their development, such as osmotic adjustment, stomatal regulation, and changes in leaf dynamics (accumulation of wax and winding), to avoid water loss (Sanjari et al., 2021); in addition, they have prolific root systems, which maximize their water absorption capacity (Ndlovu et al., 2021).

Soil salinity is one of the most significant factors for reducing crop yields. Irrigation with brackish water and the carrying of salts from deep soil layers to the surface are problems not only in Brazil, but in the world, mainly in rainfed systems (Ibrahim et al., 2020). The crop development is compromised by the effects of chloride and sodium ions that cause ionic toxicity; it occurs during the movement of these soil nutrients to the plants through the roots, which accumulate in their stems and leaves. The quantities of sodium and chloride denote the salinity level (Mariussi et al., 2019).

Growth regulators can act as elicitors in plants and, thus, stimulate genes of defense against pests, diseases, and abiotic stresses (Mao et al., 2012). These substances can be used in seed treatment and applied through leaf to make the plant to express the resistance. The number of researches on brassinosteroids has increased because of their hormonal effects on plant species. However, brassinosteroids were recently studied regarding plant response to water stress, dependent and independent of abscisic acid synthesis. Thus, the use of brassinosteroids as substances that can promote resistance to abiotic factors is important (Ahammed et al., 2015). In this sense, the objective of the present study was to assess the mitigating effect of brassinosteroids on damages caused by salt stress in sorghum.

Results

Physiological variables

The analysis of variance and mean test for chlorophyll index (SPAD), transpiration (*E*), stem weight ratio, plant height (PH), number of leaves (NL), and leaf potassium contents (K) are shown in Table 1. The interaction between the factors (salinity and brassinosteroid) were not significant, thus, the factors were analyzed individually. The salinity significantly affected transpiration, stem weight ratio, and leaf potassium contents; however, only transpiration fitted to the regression model, as shown in Figure 1.

Stem weight ratio was significantly affected by brassinosteroid application; it was 10% higher in the plants with brassinosteroid application. The other variables were not significantly affected by this factor. According to Matos et al. (2019), absence of significance for some variables in tests with abiotic stresses carried out with containers inside greenhouses (controlled environment) is common because of two factors: the short time of exposure of plants to the stresses and the existence of some plant resistance to the stress. This interpretation applies to the present study, as the sorghum plants under stress at the initial growth stage presented changes for some variables connected to activation of protection mechanisms in detriment to changes in variables connected to plant development.

The analysis of variance for calcium (Ca) and sodium (Na) contents, and Na to potassium ratio (Na/K), Ca to Na ratio (Ca/Na), total biomass, leaf and root weight ratios, and shoot to root ratio is shown in Table 2. The interaction between the factors (salinity and brassinosteroid) was significant for all these variables, denoting that the levels of one factor depends on the levels of the other and a dependency between them. However, only Ca contents, Na/K, and Ca/Na presented a significant fit to regression models.

Nutritional variables

The results of the regression analysis for transpiration (E), leaf Ca contents (Ca), Na/K, and Ca/Na are shown in Figure 1. There was no significant difference between treatments with application and non-application of brassinosteroids, thus, the mean data of transpiration and Na/K were used. The transpiration decreased sharply as the salinity of the irrigation water was increased. This result denotes the effect on the plant water status, as the decreases in the osmotic potential of the solution decrease the water potential, resulting in decreases in absorption of soil solution. In this situation, the plant reduces the stomatal opening to minimize water loss and avoid dehydration. The results are consistent with those found by Amaro et al. (2018), who reported decreases in transpiration in plants of *Jatropha curcas* L subjected to irrigation with brackish water.

Leaf Ca contents increased as the electrical conductivity of the irrigation water was increased; the plants with no application of brassinosteroids presented higher increases in Ca contents, denoting that the regulator had no positive mitigating effect on salt stress. However, the increases in Ca denote the existence of a protective mechanism in sorghum, as this element is a secondary messenger and activator of a detox mechanism in the plasma membrane that promotes sodium efflux, as reported by Taiz et al. (2017).

Discussion

According to Amombo et al. (2022), the movement of Ca from connection sites in the plasma membrane causes damages to sorghum plants and may result in a non-activation of the detox mechanism. However, the increases in Ca found in the present study denoted that there was no movement of this nutrient, thus, the decrease in Na/K with increasing salinity was probably due to activation of this detox mechanism mediated by Ca. According to Calone et al. (2020), the integrity of the plasma membrane in sorghum plants is confirmed by decreases in Na/K.

The high Ca contents in the sorghum plants are due to the plant's defense mechanism, which optimizes the efflux of salts and preserve the K contents. According to Matos et al. (2017), a Na/K lower than 1.2 indicates the resistance of plants to salt stress; in the present study, this ratio was always below 0.6, denoting the resistance of sorghum plants to salt stress due to the important Na efflux mechanism activated by Ca. The sorghum capacity to maintain adequate K contents, Na/K, and Ca/Na is a strong indicator of resistance to salt stress (Shakeri et al., 2020). The increase in Ca/Na confirms this argument, as it represents an increase in Ca in detriment of Na absorption.

The analysis of canonical variables showed a variation of 94.6% in the data and indicated the ordering of variables into two quadrants in relation to the axis 1 (Figure 2); variables whose increases represent plant protection (Ca contents and Ca/Na) are on the right, close to the salinity treatment of 16 dS m^{-1}, whereas the variables related to growth (root weight ratio, transpiration, plant height, and SPAD index) are on the left, close to zero salinity. These results confirm previous results and indicate the existence of an important defense mechanism in sorghum regarding resistance to salt stress.

The results denote that the application of brassinosteroids had a low mitigating effect on damages caused by salt stress in sorghum plants. However, the plant growth regulator was associated with high Ca/Na, i.e., high Ca contents in detriment of Na absorption; thus, further studies with longer

Table 1. Analysis of variance and mean test for chlorophyll index (SPAD), transpiration (E), stem weight ratio (SWR), plant height (PH), number of leaves (NL), and potassium (K) in sorghum grown under irrigation with brackish water and application of brassinosteroids, Ipameri, Goiás, Brazil.

		Mean squares					
Source of variation	DF	SPAD	*E* ($g\ H_2O\ day^{-1}$)	SWR (%)	PH (cm)	NL	K (ppm)
T1 (BW)	4	295.8^{ns}	6653.2*	287.4*	55.2^{ns}	8.5^{ns}	169.1*
T2 (BR)	1	1.8^{ns}	32.6^{ns}	51.96*	11.7^{ns}	2.6^{ns}	8.7^{ns}
BRs × BW	4	182.7^{ns}	1905.9^{ns}	89.2^{ns}	78.6^{ns}	13.0^{ns}	54.9^{ns}
Residue	30	1613.6	11962.5	325.1	570.9	84.0	163.2
CV (%)		15.3	26.5	15.8	17.3	19.7	15.1
Brassinosteroids		Means					
With		46.52a	74.26a	21.96a	24.67a	8.25a	15.95a
Without		46.11a	76.07a	19.68b	25.75a	8.75a	15.02a

DF = degrees of freedom; BW = brackish water treatment; BR = brassinosteroid treatment; * = significant at 5% probability; ns = not significant by the F test.

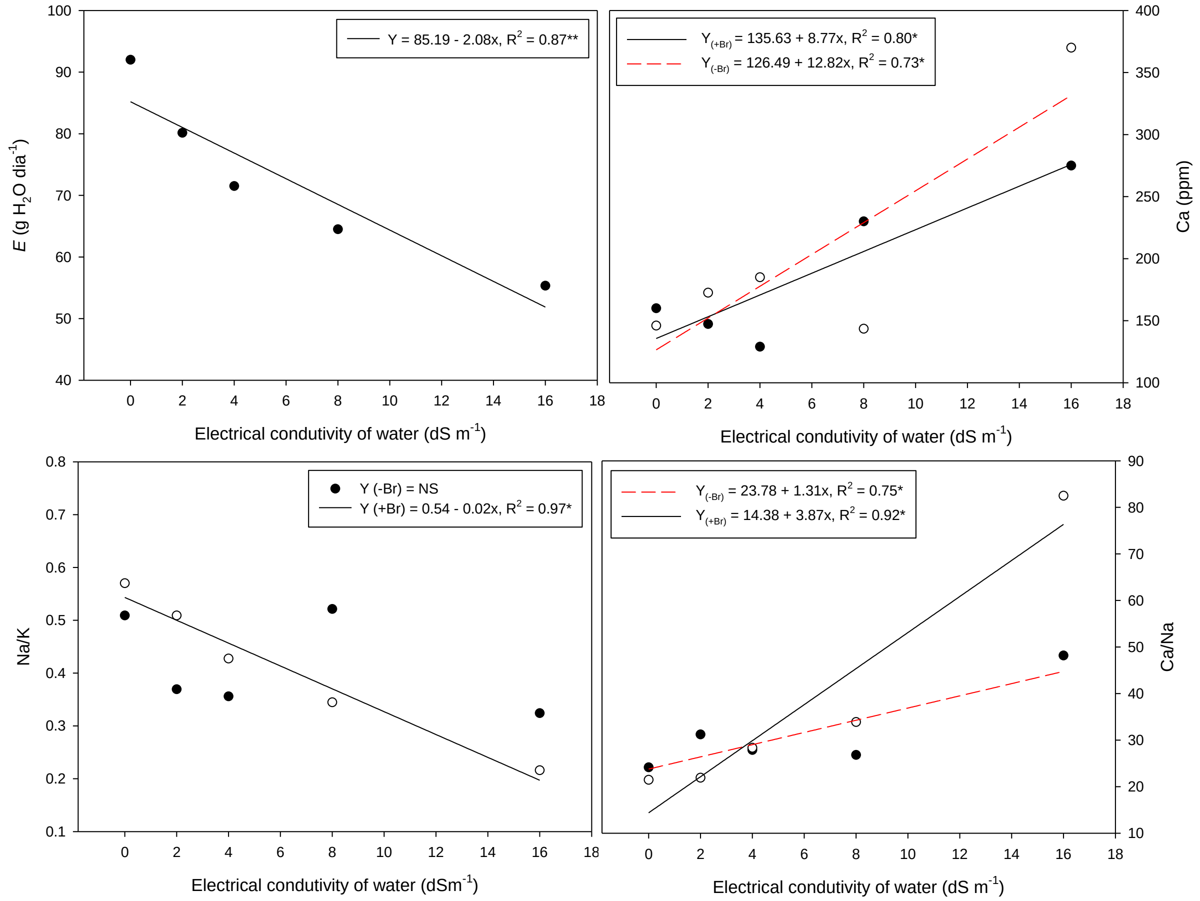

Figure 1. Regression equations for transpiration (E), leaf calcium contents (Ca), sodium to potassium ratio (Na/K), and Ca to Na ratio (Ca/Na) in sorghum plants irrigated with brackish water and subjected to application of brassinosteroids. * = significant at 5% probability by the Tukey's test. Black and red lines correspond to application and non-application of brassinosteroid, respectively.

Table 2. Analysis of variance for calcium (Ca) and sodium (Na) contents, sodium to potassium ratio (Na/K), Ca to Na ratio (Ca/Na), total biomass (TB), leaf (LWR) and root (RWR) weight ratios, and shoot to root ratio (S/R) in sorghum grown under irrigation with brackish water and application of brassinosteroids (BR), Ipameri, Goiás, Brazil.

Source of Variation	DF	Mean squares							
		Ca	Na	Na/K	Ca/Na	TB	LWR	RWR	S/R
T1 (BW)	4	16x10^{4*}	17.6*	0.31*	0.00^{ns}	38.3^{ns}	390.5*	1345.4*	1.61*
T2 (BR)	1	2295^{ns}	0.00^{ns}	0.00^{ns}	0.00*	257.8*	317.9*	626.9*	1.16*
BR × BW	4	38658*	65.9*	0.14*	0.00*	216.0*	270.6*	592.9*	0.95*
Residue	30	16893	42.4	0.79	0.00	152.2	563.8	1194.2	2.76
CV (%)	-	12.12	19.2	17.67	24.66	14.17	15.06	12.52	15.16

DF = degrees of freedom; BW = brackish water treatment; BR = brassinosteroid treatment; * = significant at 5% probability; ns = not significant by the F test.

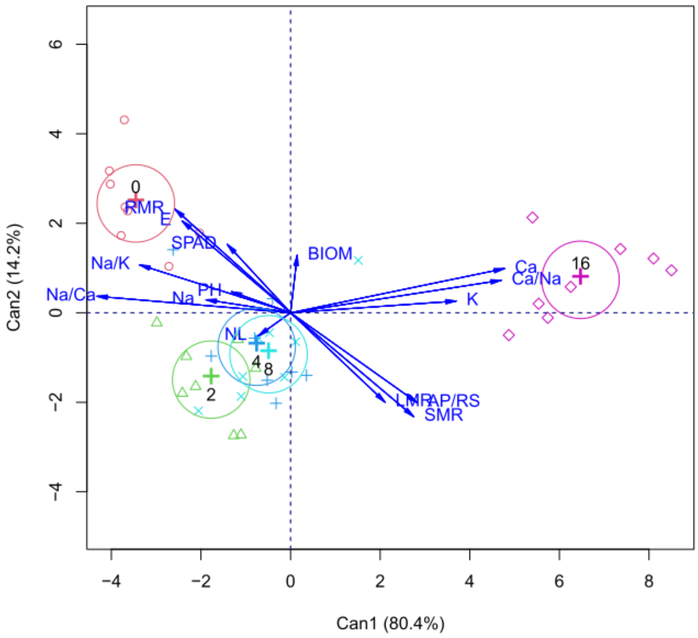

Figure 2. Analysis of canonical variables for ordering the data for root weight ratio (RWR); transpiration (E); chlorophyll index (SPAD); ratios between sodium and potassium (Na/K), sodium and calcium (Na/Ca), calcium and sodium (Ca/Na), and shoot and root (S/R); total biomass (TB); plant height (PH); number of leaves (NL); leaf sodium (Na), potassium (K), and calcium (Ca) contents; and leaf (LWR) and stem (SWR) weight ratios in sorghum plants irrigated with brackish water and subjected to application of brassinosteroids.

longer exposure times to salt stress may prove an important elicitor function for this mitigation. The sorghum plants presented no toxicity symptoms when irrigated with brackish water and little variation in growth; thus, they are at least moderately resistant to salt stress.

Material and methods

Cultivation conditions

The experiment was conducted from March to May 2022, in a greenhouse covered with transparent plastic film, with a 50% shade screen on the sides, at the State University of Goiás, in Ipameri, GO, Brazil (17°43'19''S, 48°09'35''W, and altitude of 764 m). The climate of the region is tropical, with a dry winter and a wet summer, according to the Köppen classification, presenting a mean temperature of 20 °C and two well-defined seasons: a rainy season from October to March, and a dry season from April to September (Souza et al., 2021).

Design experimental

A completely randomized design with four replications was used, in a 2×5 factorial arrangement consisted of application and non-application of brassinosteroids at concentration of 0.1 mg L^{-1} applied at the 30, 37, and 45 days after emergence, and five salinity levels of the irrigation water (electrical conductivities of 0, 2, 4, 8, and 16 dS m^{-1}). The experiment was carried out in polyethylene pots containing 5 kg of substrate, on benches, totaling 40 pots; the substrate was composed of a mixture of Typic Hapludox (Latossolo Vermelho-Amarelo Distroferrico; Santos et al., 2018), sand, and bovine manure at the proportion of 3:1:0.5, respectively.

The chemical analysis of the substrate presented the following values: pH ($CaCl_2$) 5.7; 17 g dm^{-3} of organic matter; 28.3 mg dm^{-3} of P; 0.42 cmolc dm^{-3} of K (Mehlich^{-1}); 1.7 cmolc dm^{-3} (SMP Buffer) of H + Al; 2.1 cmolc dm^{-3} of Ca; 1.3 cmolc dm^{-3} of Mg; 10.1 mg dm^{-3} of Zn; 9.86 cmolc dm^{-3} of carbon; 5.52 cmolc dm^{-3} of cation exchange capacity; and 69.20% base saturation. Soil fertilizers were applied 7 days after emergence (DAE), according to agronomic recommendations for sorghum crops (Souza et al., 2020).

The seedlings were irrigated daily with a water volume corresponding to 100% of the daily evapotranspiration. The crop coefficient (Kc) was not determined for sorghum crops grown in the region of Ipameri, thus, a Kc of 1.00 was used, as recommended by the FAO 56 (ALLEN et al., 1998) for several crops at initial developmental stages.

The water volume used was calculated by determining the reference evapotranspiration and the crop coefficient, according to the equation $ETc = ETo \times Kc$, where *ETc* is the crop evapotranspiration, *Kc* is the crop coefficient, and *ETo* is the reference evapotranspiration.

The daily ETo was calculated through the Penman-Monteith method, as recommended by the FAO (SMITH et al., 1991), using daily data of maximum and minimum air temperatures, relative air humidity, insolation, and wind speed collected by a meteorological station of the Brazilian National Institute of Meteorology (INMET) installed in Ipameri.

Three seeds of the sorghum cultivar Brevant 1G100 were sown and a thinning was carried out at 7 DAE, keeping one plant per pot. NaCl was added to the water supply to obtain the solutions with different electrical conductivities; the quantities (Q) were determined by the equation $Q\ (mg\ L^{-1}) = 640 \times CEa\ (dS\ m^{-1})$ (Rhoades et al., 2000), where *CEa* is the desired water electrical conductivity. The conductivities were verified and confirmed using a conductivity meter. The plants were irrigated with public water up to 45 DAE and, then, with brackish water up to 60 DAE, when they were evaluated.

Occurrences of pests were found during the experiment period, which included *Melanaphis sacchari*, *Rhopalosiphum maidis*, *Dalbulus maidis*, and *Spodoptera frugiperda.* The control of these pests was carried out using lambda-cyhalothrin (250 mL ha^{-1}), fenpropathrin and acetamiprid (700 mL ha^{-1}), and lufenuron (300 mL ha^{-1}), which were applied in two moments: at 25 and 45 DAE.

Growth variables

The plant height (mm) was measured from the stem base to the apex with the aid of a ruler. Stem diameter was measured at the stem base using a digital caliper. The number of leaves was counted. Leaf area (cm^2) was determined using an area meter (LI-3100, LI-COR, Lincoln, USA). Leaves, roots, and stems were separated and dried in an oven at 72 °C for 72 hours to reach a constant weight and, then, weighed separately.

The total biomass weight was obtained by summing the weights of all plant parts; the ratios between total biomass and leaf, stem, and root weights were estimated by dividing the weight of the individual organs by the total biomass weight. The chlorophyll index (SPAD) was determined in fully expanded leaves with the aid of a portable chlorophyll meter.

Transpiration rate

total daily transpiration of plants was calculated by the difference between the weights of the pots. The pots were placed in transparent plastic bags fixed with a rubber band at the plant stem, leaving only the shoot (leaves and stem) outside the bag; the pots with plant in the plastic bags were, then, weighed and, after 24 hours, weighed again. The total transpiration was estimated as the difference between the initial and final weights.

Nutritional variables

potassium, calcium, and sodium concentrations in sorghum leaves were determined in fully developed leaves. The leaves were macerated with 5 mL of distilled water, and aliquots of the solution were used for readings in a portable analyzer; the values were obtained in parts per million (ppm).

The data were subjected to analysis of variance and the differences between treatments were evaluated using the Tukey's test. Linear and quadratic regression analyses with the respective coefficients of determination (R^2) were caried out using the statistical program SigmaPlot 10.0 (SYSTAT SOFTWARE, 2006), and the program RBio (BHERING, 2017) was used for the canonical variables.

Conclusions

The application of brassinosteroids mitigated damages caused by salt stress in sorghum plants by increasing the calcium to sodium ratio. Sorghum is moderately resistant to salt stress by presenting a sodium efflux mechanism activated by calcium. Further studies with longer exposure times to salt stress are recommended for a more thorough understanding and recommendation of the use of brackish water in sorghum crops.

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