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Nitrogen sources in the formation of sour passion fruit seedlings under saline water irrigation

Fontes de nitrogênio na formação de mudas de maracujazeiro-azedo sob irrigação com águas salinas

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Highlights

Calcium nitrate and ammonium sulfate increased electrolyte leakage.
Ammonium sulfate increased plant height growth and leaf number.
Irrigation with 3.4 dS m^{-1} water produces seedlings with acceptable field quality.

Abstract

In the semiarid region of Northeast Brazil, due to irregular rainfall and high precipitation rates, water sources with high levels of dissolved salts are common, representing a limiting factor for the cultivation of species sensitive to salt stress. In this context, this study aimed to evaluate the effects of different nitrogen sources on the physiological aspects, growth, and quality of sour passion fruit seedlings irrigated with saline water. The research was conducted in a greenhouse belonging to the Agricultural Engineering Academic Unit of UFCG, in Campina Grande, Paraíba, Brazil. A randomized complete block experimental design was used in a 4 × 4 factorial arrangement. Treatments consisted of four irrigation water electrical conductivity levels (ECw: 0.4, 1.4, 2.4, and 3.4 dS m^{-1}) and four nitrogen sources (NS) (urea, calcium nitrate, ammonium sulfate, and ammonium chloride), with four replicates and two plants per plot. Irrigation with ECw levels above 0.4 dS m^{-1} increased electrolyte leakage and reduced transpiration, stomatal conductance, chlorophyll b, stem and leaf dry matter, and the Dickson quality index of seedlings, regardless of the nitrogen source supplied. Ammonium sulfate fertilization increased

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relative water content, leaf number, intercellular CO_2 concentration, CO_2 assimilation rate, instantaneous carboxylation efficiency, instantaneous water use efficiency, and seedling height. Calcium nitrate supplementation enhanced intercellular CO_2 concentration, leaf and total dry matter, and photosynthetic pigments in passion fruit seedlings. Urea fertilization increased seedling leaf area under irrigation with water salinity of 0.4 dS m^{-1}. Ammonium sulfate fertilization is recommended for passion fruit cultivation irrigated with water of 3.4 dS m^{-1} during the seedling formation stage.
Key words: Mineral nutrition. Nitrogen fertilization. *Passiflora edulis* Sims. Salt stress.

Resumo

No semiárido do Nordeste do Brasil devido a irregularidade de precipitações e elevadas taxas de precipitações é comum a ocorrência de fontes hídricas com níveis elevados de sais dissolvidos, destacando-se como um fator limitante para o cultivo de espécies sensíveis ao estresse salino. Neste contexto, objetivou-se com este estudo avaliar fontes de nitrogênio nos aspectos fisiológicos, crescimento e qualidade de mudas de maracujazeiro-azedo cultivadas sob irrigação com águas salinas. A pesquisa foi conduzida em casa de vegetação pertencente à Unidade Acadêmica de Engenharia Agrícola da UFCG, em Campina Grande – PB, utilizando-se o delineamento experimental de blocos casualizados, em esquema fatorial 4 × 4, sendo quatro níveis de condutividade elétrica da água de irrigação - CEa (0,4; 1,4; 2,4 e 3,4 dS m^{-1}) e quatro fontes de nitrogênio – FN (ureia, nitrato de cálcio, sulfato de amônio e cloreto de amônio), com quatro repetições e duas plantas por parcela. A irrigação com CEa acima de 0,4 dS m^{-1} aumentou o extravasamento de eletrólitos e, reduziu a transpiração, a condutância estomática, a clorofila b, a fitomassa seca de caule e de folhas, e o índice de qualidade de Dickson das mudas, independente da fonte nitrogenada fornecida. A adubação com sulfato de amônio aumentou o conteúdo relativo de água, o número de folhas, a concentração interna de CO_2, a taxa de assimilação de CO_2, a eficiência instantânea de carboxilação, a eficiência instantânea no uso da água e a altura de plantas das mudas. O fornecimento de nitrato de cálcio aumentou a concentração interna de CO_2, a fitomassa seca de folhas e total, e os pigmentos fotossintéticos das mudas de maracujazeiro-azedo. A adubação com ureia aumentou a área foliar das mudas sob salinidade da água de 0,4 dS m^{-1}. Recomenda-se adubação com sulfato de amônio para o cultivo de maracujazeiro-azedo irrigado com água de 3,4 dS m^{-1} durante a fase de formação de mudas.
Palavras-chave: Adubação nitrogenada. Estresse salino. Nutrição mineral. *Passiflora edulis* Sims.

Introduction

The passion fruit (*Passiflora edulis* Sims), belonging to the family Passifloraceae, is a fruit tree native to Tropical America and cultivated commercially in several countries with predominantly tropical and subtropical climates (B. R. Lima et al., 2020a). This species stands out for the quality of its fruits, which can be consumed fresh or processed into various byproducts for the agricultural industry (Santos et al., 2017).

Despite its economic importance in Northeast Brazil, water scarcity remains a limiting factor for the expansion of irrigated fruit cultivation. This constraint results from irregular rainfall, ranging from 240 to 800 mm per year, combined with high temperatures and evapotranspiration rates, which accelerate the reduction of water in reservoirs (Melo et al., 2019). Consequently, the use of saline water for irrigation becomes necessary, especially during dry seasons, which prevail throughout most of the year in the semiarid region of Northeast Brazil. However, irrigation with high-salinity water promotes ion accumulation in the root zone, increasing the risk of soil salinization (Sousa et al., 2020).

Irrigation with saline water can induce physiological and morphological alterations due to osmotic effects, which inhibit growth by reducing water and nutrient uptake; ionic effects, resulting from excessive ion accumulation in plant tissues that causes toxicity; and nutritional imbalance, arising from the preferential absorption of toxic ions over essential nutrients (G. S. de Lima et al., 2019; Mendonça et al., 2022).

To mitigate the deleterious effects of salt stress, alternative management strategies are required. Nitrogen fertilization represents a promising approach to alleviating salt stress (Nobre et al., 2012; A. M. da S. Silva Neta et al., 2020). Nitrogen is a macronutrient involved in plant metabolism and development, as it participates in cell division and expansion within the photosynthetic tissues and is a fundamental component of amino acids, proteins, nucleic acids, and chlorophyll (M. H. da Silva et al., 2024; Zayed et al., 2023). Nevertheless, the beneficial effects of nitrogen fertilization depend on the fertilizer source used.

Several studies have investigated the effects of saline irrigation on passion fruit during the seedling formation phase (Cavalcante et al., 2011; A. A. R. da Silva et al., 2019; Lima et al., 2020b; A. M. da S. Silva Neta et al., 2022). M. A. F. Bezerra et al. (2019), evaluating nitrogen fertilization as a means of mitigating salt stress in passion fruit, concluded that seedlings with the highest quality index were obtained under water salinity of 2.1 and 2.5 dS m^{-1} when fertilized with ammonium sulfate and urea, respectively. M. B. Pereira et al. (2024) found that nitrogen and potassium fertilization rates between 100% and 120% improved absolute growth rates in height and stem diameter, chlorophyll b content, shoot dry matter, and total dry matter of passion fruit seedlings subjected to salt stress.

However, studies evaluating the specific effects of different nitrogen sources on mitigating salt stress in passion fruit remain scarce. Based on this gap, the present study was conducted under the hypothesis that the effectiveness of nitrogen fertilization in reducing salt stress in passion fruit depends on the fertilizer source, as each has distinct physical and chemical properties. Therefore, this study aimed to evaluate the effects of different nitrogen sources on the physiological aspects, growth, and quality of passion fruit seedlings irrigated with saline water during the seedling formation phase.

Material and Methods

The experiment was conducted in a greenhouse from September to December 2023 at the Agricultural Engineering Academic Unit (UAEA) of the Federal University of Campina Grande (UFCG), in Campina Grande, Paraíba, Brazil, located at 7º 15′18″ S, 35º 52′28″ W, and at an altitude of 550 m. The climate of the region is classified as tropical SA with a dry season (Alvares et al., 2013). Air temperature (maximum and minimum) and relative humidity inside the greenhouse during the experimental period were monitored using a thermohygrometer, as shown in Figure 1.

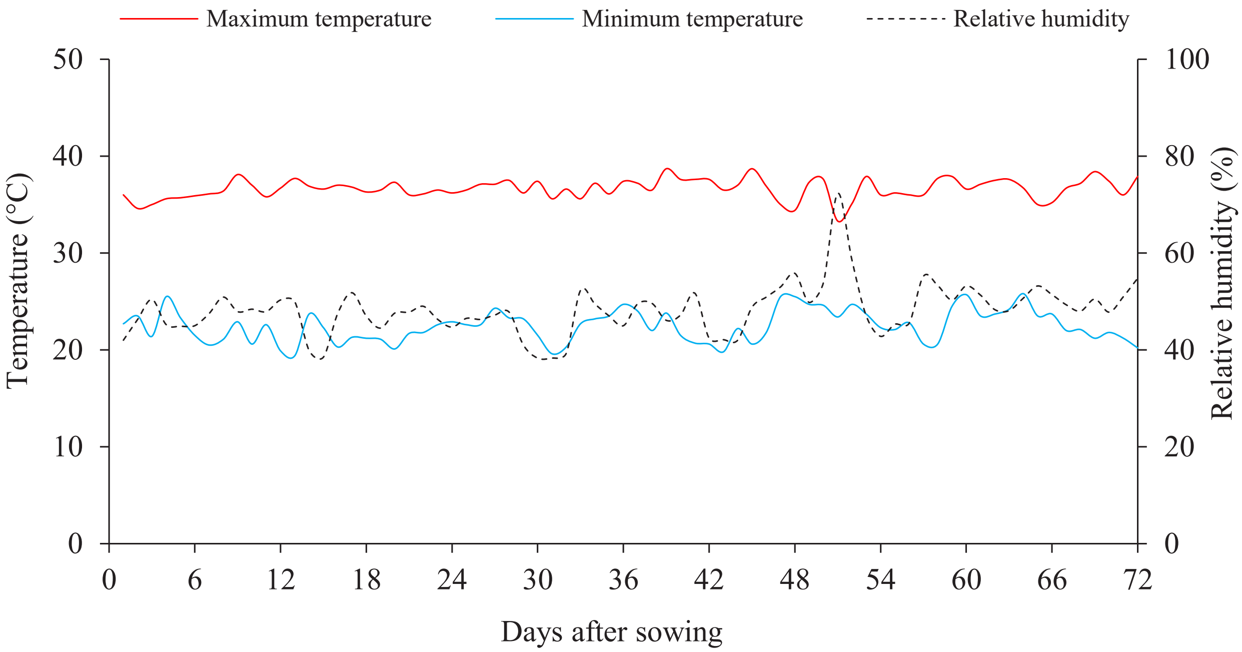

Figure 1. Maximum and minimum temperatures and relative humidity inside the greenhouse from September to December 2023.

Treatments consisted of four irrigation water electrical conductivity levels (ECw) (0.4, 1.4, 2.4, and 3.4 dS m^{-1}) and four nitrogen sources (NS) (urea, calcium nitrate, ammonium sulfate, and ammonium chloride), arranged in a 4 × 4 factorial scheme and distributed in a randomized complete block design with four replicates and two plants per plot. The ECw levels were established according to the study of Andrade et al. (2019).

Passion fruit seedlings were obtained from seeds of the cultivar BRS Gigante Amarelo (BRS GA1). Sowing was performed using three seeds spaced equidistantly at a depth of 1 cm in 10 × 25 cm polyethylene bags with a capacity of 3 kg. The bags were filled with a substrate composed of sandy loam soil, sand, and aged cattle manure at a 2:1:1 ratio (v/v/v). Ten days after sowing (DAS), thinning was carried out, leaving one plant per bag the one showing the greatest vigor.

The soil used was collected at a depth of 0-20 cm in an area of the municipality of Lagoa Seca, Paraíba. Its physical and chemical properties (Table 1) were determined according to the methodology of Teixeira et al. (2017). The bags were arranged equidistantly and placed on benches at a height of 0.80 m above the ground.

Table 1
Chemical and physical-hydric characteristics of the soil used in the experiment

Chemical characteristics								
pH (H_2O)	OM	P	K^+	Na^+	Ca^+	Mg^{2+}	Al^{3+}	H^+
1:2.5	g dm^{-3}	mg dm^{-3}	.....................cmolc kg^{-1}.....................					
5.40	17.62	2.92	0.28	0.04	1.87	1.70	0.20	2.85
Chemical characteristics				Physical characteristics				
EC_{se}	CEC	SAR_{se}	ESP	Particle fraction (g kg^{-1})			Moisture (dag kg^{-1})	
dS m^{-1}	$cmol_c$ kg^{-1}	(mmol L^{-1})$^{0.5}$	%	Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
0.72	6.94	0.03	0.58	675.2	221.8	103	12.94	5.32

pH - potential of hydrogen; OM - organic matter, Walkley-Black wet digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl, pH 7.0; Na^+ and K^+ extracted using 1 M NH_4OAc, pH 7.0; Al^{3+} and H^+ extracted with 0.5 M CaOAc, pH 7.0; EC_{se} - electrical conductivity of the saturation extract; CEC - cation-exchange capacity; SAR_{se} - sodium adsorption ratio of the saturation extract; ESP - exchangeable sodium percentage.

Irrigation waters were prepared by dissolving sodium chloride (NaCl), calcium chloride ($CaCl_2 \cdot 2H_2O$), and magnesium chloride ($MgCl_2 \cdot 6H_2O$) in an equivalent ratio of 7:2:1 the predominant ratio in water sources used for irrigation in the northeastern region of Brazil (L. G. de A. Silva Jr. et al., 1999) using water from the local supply system of Campina Grande, Paraíba. The relationship between ECw and salt concentration (Richards, 1954) was used to prepare the saline waters, as expressed by Eq. 1:

$$A = 10 \times ECw \qquad (1)$$

where A - amount of salts to be applied (mmolc L^{-1}); ECw - electrical conductivity of the water (dS m^{-1}).

After preparation, the irrigation waters were analyzed and their ECw adjusted as desired. Before sowing, soil moisture was raised to field capacity using water with the lowest salinity level (0.4 dS m^{-1}). Irrigation was then performed manually every day at 17:00 h, applying to each bag the volume of water necessary to maintain soil moisture near field capacity. Irrigation with the respective saline waters began when the seedlings developed their first pair of definitive leaves. The volume applied to each bag was determined using the water balance method, as shown in Eq. 2:

$$VI = \frac{(Vp-Vd)}{(1-LF)} \qquad (2)$$

where VI - volume of water applied in the irrigation event (mL); Vp - volume applied in the previous irrigation event (mL); Vd - volume of water drained after the previous irrigation event (mL); LF - leaching fraction (0.10), adopted to prevent excessive salt accumulation in the soil.

NPK fertilization was performed according to the recommendations of Novais et al. (1991), applying 100, 150, and 300 mg kg^{-1} of soil of N, K_2O, and P_2O_5, respectively. Nitrogen was supplied according to each treatment [urea (N1), calcium nitrate (N2), ammonium sulfate (N3), and ammonium chloride (N4)] and applied via irrigation water in three equal splits at 34, 48, and 62 DAS. A nitrification inhibitor (dicyandiamide) was added to ammoniacal sources at 10% of total nitrogen (NH_4^+) to minimize nitrogen transformation in the soil.

Phosphorus and potassium fertilization were split into two applications: P_2O_5 at 30 and 44 DAS, and K_2O at 32 and 46 DAS, using monoammonium phosphate (MAP) and potassium sulfate (K_2SO_4) as sources. Micronutrient fertilization was carried out via foliar application of 0.5 g L^{-1} of Dripsol Micro Rexene® Equilíbrio [1.2% (Mg); 0.85% (B); 3.4% (Fe); 4.2% (Zn); 3.2% (Mn); 0.5% (Cu); 0.06% (Mo)] at 15 and 30 days after emergence.

At 69 DAS, the following growth and physiological parameters were evaluated: plant height (PH), measured from the collar to the insertion of the apical meristem; stem diameter (SD), measured 5 cm above the collar; leaf area (LA); relative water content (RWC); and electrolyte leakage (%EL) in leaf blades. Chlorophyll a (*Cla*), b (*Clb*), total chlorophyll (*Clt*), and carotenoid contents were determined, as well as leaf gas exchange parameters, including stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), CO_2 assimilation rate (A, μmol CO_2 m^{-2} s^{-1}), intercellular CO_2 concentration (Ci, μmol CO_2 mol^{-1}), instantaneous water use efficiency (*WUEi = A/E*) [(μmol CO_2 m^{-2} s^{-1})/(mmol H_2O m^{-2} s^{-1})], and instantaneous carboxylation efficiency (*CEi = A/Ci*) [(μmol CO_2 m^{-2} s^{-1})/(μmol CO_2 mol^{-1})]. Dry matter accumulation and the Dickson quality index (DQI) were also determined.

Leaf area (LA) was obtained by measuring the length and width of each leaf with a ruler and calculating it according to Cavalcante et al. (2002), as shown in Eq. 3:

$$LA = \sum 0.81 * x \text{ ....(3)}$$

where LA = leaf area (cm^2); x = product of leaf length and width (cm^2).

The relative water content (RWC) was determined using 10 leaf discs (diameter 113 cm^2) collected from leaves located in the middle third of each plant. The leaf discs were immediately weighed to prevent water loss, yielding the fresh weight (FW) values. The samples were then placed in plastic bags, immersed in distilled water, and stored for 24 h. After this period, the discs were removed, gently dried with paper towels to remove excess water, and weighed to obtain the turgid weight (TW). They were then placed in an oven at approximately 65 ± 3 ºC until reaching a constant weight to determine the dry weight (DW). The RWC was calculated according to Weatherley (1950), using Eq. 4:

$$RWC = \frac{FW - DW}{TW - DW} \times 100 \text{.......................................(4)}$$

where RWC = relative water content (%); FW = fresh weight of leaf discs (g); TW = turgid weight of discs (g); DW = dry weight of discs (g).

Electrolyte leakage (EL) from the leaf blades was determined according to Scotti-Campos et al. (2013). Four leaf discs with a total area of 113 cm² were placed in beakers containing 50 mL of double-distilled water and sealed hermetically with aluminum foil. The beakers were maintained at 25 ºC for 24 h, after which the initial electrical conductivity (Ci) was measured. Subsequently, the beakers were placed in a forced-air oven and heated to 80 ºC for 120 min. After cooling, the final electrical conductivity (Cf) was measured, and the percentage of electrolyte leakage was calculated using Eq. 5:

$$EL\% = \frac{Ci}{Cf} \text{ x } 100 \text{..(5)}$$

where EL% = electrolyte leakage (%); Ci = initial electrical conductivity (dS m^{-1}); Cf = final electrical conductivity (dS m^{-1}).

Photosynthetic pigment contents (chlorophyll a, b, total chlorophyll, and carotenoids) were determined according to the methodology of Porra et al. (1989). Extracts were prepared using 10 leaf discs (total area of 113 cm^2) from the third fully expanded leaf below the apex. Each sample was immersed in 5.0 mL of dimethyl sulfoxide and kept in the dark for 48 h. The extracts were then analyzed in a spectrophotometer at wavelengths of 480, 649, and 665 nm. Chlorophyll and carotenoid contents were calculated using Eqs. 6, 7, and 8, respectively, with results expressed in mg g^{-1} FW.

$$\text{Clorophyll a} = 12.19\text{ABS\_665} - 3.45\text{ABS\_649} \ldots\ldots\ldots\ldots \quad (6)$$

$$\text{Clorophyll b} = 21.99\text{ABS}_{649} - 5.32\text{ABS}_{665} \ldots\ldots\ldots\ldots (7)$$

$$\text{Carotenoids} = ((1000\text{ABS}_{480} - 2.86\text{Cl}\ a - 129.2\ \text{Cl}\ b)/221)) \ldots\ldots (8)$$

Gas exchange measurements were taken between 07:00 and 10:00 h using a portable infrared CO_2 analyzer (IRGA, model LCPro+, ADC BioScientific Ltd.). Measurements were performed under natural air temperature and CO_2 concentration, with an artificial radiation source of 1,200 μmol m^{-2} s^{-1}.

To determine the accumulation of stem (SDB), leaf (LDB), and root (RDB) dry biomass, plants were cut close to the soil surface and separated into leaves, stems, and roots. Each plant component was placed in labeled paper bags and dried in a forced-air oven at 65 ºC until constant weight. The material was then weighed on a precision scale (0.01 g) to obtain LDB, SDB, and RDB (g per plant). Shoot dry biomass was determined by summing LDB and SDB, and total dry biomass (TDB) by summing all plant components.

The Dickson Quality Index (DQI) was calculated using growth data according to Dickson et al. (1960), as shown in Eq. 9:

$$\text{DQI} = \frac{(\text{TDB})}{\left(\frac{\text{PH}}{\text{SD}}\right) + \left(\frac{\text{SDB}}{\text{RDB}}\right)} \ldots\ldots\ldots\ldots (9)$$

where DQI = Dickson Quality Index.

The data obtained were tested for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett test), and subsequently subjected to analysis of variance (ANOVA) using the F-test ($p \leq 0.05$). When significant effects were detected, polynomial regression analysis ($p \leq 0.05$) was applied for the salinity levels, and Tukey's test ($p \leq 0.05$) for the nitrogen sources, using the statistical software SISVAR-ESAL version 5.6 (Ferreira, 2019).

Results and Discussion

A significant interaction between irrigation water salinity levels and nitrogen sources (ECw × NS) was observed for electrolyte leakage (EL) in the leaf blades. Nitrogen sources also had a significant effect on the relative water content (RWC) in the leaves of 'BRS GA1' sour passion fruit seedlings at 69 DAS (Table 2).

Table 2
Summary of analysis of variance for relative electrolyte leakage (EL) and water content (RWC) in the leaf blade of 'BRS GA1' sour passion fruit plants grown under irrigation with different water salinity levels and different nitrogen sources, 69 days after sowing

Source of variation	DF	Mean square	
		EL	RWC
Salinity level (SL)	3	148.6322**	5.3085^{ns}
Linear regression	1	403.5837**	0.5951^{ns}
Quadratic regression	1	39.7372*	11.6110^{ns}
Nitrogen source (NS)	3	265.6625**	112.1659*
Interaction (SL × NS)	9	19.4851*	80.0765^{ns}
Blocks	3	12.5987^{ns}	5.1513^{ns}
Residual	45	9.2188	36.9857
CV (%)		12.32	7.69

DF - degree of freedom; CV - coefficient of variation; *, ** Significant at the 0.05 and 0.01 probability levels, respectively; ^{ns} Not significant.

For EL in the leaf blades (Figure 2A), urea fertilization did not show a satisfactory fit to the tested models, with an average value of 21.77%. Plants fertilized with nitrogen sources N2 and N4 showed a linear increase in electrolyte leakage, with increments of 14.67% and 8.64%, respectively, per unit increase in ECw. Plants fertilized with N3 exhibited the highest EL value (33.06%) under irrigation with 3.1 dS m^{-1} water. Those fertilized with calcium nitrate (N2) showed the lowest EL values. Under irrigation with 3.4 dS m^{-1} water, plants fertilized with calcium nitrate (N2), ammonium sulfate (N3), and ammonium chloride (N4) had higher EL values than those fertilized with urea (N1).

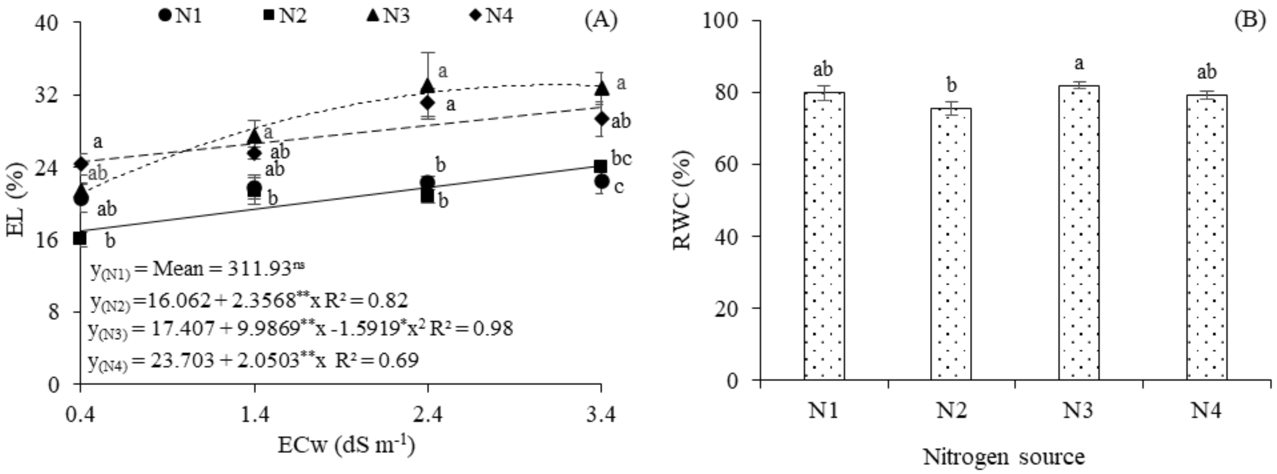

Figure 2. Electrolyte leakage in the leaf blade (EL; A) of 'BRS GA1' sour passion fruit seedlings as a function of the interaction between irrigation water salinity levels (ECw) and nitrogen sources; and relative water content in the leaf blade (RWC; B) as a function of nitrogen sources, 69 days after sowing.
^{ns}, *, ** Not significant ($p > 0.05$) and significant at $p \leq 0.05$ and ≤ 0.01 by the F-test, respectively. Vertical bar represents the standard error of the mean (n = 3); N1- Urea, N2- calcium nitrate, N3- ammonium sulfate, N4- ammonium chloride. Means followed by different letters indicate significant differences between nitrogen sources by Tukey's test ($p \leq 0.05$).

Salinity can cause nutritional imbalances in plants by inhibiting nutrient absorption, including that of calcium, which is a crucial element for cell wall formation. This imbalance can lead to increased EL at high salinity levels (I. L. Bezerra et al., 2022). However, in the present study, calcium nitrate fertilization reduced the percentage of EL in plants, which may be associated with enhanced calcium absorption (Wdowiak et al., 2024). Furthermore, it can be inferred that the increased electrolyte leakage observed in the leaf blades did not result in membrane damage, since electrolyte leakage exceeds 50% when cell membranes are compromised (Sullivan et al., 1971).

Regarding the effects of nitrogen sources on relative water content (Figure 2B), plants fertilized with ammonium sulfate (N3) differed significantly from those fertilized with calcium nitrate (N2). Comparing the RWC of plants fertilized with N1 and N4, no significant differences were observed among them or in relation to the other N sources. The increase in RWC may be related to the acclimation mechanism of passion fruit plants under brackish water irrigation, induced by nitrogen supply. This nutrient is a structural component of several organic compounds, such as enzymes, proteins, amino acids, and nucleic acids, which contribute to the osmotic adjustment of plants under saline conditions, enhancing water absorption (Roque et al., 2022).

A significant interaction effect between irrigation water salinity levels and nitrogen sources (ECw × NS) was observed for intercellular CO_2 concentration (*Ci*), CO_2

assimilation rate (*A*), instantaneous water use efficiency (*WUEi*), and instantaneous carboxylation efficiency (*CEi*) of sour passion fruit seedlings at 69 days after sowing. Irrigation water salinity also significantly affected transpiration (*E*) and stomatal conductance (*gs*) of 'BRS GA1' sour passion fruit plants at 69 DAS (Table 3).

Table 3
Summary of analysis of variance for intercellular CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*), CO_2 assimilation rate (*A*), instantaneous water use efficiency (*WUEi*), and instantaneous carboxylation efficiency (*CEi*) of sour passion fruit 'BRS GA1' cultivated under irrigation with different water salinity levels and different nitrogen sources, at 69 days after sowing

Source of variation	DF	Mean square					
		Ci	E	gs	A	WUEi	CEi
Salinity level (SL)	3	1790.04**	2.20**	0.0348**	93.24**	3.03**	0.0012**
Linear regression	1	5128.16**	5.87**	0.1040**	253.36**	8.21**	0.0035**
Quadratic regression	1	46.13^{ns}	0.16^{ns}	0.0003^{ns}	0.27^{ns}	0.06^{ns}	0.0000^{ns}
Nitrogen source (NS)	3	360.55^{ns}	0.04^{ns}	0.0041^{ns}	23.95**	2.68**	0.0002**
Interaction (SL × NS)	9	1266.25**	0.32^{ns}	0.0081^{ns}	29.58**	3.07**	0.0003**
Blocks	3	128.59^{ns}	0.14^{ns}	0.0014^{ns}	0.49^{ns}	0.26^{ns}	0.0000^{ns}
Residual	45	319.39	0.23	0.0064	0.94	0.23	0.0000
CV (%)		5.81	13.95	28.68	10.31	17.87	14.90

DF - degree of freedom; CV - coefficient of variation; *, ** Significant at the 0.05 and 0.01 probability levels, respectively; ^{ns} Not significant.

The intercellular CO_2 concentration (*Ci*) increased with irrigation water salinity when calcium nitrate and ammonium sulfate were used (Figure 3A). Comparing plants irrigated with ECw of 3.4 dS m^{-1} to those receiving 0.4 dS m^{-1}, increases of 22.28 and 8.09% were observed under fertilization with calcium nitrate (N2) and ammonium sulfate (N3), respectively. No significant differences in *Ci* were found among nitrogen sources when irrigation water ECw ranged from 1.4 to 3.4 dS m^{-1}. The absence of a significant effect of N rates on *Ci* may be related to the salt concentration in the water, which, at high ECw levels, likely exerts a similar influence on CO_2 diffusion into the substomatal chamber of the sour passion fruit plants.

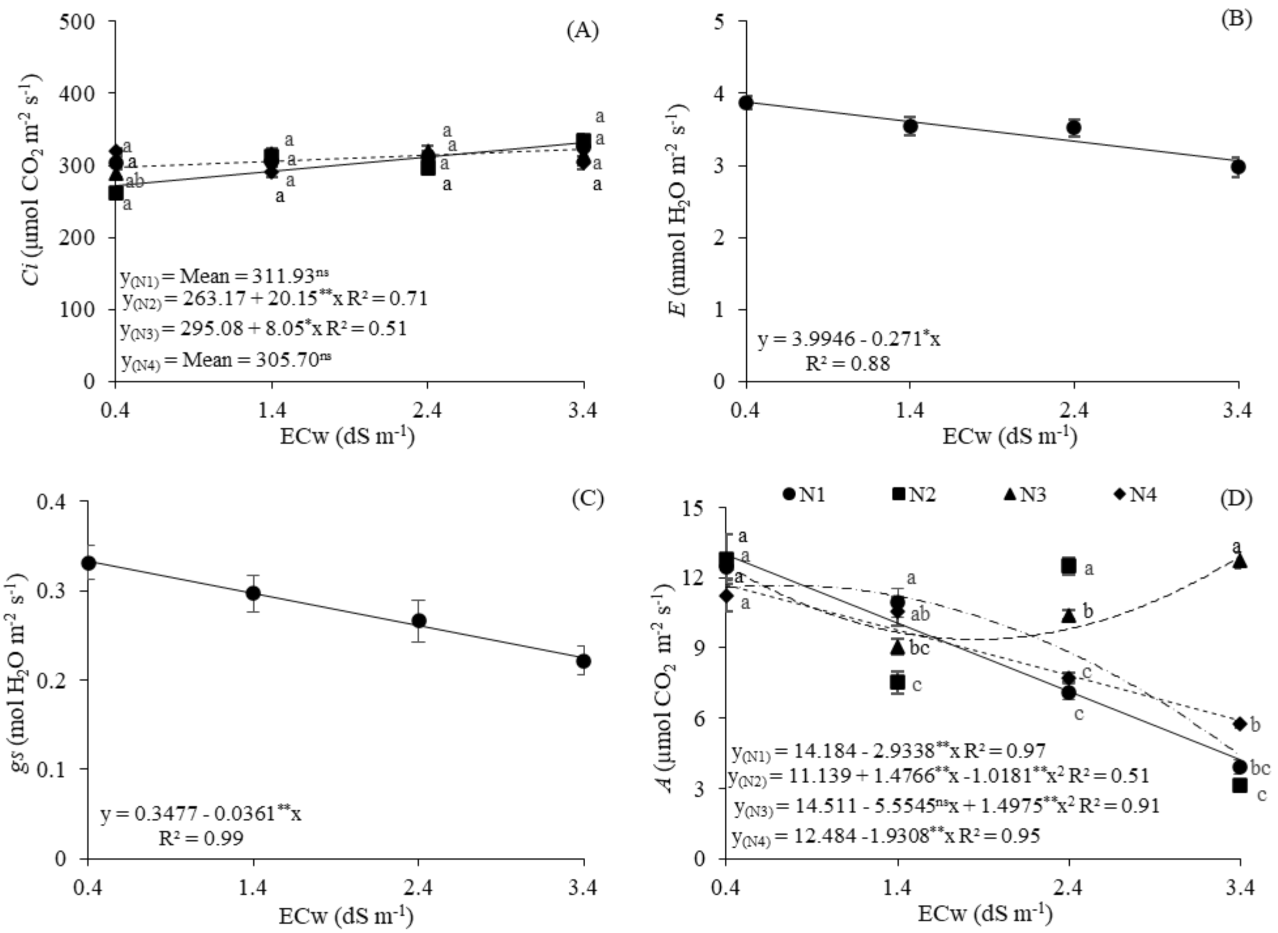

Figure 3. Intercellular CO_2 concentration (*Ci*; A) and CO_2 assimilation rate (*A*; D) of sour passion fruit 'BRS GA1' plants as a function of the interaction between the electrical conductivity levels of the irrigation water (ECw) and nitrogen sources; and transpiration (*E*; B) and stomatal conductance (*gs*) as a function of ECw levels, at 69 days after sowing.
^{ns}, *, ** Not significant ($p > 0.05$) and significant at $p \leq 0.05$ and ≤ 0.01 by the F-test, respectively. Vertical bar represents the standard error of the mean (n = 3); N1- Urea, N2- calcium nitrate, N3- ammonium sulfate, N4- ammonium chloride. Means followed by different letters indicate significant differences between nitrogen sources by Tukey's test ($p \leq 0.05$).

For plants fertilized with urea (N1) and ammonium chloride (N4) (Figure 3A), the data did not fit satisfactorily to the regression models tested, yielding mean values of 311.93 and 305.70 µmol CO_2 m^{-2} s^{-1}. Nitrogen sources N1, N3, and N4 did not differ significantly from each other. Nitrogen is involved in the synthesis of enzymes and proteins involved in photosynthesis, including ATP synthase and RuBisCO, which are crucial for efficient carbon fixation in plants (Wanderley et al., 2020). Therefore, the observed increase in intercellular CO_2 concentration may indicate non-stomatal limitations, meaning that CO_2 absorbed from the atmosphere was not used for sugar synthesis during photosynthesis, leading to its accumulation (Freire et al., 2014).

Transpiration decreased linearly with increasing water salinity, with a 6.78% reduction per unit increase in ECw, corresponding to a 22.92% decrease between plants irrigated with the lowest and highest salinity levels (Figure 3B). Osmotic stress caused by salt accumulation in the root zone induces partial stomatal closure, reducing water loss through transpiration (E. M. da Silva et al., 2018). A. A. R. da Silva et al. (2019), evaluating gas exchange in passion fruit plants irrigated with saline water (0.7, 1.4, 2.1, and 2.8 dS m^{-1}), observed that ECw up to 1.4 dS m^{-1} did not limit leaf transpiration.

For stomatal conductance (Figure 3C), water salinity caused a 10.38% reduction per unit increase in ECw. Comparing plants irrigated with ECw of 3.4 dS m^{-1} to those receiving 0.4 dS m^{-1}, a 32.50% decrease was observed. Partial stomatal closure is a mechanism to minimize water loss and maintain water balance, also reducing the uptake of toxic ions (Dias et al., 2019). A. A. R. da Silva et al. (2019), evaluating the growth of yellow passion fruit seedlings under saline irrigation, found that increasing water EC from 0.7 to 2.8 dS m^{-1} resulted in a 50% reduction in *E*.

The CO_2 assimilation rate decreased linearly in plants fertilized with urea and ammonium chloride, by 20.68 and 15.46% per unit increase in ECw, respectively. This represented reductions of 67.64 and 49.45% between plants irrigated with ECw of 0.4 and 3.4 dS m^{-1} (Figure 3D). On the other hand, plants fertilized with calcium nitrate and ammonium sulfate reached maximum estimated values of 11.67 and 12.93 μmol CO_2 m^{-2} s^{-1} under ECw of 0.7 and 3.4 dS m^{-1}, respectively. The N supplied to the passion fruit plants through calcium nitrate and ammonium sulfate likely enhanced nitrogen uptake and, consequently, increased CO_2 assimilation rates, as nitrogen directly influences photosynthesis by being an integral component of the chlorophyll molecule (M. G. Silva et al., 2004).

The instantaneous carboxylation efficiency (Figure 4A) of sour passion fruit seedlings fertilized with N1, N2, and N4 decreased linearly with increasing irrigation water salinity, with reductions of 21.32, 20.15, and 14.88% per unit increase in ECw, respectively. In relative terms, plants irrigated with ECw of 3.4 dS m^{-1} exhibited reductions in *CEi* of 69.93, 65.77, and 47.49% compared to those grown under the lowest saline level (0.4 dS m^{-1}), when fertilized with N1, N2, and N4, respectively. Conversely, plants fertilized with ammonium sulfate (N3) showed a maximum estimated value of 0.0441 [(μmol CO_2 m^{-2} s^{-1})/(μmol CO_2 m^{-2} s^{-1})] under irrigation with ECw of 0.4 dS m^{-1}. The reduction in instantaneous carboxylation efficiency may be associated with the inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity due to salt accumulation in the leaf tissues, particularly Na^+ and Cl^- (E. N. da Silva et al., 2011).

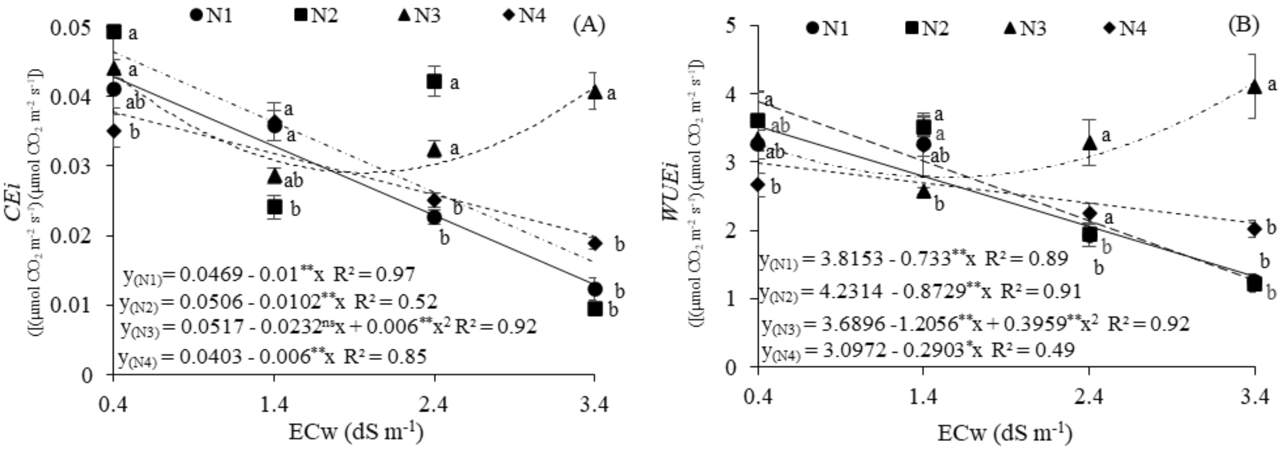

Figure 4. Instantaneous carboxylation efficiency (*CEi*; A) and instantaneous water use efficiency (*WUEi*; B) of 'BRS GA1' sour passion fruit plants as a function of the interaction between water electrical conductivity levels (ECw) and nitrogen sources, 69 days after sowing.
^{ns}, *, ** Not significant ($p > 0.05$) and significant at $p \leq 0.05$ and ≤ 0.01 by the F-test, respectively. Vertical bar represents the standard error of the mean (n = 3); N1- Urea, N2- calcium nitrate, N3- ammonium sulfate, N4- ammonium chloride. Means followed by different letters indicate significant differences between nitrogen sources by Tukey's test ($p \leq 0.05$).

Regarding instantaneous water use efficiency (Figure 4B), plants fertilized with N1, N2, and N4 showed a linear decrease of 19.21, 20.62, and 9.37% per unit increase in ECw, respectively. Comparing plants irrigated with ECw of 3.4 dS m-^{1} to those receiving 0.4 dS m^{-1}, reductions in *WUEi* of 62.43, 67.45, and 29.38% were observed for N1, N2, and N4, respectively. Plants fertilized with N3 exhibited a maximum estimated value of 4.1013 [(μmol CO_2 m^{-2} s^{-1})/(μmol CO_2 m^{-2} s^{-1})] under ECw of 3.4 dS m^{-1}.

Comparing the effects of nitrogen sources at each salinity level (Figure 4B), significant differences were observed across all ECw values. Under 0.4 dS m^{-1}, plants fertilized with N2 were statistically superior to those with N4. At 1.4 dS m^{-1}, significant differences were observed between N1 and N2 compared to N3. When plants were irrigated with 2.4 dS m^{-1} water, N3 and N4 resulted in higher *WUEi* than N1 and N2. Under 3.4 dS m^{-1}, plants fertilized with N3 differed significantly from the other N sources. The increase in instantaneous water use efficiency may be attributed to nitrogen supplied via ammonium sulfate, which enhances the synthesis of organic compounds such as amino acids, proteins, coenzymes, nucleic acids, vitamins, and chlorophyll (Lima et al., 2019).

A significant effect of irrigation water salinity was observed on chlorophyll b content in 'BRS GA1' passion fruit plants at 69 DAS (Table 4). Nitrogen sources significantly influenced the chlorophyll a, chlorophyll b, and carotenoid contents of passion fruit seedlings, but the interaction between factors (ECw × NS) did not significantly affect any of these variables.

Table 4
Summary of analysis of variance for chlorophyll a (Cl *a*), b (Cl *b*), and carotenoid contents of 'BRS GA1' sour passion fruit grown under irrigation with different water salinity levels and different nitrogen sources, 69 days after sowing

Source of variation	DF	Mean square		
		Ci	E	gs
Salinity level (SL)	3	17.3140^{ns}	4.9188*	1.3176^{ns}
Linear regression	1	12.2109^{ns}	10.7055*	2.2713^{ns}
Quadratic regression	1	1.6224^{ns}	0.4405^{ns}	0.4590^{ns}
Nitrogen source (NS)	3	58.3198**	10.0537**	3.9337**
Interaction (SL × NS)	9	6.1221^{ns}	2.2797^{ns}	1.7419^{ns}
Blocks	3	15.7727^{ns}	2.2095^{ns}	4.3548**
Residual	45	7.9887	1.6953	0.8702
CV (%)		10.85	21.20	12.43

DF - degree of freedom; CV - coefficient of variation; *, ** Significant at the 0.05 and 0.01 probability levels, respectively; ^{ns} not significant.

Regarding chlorophyll a content (Figure 5A), plants fertilized with calcium nitrate (N2) exhibited the highest values (28.48 mg g^{-1} FW), differing significantly from those fertilized with N3 and N4. However, no significant differences were observed among plants fertilized with N1, N3, and N4. The increase in chlorophyll a content due to calcium nitrate fertilization under saline conditions is related to stimulation of N uptake, since plants have a higher affinity for nitrate than for chloride. Under salinity stress, Cl^-/NO_3^- competition occurs, and increased NO_3^- uptake reduces the Cl^-/N ratio in the leaves, restoring nutritional balance and mitigating the adverse effects of salinity (Blanco et al., 2008).

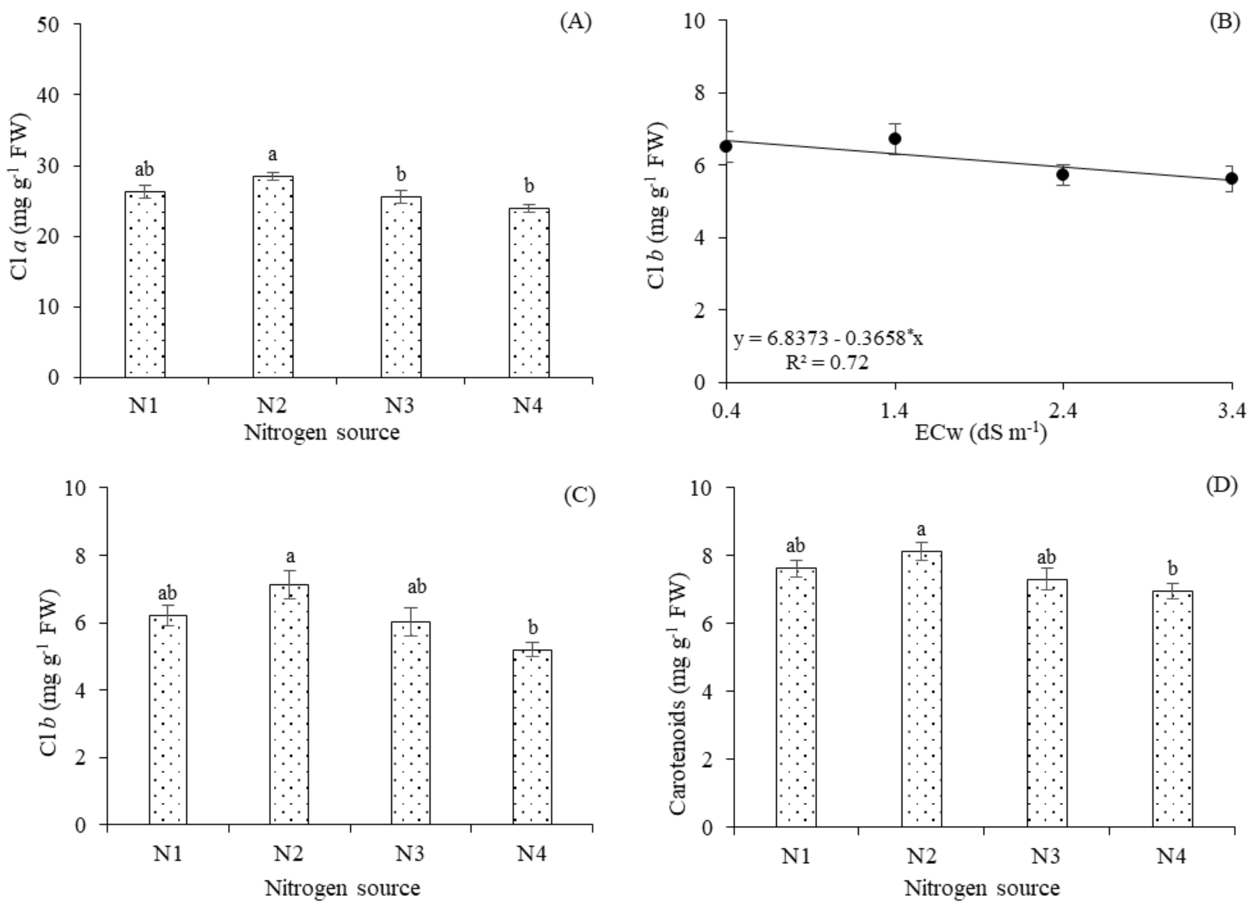

Figure 5. Chlorophyll a (Cl *a*; A), b (Cl *b*; C), and carotenoid (D) levels of 'BRS GA1' sour passion fruit plants as a function of nitrogen sources; and chlorophyll b (Cl *b*; B) levels of 'BRS GA1' sour passion fruit plants as a function of irrigation water salinity (ECw) levels, at 69 days after sowing.
* Significant at $p \leq 0.05$ by the F-test, respectively. Vertical bar represents the standard error of the mean (n = 3); N1- Urea, N2- calcium nitrate, N3- ammonium sulfate, N4- ammonium chloride. Means followed by different letters indicate significant differences between nitrogen sources by Tukey's test ($p \leq 0.05$).

Chlorophyll b (Cl b) content decreased with increasing water salinity, showing a reduction of 5.35% per unit increase in ECw (Figure 5B). Comparing plants irrigated with ECw of 0.4 and 3.4 dS m^{-1}, a 16.40% decrease (6.69 mg g^{-1} FW) was observed. Photosynthetic pigments play an essential role in light energy assimilation, and their concentrations are often reduced under salt stress (Nigam et al., 2022).

Regarding the effect of nitrogen sources on Cl b (Figure 5C), plants fertilized with calcium nitrate (N2) showed the highest Cl b content (7.12 mg g^{-1} FW), differing significantly from those fertilized with N4. No significant differences were observed among N1, N2, and N3. The increase in Cl b levels under calcium nitrate fertilization may be attributed to nitrate-chloride competition, since high nitrate concentrations in the root zone can inhibit chloride uptake, reducing its toxicity (R. F. Pereira, 2014).

The highest carotenoid levels (8.12 mg g^{-1} FW) were recorded in plants fertilized with N2, which was 1.17 mg g^{-1} FW higher than those fertilized with N4. The increase in carotenoid levels under calcium nitrate fertilization may be related to its greater availability compared to ammoniacal sources such as ammonium chloride (N4), as nitric sources applied via irrigation readily release NO_3-, which is directly absorbed by plants (Fassbender, 1986). Moreover, nitrates are not adsorbed to soil particles, remaining mobile and available for uptake. Conversely, ammoniacal forms tend to be adsorbed onto soil colloids, reducing fertilizer mobility and bioavailability, which may have limited plant physiological performance (Coelho et al., 2004).

Nitrogen sources significantly affected plant height (PH), number of leaves (NL), and leaf area (LA) of 'BRS GA1' sour passion fruit plants at 69 DAS (Table 5). However, water salinity and the interaction between factors (ECw × NS) did not significantly influence these variables.

Table 5
Summary of analysis of variance for plant height (PH), number of leaves (NL), stem diameter (SD), and leaf area (LA) of 'BRS GA1' sour passion fruit grown under irrigation with different water salinity levels and different nitrogen sources, at 69 days after sowing

Source of variation	DF	Mean square			
		PH	NL	SD	LA
Salinity level (SL)	3	7.2694^{ns}	2.5989^{ns}	0.1856^{ns}	54.4515^{ns}
Linear regression	1	6.8298^{ns}	1.6531^{ns}	0.2147^{ns}	8.7582^{ns}
Quadratic regression	1	11.5855^{ns}	3.5156^{ns}	0.0735^{ns}	93.3639^{ns}
Nitrogen source (NS)	3	76.3581*	3.9322*	0.3674^{ns}	239.1886**
Interaction (SL × NS)	9	173.0219^{ns}	2.5156^{ns}	0.2873^{ns}	79.3485^{ns}
Blocks	3	4.5474^{ns}	1.5572^{ns}	0.6904*	17.6409^{ns}
Residual	45	26.4813	1.2461	0.2238	51.7831
CV (%)		16.04	10.16	12.61	15.64

DF - degree of freedom; CV - coefficient of variation; *, ** significant at the 0.05 and 0.01 probability levels, respectively; ^{ns} not significant.

Ammonium sulfate (N3) fertilization increased the plant height growth (Figure 6A) of sour passion fruit plants at 69 DAS. Plants fertilized with N3 showed statistically higher height growth compared to those cultivated with N4 (Figure 6A). However, no significant differences were observed among the N1, N2, and N4 treatments. Regarding the number of leaves (Figure 6B), there were no significant differences among nitrogen sources. For leaf area (Figure 6C), plants fertilized with N1 differed significantly from those receiving N3. When comparing the leaf area of plants fertilized with N1, N2, and N4, no significant differences were detected. M. A. F. Bezerra et al. (2019), when evaluating the effect of nitrogen as a mitigator of salt stress in yellow passion fruit seedlings, concluded that nitrogen fertilization favors plant height, leaf number, and leaf area, thereby promoting seedling quality.

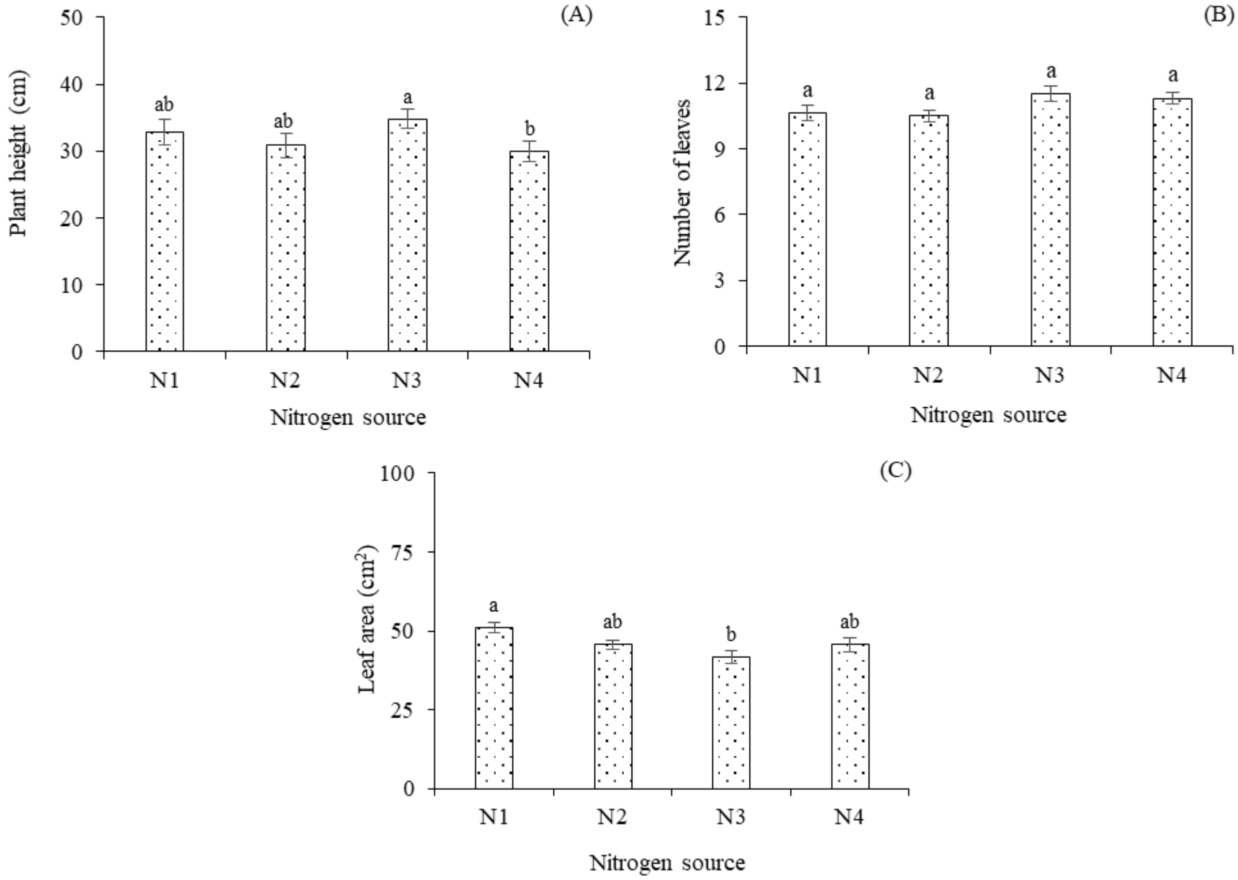

Figure 6. Plant height (A), number of leaves (B), and leaf area (C) of 'BRS GA1' sour passion fruit plants as a function of nitrogen sources at 69 days after sowing.
N1- Urea, N2- calcium nitrate, N3- ammonium sulfate, N4- ammonium chloride. Means followed by different letters indicate significant differences between nitrogen sources by Tukey's test ($p \leq 0.05$).

The interaction between irrigation water salinity levels and nitrogen sources (ECw × NS) significantly affected stem dry biomass (SDB), total dry biomass (TDB), and the Dickson quality index (DQI) of sour passion fruit seedlings. Irrigation water salinity levels significantly influenced leaf dry biomass (LDB), and nitrogen sources individually affected the LDB (Table 6) of 'BRS GA1' sour passion fruit plants at 72 DAS.

Table 6

Summary of analysis of variance for leaf (LDB), stem (SDB), and root (RDB) dry biomass and Dickson quality index of 'BRS GA1' sour passion fruit grown under irrigation with different water salinity levels and different nitrogen sources, at 72 days after sowing

Source of variation	DF	Mean square				
		LDB	SDB	RDB	TDM	DQI
Salinity level (SL)	3	1.1948**	0.1251^{ns}	0.0170ns	0.6448^{ns}	0.007*
Linear regression	1	0.1683^{ns}	0.1674^{ns}	0.0344ns	0.0362^{ns}	0.011*
Quadratic regression	1	3.3948**	0.1892*	0.0010^{ns}	1.8837*	0.004^{ns}
Nitrogen source (NS)	3	0.6980*	0.3470**	0.0425^{ns}	1.8254**	0.0008^{ns}
Interaction (SL × NS)	9	0.3783^{ns}	0.2569**	0.0409^{ns}	1.0216^{ns}	0.005**
Blocks	3	0.2038^{ns}	0.0880^{ns}	0.0822**	0.5403^{ns}	0.0005^{ns}
Residual	45	0.2347	0.0462	0.0165	0.2749	0.0008
CV (%)		22.00	20.10	24.15	13.78	12.40

DF - degree of freedom; CV - coefficient of variation; *, ** significant at the 0.05 and 0.01 probability levels, respectively; ^{ns} not significant.

For LDB (Figure 7A), the maximum estimated value (2.5 g per plant) was observed in plants irrigated with water at 3.4 dS m^{-1}. Among the nitrogen sources, calcium nitrate (N2) promoted the greatest accumulation of LDB (2.5 g per plant), although this result did not differ statistically from the other nitrogen sources (Figure 7B). A. M. da S.Silva Neta et al. (2022), when evaluating biomass accumulation and seedling quality of sour passion fruit cv. BRS Rubi do Cerrado under saline irrigation (0.3, 1.1, 1.9, 2.7, and 3.5 dS m^{-1}), reported a 34.67% reduction in stem dry biomass in plants subjected to irrigation with increased electrical conductivity from 0.3 to 3.5 dS m^{-1}.

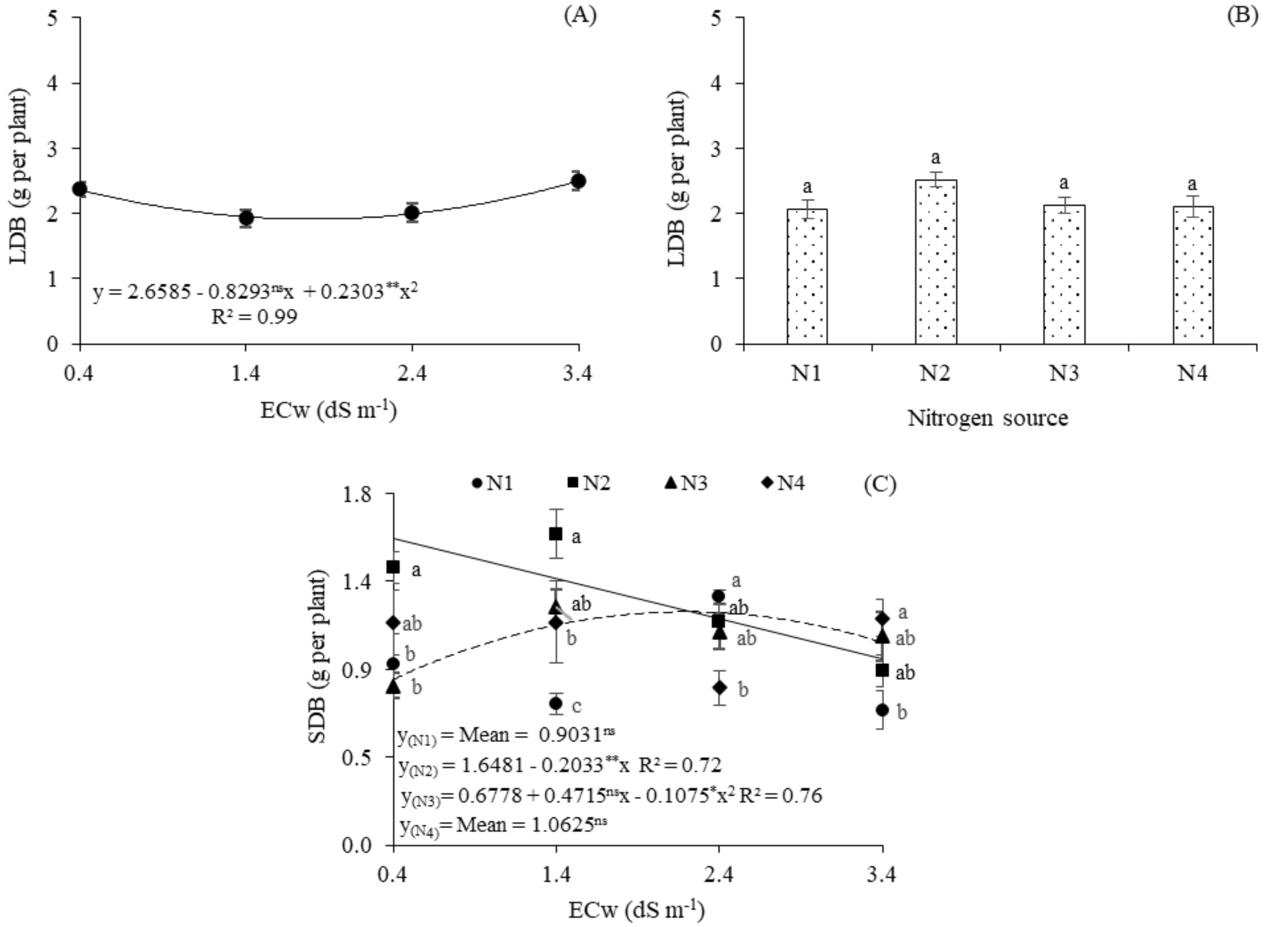

Figure 7. Leaf dry biomass (LDB) 'BRS GA1' passion fruit plants as a function of the electrical conductivity of irrigation water (ECw; A) and nitrogen sources (B); and stem dry biomass (SDB; C) as a function of the interaction between ECw levels and nitrogen sources, at 72 days after sowing.
* Significant at $p \leq 0.05$ by the F-test, respectively. Vertical bar represents the standard error of the mean (n = 3). N1- Urea, N2- calcium nitrate, N3- ammonium sulfate, N4- ammonium chloride. Means followed by different letters indicate significant differences between nitrogen sources by Tukey's test ($p \leq 0.05$).

For SDB, N2 fertilization resulted in a 12.33% decrease per unit increase in ECw, corresponding to a reduction of 0.60 g per plant between the water salinity levels of 3.4 and 0.4 dS m^{-1}. Under N3 fertilization, the maximum value (1.19 g per plant) was observed at an ECw of 2.1 dS m^{-1}. SDB data for plants fertilized with N1 and N4 did not fit satisfactorily to the regression models tested. The reduction in stem dry biomass accumulation can be attributed to decreased water availability, which induces stomatal closure, reduces CO_2 assimilation (G. S. de Lima et al., 2020c), and limits water and nutrient uptake. According to G. S. de Lima et al. (2020b), under salt stress conditions, the reduced osmotic potential of the soil solution hampers water and nutrient absorption by plants, leading to morphological and anatomical changes.

The total dry biomass (Figure 8A) of plants fertilized with N2 was higher than that obtained with other nitrogen sources. No significant differences in TDB were found among plants fertilized with N1, N3, and N4. The increase in TDB observed in plants receiving N2 may be associated with higher photosynthetic rates (Marschner, 2012).

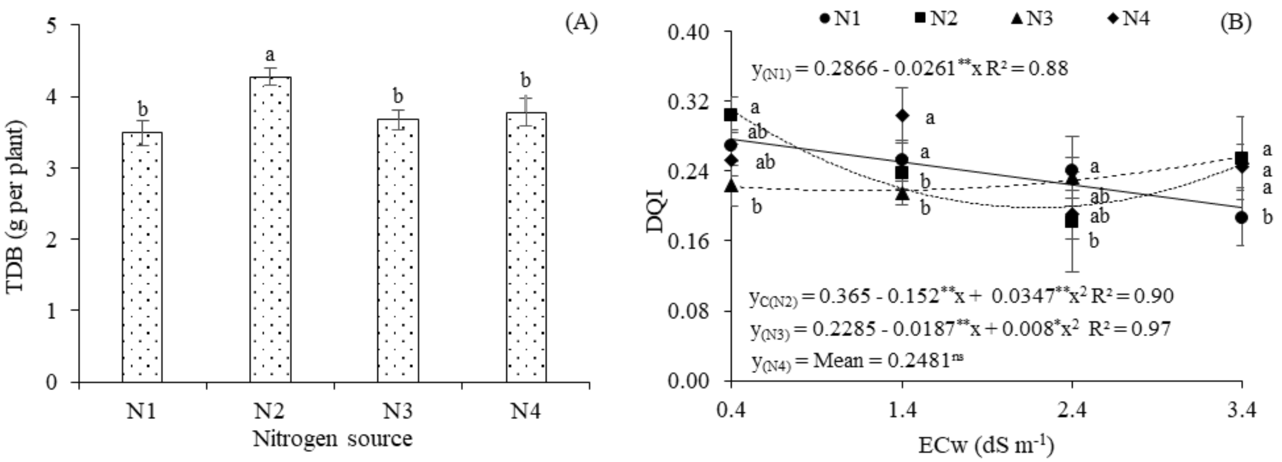

Figure 8. Total dry biomass (TDB; A) of 'BRS GA1' sour passion fruit seedlings as a function of nitrogen sources; Dickson quality index (DQI; B) as a function of the interaction between irrigation water salinity levels (ECw) and nitrogen sources, 72 days after sowing.
ns, ** Not significant and significant at $p \leq 0.01$ by the F-test, respectively. Vertical bar represents the standard error of the mean (n = 3). N1- Urea, N2- calcium nitrate, N3- ammonium sulfate, N4- ammonium chloride. Means followed by different letters indicate significant differences between nitrogen sources by Tukey's test ($p \leq 0.05$).

For the Dickson Quality Index (Figure 8B), plants fertilized with urea (N1) showed a linear reduction of 9.10% per unit increase in ECw. In relative terms, a DQI decrease of 28.35% was observed between plants irrigated with ECw of 0.4 and 3.4 dS m^{-1}. Plants fertilized with calcium nitrate (N2) and ammonium sulfate (N3) exhibited maximum estimated DQI values of 0.31 and 0.25, respectively, under ECw of 0.4 and 3.4 dS m^{-1}. Conversely, data for plants grown with ammonium chloride (N4) fitted polynomial regression models. When decomposing nitrogen sources at each ECw level, it was observed that under irrigation with 0.4 dS m^{-1} water, plants fertilized with N2 exhibited higher DQI than those receiving N3, though they did not differ significantly from those grown with N1 or N4. Under 1.4 dS m^{-1} irrigation, significant differences were observed between N1 and N4 compared to N2 and N3. At 2.4 dS m^{-1}, plants fertilized with N1 differed significantly from those cultivated with N2. Under 3.4 dS m^{-1} irrigation, fertilization with N2, N3, and

N4 resulted in higher DQI values (0.25, 0.25, and 0.24, respectively) compared to urea (N1), which had the lowest value (0.1862). Despite biomass reduction, sour passion fruit seedlings irrigated with 3.4 dS m^{-1} water and fertilized with N2, N3, or N4 maintained DQI values above 0.2, indicating agronomic quality and suitability for field establishment (Oliveira et al., 2013). M. A. F. Bezerra et al. (2019) also observed a reduction in DQI when the electrical conductivity of irrigation water increased from 0.3 to 4.0 dS m^{-1} in yellow passion fruit seedlings.

Conclusions

Irrigation water electrical conductivity above 0.4 dS m^{-1} increases electrolyte leakage and reduces transpiration, stomatal conductance, chlorophyll b, stem and leaf dry biomass, and the Dickson quality index of 'BRS GA1' sour passion fruit seedlings, regardless of the nitrogen source.

Ammonium sulfate fertilization increases relative water content, the number of leaves, intercellular CO_2 concentration, CO_2 assimilation rate, instantaneous carboxylation efficiency, instantaneous water use efficiency, and plant height in 'BRS GA1' sour passion fruit plants under water salinity of 0.4 dS m^{-1}.

Calcium nitrate supplementation enhances intercellular CO_2 concentration, leaf and total dry biomass, and photosynthetic pigments in 'BRS GA1' sour passion fruit seedlings under saline irrigation.

Ammonium sulfate fertilization is recommended for sour passion fruit plants irrigated with 3.4 dS m^{-1} water during the seedling formation phase.

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