

## Morphophysiology of the passion fruit 'BRS Rubi do Cerrado' irrigated with saline waters and nitrogen fertilization

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### Abstract

This study aimed to evaluate the gas exchanges and growth of the purple passion fruit cultivar 'BRS Rubi do Cerrado' as a function of the salinity levels of the irrigation water and nitrogen fertilization. The research was conducted in pots adapted as drainage lysimeters, placed within a plant nursery, using a Regolithic Neosol of sandy texture, in the municipality of Pombal-PB, Brazil. A randomized block design was used, testing five levels of electrical conductivity of irrigation water (0.3, 1.1, 1.9, 2.7, and 3.5 dS m<sup>-1</sup>) associated with four doses of nitrogen (50, 75, 100, and 125% of the recommendation). The irrigation water salinity above 0.3 dS m<sup>-1</sup> compromised the leaf area and the relative water content of the purple passion fruit 'BRS Rubi do Cerrado'. High doses of nitrogen enhance the deleterious effects of irrigation water salinity on stomatal conductance, transpiration, internal CO<sub>2</sub> concentration, CO<sub>2</sub> assimilation rate, number of leaves, stem diameter, and height of purple passion fruit plants. When waters with salinity levels of up to 1.3 dS m<sup>-1</sup> are used, the dose of 125 mg of N kg<sup>-1</sup> of soil is recommendation for providing increases in the CO<sub>2</sub> assimilation rate of the purple passion fruit 'BRS Rubi do Cerrado' at 70 days after sowing (DAS). Water salinity increases electrolyte leakage, regardless of nitrogen doses.

**Keywords:** salt stress, nitrogen, *Passiflora edulis* Sims

### Introduction

Salt stress became of the main abiotic stresses that restrict crop productivity in several areas of the world, especially in arid and semiarid regions, such as those found in the Northeast of Brazil, where most of the water used in irrigation possesses high contents of dissolved salts (Zorb et al., 2019). However, the use of water with salt excess may inhibit plant growth and production due to the reduction in the osmotic potential of the soil solution, besides causing toxicity, nutritional imbalance, or both (Islam et al., 2017).

The accumulation of ions at toxic levels interferes with physiological processes, compromising the rates of transpiration, photosynthesis and internal CO<sub>2</sub> concentration, starch metabolism, and nitrogen fixation (Gong et al., 2018). By compromising the physiological and metabolic processes of plants, they may lead to a decrease in leaf area, increase in leaf thickness and

succulence, leaf abscission, and decrease in internode length, with a consequent reduction in the production of plant biomass and crop yield (Win et al., 2018). However, the way how plants tolerate salinity and the success of their strategies varies widely among species (Flower & Colmer, 2008).

Brazil is highlighted as the largest producer and consumer of yellow passion fruit in the world, with a production of 602,651 tons in 2018, in an area of 42,731 ha, of which 62.3% are in the Northeast region (IBGE, 2019). According to Ayers & Westcot (1999), the yellow passion fruit is considered sensitive to salinity, with a threshold salinity level of 1.3 dS m<sup>-1</sup>, occurring a potential loss of its yield. However, the reference values of purple passion fruit tolerance to salinity in the early stage of development and their reflections on physiology and growth are still incipient and need to be conveniently researched.

The increase in nitrogen fertilization can minimize the deleterious effects of salinity on plants since this element is a vital component of several compounds, including amino acids, amides, and proteins involved in the tolerance of the plants to salinity (Arghavani et al., 2017; Lima et al., 2018). However, the relationship between salinity and N metabolism is very complex, depending on the degree and duration of salt stress, plant species, growth stage, number, type, and form of N in the rhizosphere (Teh et al., 2016).

In spite of the studies already developed with this crop, the need for more data on the interactive effect between irrigation water salinity and nitrogen fertilization of purple passion fruit is evident. In this context, this study aimed to evaluate the effects of irrigation water salinity and nitrogen fertilization on the gas exchanges and growth of the purple passion fruit cultivar 'BRS Rubi do Cerrado'.

**Material and Methods**

The experiment was developed in the period from December 2019 to March 2020, under plant nursery conditions, in the experimental area of the Center of Sciences and Agrifood Technology – CCTA of the Federal University of Campina Grande - UFCG, located in the municipality of Pombal, Paraíba, in the geographic

coordinates 06°46'20" S, 37°48'01" W, and mean elevation of 1,194 m.

The experimental design was in randomized blocks, in a 5 x 4 factorial arrangement, with treatments consisting of the combinations of five levels of electrical conductivity of irrigation water - EC<sub>w</sub> (0.3, 1.1, 1.9, 2.7, and 3.5 dS m<sup>-1</sup>) and four doses of nitrogen - ND (N1 -50; N2 -75; N3 -100, and N4 -125%) of the dose indicated for the in-pot assay, according to Novais et al. (1991). The combined factors resulted in 20 treatments, with four replications and three plants per plot, totaling 240 plants.

The purple passion fruit seedlings were obtained via seeds, using the cultivar 'BRS Rubi do Cerrado', by sowing two seeds per polyethylene bag with a capacity of 1.5 dm<sup>3</sup>, filled with a substrate prepared with a mixture of Regolithic Neosol of sandy texture constituted by 75% of soil, 25% of sand, and 25% of organic compost (bovine manure) at a 2:1:1 ratio, with a total mass of 1.7 Kg of the substrate, whose chemical and physical characteristics (Table 1) were obtained according to the methodologies proposed by Claessen (1997). Thinning was performed 10 days after sowing (DAS) by allowing only one plant per bag (the one with greater physiological vigor). The bags were arranged on a metal workbench at 0.8m from the ground.

**Table 1.** Physical and chemical characteristics of the substrate used in the experiment, before the application of the treatments, Pombal, PB, 2020.

pH H <sub>2</sub> O) (1:2.5)	OM g kg <sup>-1</sup>	P (mg kg <sup>-1</sup> )	Chemical characteristics					
			K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H <sup>+</sup>
5.58	2.93	39.2	0.23	1.64	9.07	2.78	0.00	8.61
..... Chemical characteristics.....			Physical characteristics					
EC <sub>se</sub> (dS m <sup>-1</sup> )	CEC cmol <sub>c</sub> kg <sup>-1</sup>	SAR (mmol L <sup>-1</sup> ) <sup>0.5</sup>	ESP %	Size fraction (g kg <sup>-1</sup> )			Water content (dag kg <sup>-1</sup> )	
2.15	22.33	0.67	7.34	Sand	Silt	Clay	33.42 kPa <sup>1</sup>	1519.5 kPa <sup>2</sup>
				572.70	100.70	326.60	25.91	12.96

pH – Hydrogen potential, OM – Organic matter: Walkley-Black Wet Digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1 M KCl at pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted with 1 M NH<sub>4</sub> OAc at pH 7.0; Al<sup>3+</sup>+H<sup>+</sup> extracted with 0.5 M CaOAc at pH 7.0; EC<sub>se</sub> – Electrical conductivity of the saturation extract; CEC – Cation exchange capacity; SAR – Sodium adsorption rate of the saturation extract; ESP – Exchangeable sodium percentage; <sup>1,2</sup> referring to the limits of field capacity and permanent wilting point, respectively.

The water with the lowest electrical conductivity (0.3 dS m<sup>-1</sup>) was originated from the local supply system. In contrast, the remaining salinity levels (1.1, 1.9, 2.7, and 3.5 dS m<sup>-1</sup>) were prepared from the dissolution of sodium chloride (non-iodized) in the supply water, considering the relationship between the EC<sub>w</sub> and the concentration of salts (10\*meq L<sup>-1</sup> = 1 dS m<sup>-1</sup> of EC<sub>w</sub>) extracted from Rhoades et al. (1992). After the preparation, the waters were stored in plastic containers with a capacity of 200 L, one for each EC<sub>w</sub> level studied, which were adequately protected in order to avoid evaporation, the inflow of rainwater, and contamination with materials that could compromise quality.

At sowing, field capacity was induced with low-

salinity water (0.3 dS m<sup>-1</sup>) through capillary saturation, followed by free drainage. At 30 days after sowing (DAS), irrigation was initiated according to the distinct salt levels, with the quantity being applied according to the water need of the plants, which was determined by water balance by obtaining the following terms as a base: consumed volume, considering the water volume applied to the plants in the previous day; and drained volume, quantified in the morning of the next day, for the leaching fraction, to be estimated at 20%, aiming at maintaining part of the salts accumulated in the root zone, originated from the irrigation water.

The nitrogen doses were provided using urea (45% of N), according to the recommendation by Novais

et al. (1991), by applying 0.015, 0.0234, 0.0315, and 0.0391 g of urea per plant for the doses of 50, 75, 100, and 125%, respectively, beginning at 30 DAS, divided into four weekly applications. The fertilizations with phosphorus and potassium were performed according to the recommendation for the fertilization of in-pot assays, as contained in Novais et al. (1991), by using the amounts of 300 and 150 mg of  $P_2O_5$  and  $K_2O$   $kg^{-1}$  of soil, respectively, in the form of monoammonium phosphate - MAP (52% of  $P_2O_5$ ) and potassium chloride (60%  $K_2O$ ), applied in topdressing, via irrigation water, at 30, 40, 50, and 60 DAS. It is highlighted that the nitrogen provided by the MAP was discounted in all N doses studied. Furthermore, low-salinity water (0.3 dS  $m^{-1}$ ) was used in nutrient applications. To improve plant nutrition and supply possible micronutrient deficiencies, foliar fertilization was performed at 45 DAS using Ubyfol, which contained: Mg - 1.2%; B - 0.85%; Zn - 4.2%; Fe - 3.4%; Mn - 3.2%; Cu - 0.5%, and Mo - 0.06%.

The gas exchange evaluations were performed at 70 DAS based on the measurement of stomatal conductance ( $g_s$ ) ( $mol\ m^{-2}\ s^{-1}$ ), transpiration ( $E$ ) ( $mmol\ H_2O\ m^{-2}\ s^{-1}$ ),  $CO_2$  assimilation rate ( $A$ ) ( $\mu mol\ m^{-2}\ s^{-1}$ ), and internal  $CO_2$  concentration ( $C_i$ ) ( $\mu mol\ mol^{-1}$ ). Based on these data, the instantaneous water-use efficiency was estimated ( $WUE_i$ ) ( $A/E$ ) [ $(\mu mol\ m^{-2}\ s^{-1})\ (mmol\ H_2O\ m^{-2}\ s^{-1})^{-1}$ ]. These evaluations were made with a plant gas exchange determiner containing an infrared gas analyzer - IRGA, model LCpro - SD, made by ADC Bioscientific, UK. The readings were performed at 7:00 a.m. in the third fully-expanded leaf from the apical bud, conducted under natural conditions of air temperature,  $CO_2$  concentration, and using an artificial source of radiation with 1,200  $\mu mol\ m^{-2}\ s^{-1}$ .

The growth of the passion fruit cultivar 'BRS Rubi do Cerrado' was evaluated at 70 DAS through the determination of the number of leaves (NL), leaf area (LA), stem diameter (SD), and plant height (PH). In the same period, the relative water content (RWC) and the electrolyte leakage (EL) were also determined. For the quantification of the NL, only the photosynthetically active leaves with a minimum length of 3 cm were considered; the LA was obtained according to the methodology by Cavalcante et al. (2002); the SD was determined at 3 cm from the base of the plants, using a digital pachymeter, and the PH was defined by measuring the distance between the base of the plant and the insertion of the apical meristem.

The evaluation of the water status of the passion fruit, at 70 DAS, was performed in three fully-expanded leaves through the determination of the RWA in the leaf blade. For that purpose, leaf discs (113  $mm^2$ ) were

perforated immediately after the collection of the leaves, which were then weighed in an analytical balance for the obtainment of the fresh matter (FM); afterward, the samples were put in plastic bags, immersed in distilled water, and left to rest for 90 minutes, thus obtaining the turgid mass (TM); afterward, the samples were taken to a forced-air oven at 65 °C for the obtainment of the dry matter (MS), for 24 hours; the RWA was obtained according to Barrs & Weatherley (1962), using Eq. 1:

$$RWA = \frac{FM-DM}{TM-DM} \times 100 \quad \text{Eq. 1}$$

Where: RWA = relative water content (%);

FM = leaf fresh mass (g);

TM = leaf turgid mass (g);

DM = leaf dry mass (g).

At 70 DAS, aiming at evaluating the damage in the cell membrane under salt stress conditions, the EL was determined. For that purpose, 5 leaf discs with a 113  $mm^2$  area were also collected from the middle-third of the plants, washed with distilled water, aiming at the removal of other electrolytes adhered to the leaves, and placed in beakers with 50 mL of distilled water, which were then hermetically sealed with aluminum foil. The beakers were kept at 25 °C for 90 min, and then the determination of the initial electrical conductivity ( $EC_i$ ) was performed; afterward, the beakers were put in a forced-air oven at 80 °C for 90 min, after which period the final electrical conductivity was determined ( $EC_f$ ). In this manner, the EL was obtained according to Scotti Campos & Thu Pham Thi (1997), using Eq. 2:

$$EL = \frac{EC_i}{EC_f} \times 100 \quad \text{Eq. 2}$$

Where: EL = electrolyte leakage (%);

$EC_i$  = initial electrical conductivity (dS  $m^{-1}$ );

$EC_f$  = final electrical conductivity (dS  $m^{-1}$ ).

The data obtained were evaluated by analysis of variance through the F-test at 0.05 and 0.01 of probability, and in the cases of significance, linear and quadratic polynomial regression analyses were performed for the factors of water salinity levels and nitrogen doses, using the statistical software SISVAR (Ferreira, 2011).

## Results and Discussion

In the analysis of variance, a significant response was observed for the interaction between the salinity levels of the irrigation water (SL) and the nitrogen doses (ND) on stomatal conductance ( $g_s$ ), transpiration ( $E$ ), internal  $CO_2$  concentration ( $C_i$ ), and  $CO_2$  assimilation rate ( $A$ ). For the instantaneous water-use efficiency ( $WUE_i$ ), a significant response was only verified for the factor of nitrogen doses, both at 1% of significance.

Figure 1A exhibits a significant effect of the interaction between salinity levels and nitrogen fertilization

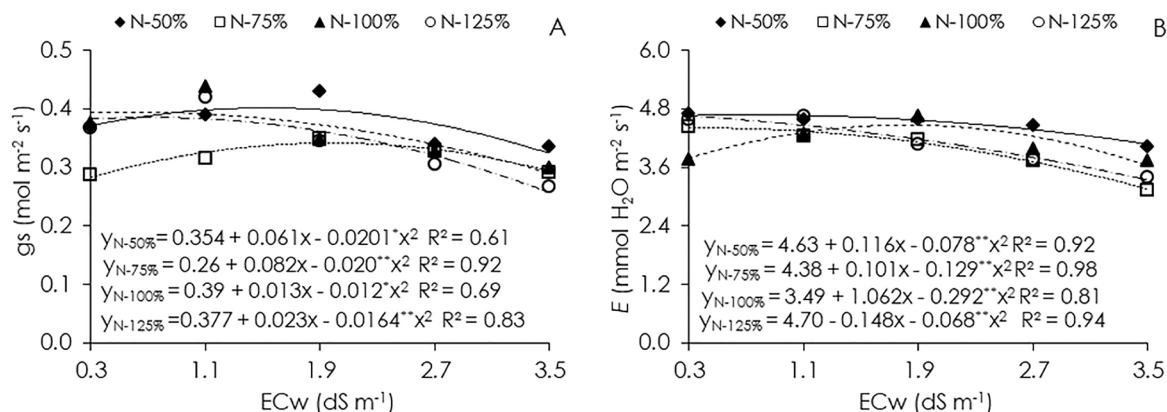
**Table 2.** Summary of the analysis of variance for stomatal conductance ( $g_s$ ) ( $\text{mol m}^{-2} \text{s}^{-1}$ ), transpiration ( $E$ ) ( $\text{mmol of H}_2\text{O m}^{-2} \text{s}^{-1}$ ), internal  $\text{CO}_2$  concentration ( $C_i$ ) ( $\mu\text{mol mol}^{-1}$ ),  $\text{CO}_2$  assimilation rate ( $A$ ) ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), and instantaneous water-use efficiency ( $\text{WUE}_i$ ) [ $(\mu\text{mol m}^{-2} \text{s}^{-1}) / (\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1})$ ] of the passion fruit cultivar 'BRS Rubi do Cerrado' subjected to different salinity levels of the irrigation water and doses of nitrogen at 70 DAS, Pombal, PB, 2020.

Source of variation	DF	Mean squares				
		$g_s$	$E$	$C_i$	$A$	$\text{WUE}_i$
Saline levels (SL)	4	0.012**	2.184**	12897.643**	91.582**	0.0003 <sup>ns</sup>
Linear regression	1	0.043**	8.404**	29484.900**	349.310**	0.0009 <sup>ns</sup>
Quadratic regression	1	0.004*	0.003 <sup>ns</sup>	12993.017**	2.526 <sup>ns</sup>	0.00007 <sup>ns</sup>
N doses (ND)	3	0.0106*	1.041**	2822.483**	57.983**	0.001**
Linear regression	1	0.0003 <sup>ns</sup>	1.036**	6593.440**	144.600**	0.003**
Quadratic regression	1	0.003 <sup>ns</sup>	1.415**	980.000 <sup>ns</sup>	22.641 <sup>ns</sup>	0.001**
Interaction (SL x ND)	12	0.006*	0.321**	1301.368**	18.105**	0.0005 <sup>ns</sup>
Blocks	3	0.017 <sup>ns</sup>	0.115 <sup>ns</sup>	290.683 <sup>ns</sup>	0.676 <sup>ns</sup>	0.0006 <sup>ns</sup>
Residual	57	0.002	0.105	434.657	5.755	0.0003
CV (%)		14.76	7.83		8.10	24.82
Mean		0.345	4.147	257.475	17.876	0.072

<sup>ns</sup>, \*\*, respectively not significant, significant at  $p < 0.01$ , and significant at  $p < 0.05$ ; DF - Degree of freedom; CV - Coefficient of variation

on stomatal conductance ( $g_s$ ), in which it can be seen that the data presented a better adjustment to quadratic and decreasing equations with the increment in the salt levels for the doses of 50, 75, 100, and 125 mg of  $\text{N kg}^{-1}$  of soil, indicating that the highest values for  $g_s$  (0.396, 0.344, 0.393, and 0.378  $\text{mol m}^{-2} \text{s}^{-1}$ ) were obtained, respectively, with the  $\text{EC}_w$  of 1.5, 2.1, 0.6, and 0.7  $\text{dS m}^{-1}$ . Although presenting the same trend, the reduction in the  $g_s$  as a consequence of the increase in irrigation water salinity was higher in the plants that received the doses of 100 and 125 mg of  $\text{N kg}^{-1}$  of soil, which presented reductions of 30.51 and 33.51%, respectively, in the highest salinity, compared to the results found for the salinity of 0.3  $\text{dS}$

$\text{m}^{-1}$ . This reduction in the  $g_s$  can be attributed to stomatal closure in order to avoid excessive water loss, avoiding the harmful effect of stomatal opening with the increase in the resistance to  $\text{CO}_2$  diffusion (Praxedes et al., 2014; Silva et al., 2019). On the other hand, the increase in transpiration ( $E$ ), observed particularly at the dose of 125 mg of  $\text{N kg}^{-1}$  of soil up to the electrical conductivity of 1.3  $\text{dS m}^{-1}$ , probably resulted from the beneficial effect promoted by nitrogen, which may be associated to its role in the higher accumulation of organic compounds containing N, such as proline, which can increase the cell osmotic adjustment ability, increasing the tolerance to water and salt stress (Perveen & Nazir, 2018).



**Figure 1.** Stomatal conductance –  $g_s$  (A) and transpiration –  $E$  (B) of the passion fruit cultivar 'BRS Rubi do Cerrado' as a function of the electrical conductivity of irrigation water -  $\text{EC}_w$  and doses of nitrogen fertilization – ND at 70 DAS, Pombal, PB, 2020.

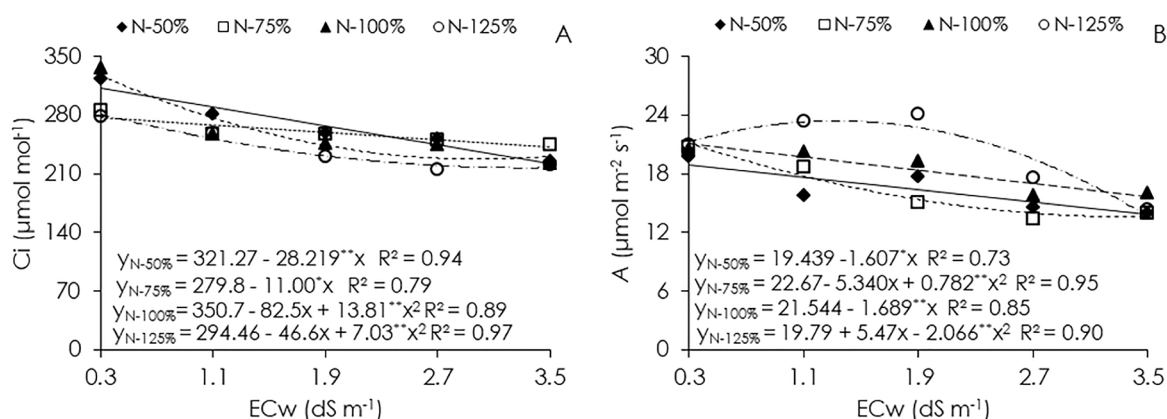
As a consequence of the partial stomatal closure, leaf transpiration ( $E$ ) was equally reduced, verifying in Figure 1B an interaction between the factors (SL x ND) on the  $E$  of the passion fruit cultivar 'BRS Rubi do Cerrado' at 70 DAS, with a quadratic effect for the doses of 50, 75, 100, and 125 mg of  $\text{N kg}^{-1}$  of soil, indicating that the highest values of  $E$  (4.67, 4.39, 4.45, and 4.58  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) were obtained in the plants irrigated with  $\text{EC}_w$  of

0.9, 0.6, 1.8, and 0.6  $\text{dS m}^{-1}$ , respectively. It is worth noting that the maximum value of  $E$ , 4.45  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ , was obtained by applying the water with 1.8  $\text{dS m}^{-1}$  associated with the dose of 100 of  $\text{N kg}^{-1}$  of soil (Figure 1B). Nitrogen fertilization at the recommendation dose probably reduced the effect of salinity on the purple passion fruit since the accumulation of these solutes increases the osmotic adjustment ability of plants, increasing the crop

resistance to salt stress (Ding et al., 2010).

At 70 DAS, the internal  $\text{CO}_2$  concentration ( $C_i$ ) was affected by the interaction between the studied factors (SL x ND) (Table 2), and according to the regression equation (Figure 2A), in relation to the nitrogen doses of 100 and 125 mg of  $\text{N kg}^{-1}$  of soil, the model to which the data best adjusted was the quadratic model, through which it was noted that the ECw of  $0.3 \text{ dS m}^{-1}$  promoted the maximum concentration of  $\text{CO}_2$  in the plants ( $327.19$  and  $281.11 \mu\text{mol mol}^{-1}$ ), respectively, and from this level that was a reduction of the  $C_i$ . According to the regressions of the remaining doses of 50 and 75 mg of  $\text{N kg}^{-1}$  of soil (Figure 2A), the  $C_i$  was linearly affected with reductions of 8.78

and 3.93%, respectively, per unitary increase of the ECw, that is, reductions of  $90.3$  and  $35.2 \mu\text{mol mol}^{-1}$  in the  $C_i$  of the plants irrigated with the water with the highest salinity ( $3.5 \text{ dS m}^{-1}$ ), in comparison with those irrigated with the water with the lowest salinity ( $0.3 \text{ dS m}^{-1}$ ). These reductions observed in the  $g_s$ ,  $E$ , and  $C_i$  may be the consequence of the osmotic effect caused by the excess of salts in the irrigation water, increasing the concentration of salts in the soil, compromising water absorption by the roots, leading the purple passion fruit 'BRS Rubi do Cerrado' to reduce its stomatal opening to avoid water loss, and consequently reducing transpiration. These results were in agreement with those obtained by Liu et al. (2014).



**Figure 2.** Internal  $\text{CO}_2$  concentration –  $C_i$  (A) and  $\text{CO}_2$  assimilation rate –  $A$  (B) of the passion fruit cultivar 'BRS Rubi do Cerrado' as a function of the electrical conductivity of irrigation water - ECw and doses of nitrogen fertilization - ND at 70 DAS, Pombal, PB, 2020.

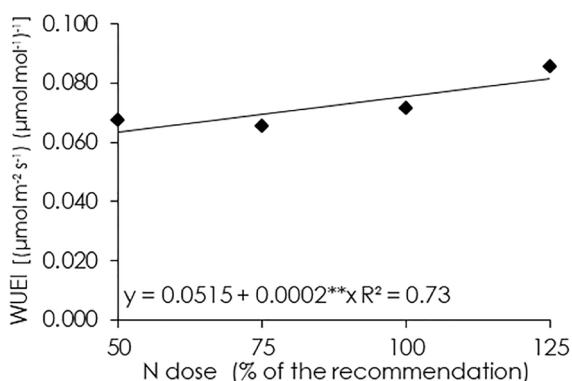
As a function of the reductions observed in stomatal conductance, transpiration rate, and internal  $\text{CO}_2$  concentration, the  $\text{CO}_2$  assimilation rate was compromised when the plants were irrigated with high-salinity water ( $3.5 \text{ dS m}^{-1}$ ). According to the regression studies (Figure 2B), a quadratic effect is verified for the doses of 75 and 125 mg of  $\text{N kg}^{-1}$  of soil, indicating that the highest values of the  $\text{CO}_2$  assimilation rate ( $21.13$  and  $23.40 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) were obtained, respectively, with the ECw levels of  $0.3$  and  $1.3 \text{ dS m}^{-1}$ . In relation to the plants subjected to the doses of 50 and 100 mg of  $\text{N kg}^{-1}$  of soil (Figure 2B), there was a linear decreasing effect in the  $A$ , equivalent to 8.26 and 7.83%, respectively, per unitary increase of the ECw, that is, reductions of 27.12 and 25.69% in the  $\text{CO}_2$  assimilation rate of the plants irrigated with ECw of  $3.5 \text{ dS m}^{-1}$ , when compared with those under ECw of  $0.3 \text{ dS m}^{-1}$ .

This decrease in the photosynthetic rates in plants under salt stress occurred mainly due to decreased water potential (Parihar et al., 2015). However, Andrade et al. (2019), evaluating the exogenous application of hydrogen peroxide on the morphophysiology of yellow passion fruit irrigated with saline water, observed a more

pronounced reduction (44%) in the  $\text{CO}_2$  assimilation rates when the plants were irrigated with the water of  $2.8 \text{ dS m}^{-1}$ , compared to the plants that received the water of  $0.7 \text{ dS m}^{-1}$ . It is highlighted that the combination of the dose of  $125 \text{ mg of N kg}^{-1}$  soil and the water salinity of  $1.3 \text{ dS m}^{-1}$  resulted in a positive impact on the photosynthetic rate, observed in the  $\text{CO}_2$  assimilation rate of the purple passion fruit 'BRS Rubi do Cerrado' at 70 DAS. This improvement may result from the increase in the synthesis of photosynthetic pigments and the rigidity of the cell wall in these treatments (Chen et al., 2010).

The instantaneous water-use efficiency (WUEi) in the passion fruit 'BRS Rubi do Cerrado' increased linearly as a function of the nitrogen doses, and according to the regression equation (Figure 3), an increment of 9.70% is verified per increase of 25% in the dose of N. By comparing the plants subjected to fertilization with 125% of N in relation to the plants that received 50% of the N recommendation, it is possible to verify an increment in the WUEi of  $0.015 (\mu\text{mol m}^{-2} \text{ s}^{-1}) (\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1})^{-1}$ . The WUEi increase in the passion fruit plants reflects the role of nitrogen in plant metabolism through its structural role in the synthesis of amino acids, proteins, coenzymes, nucleic

acids, vitamins, and chlorophyll – organic compounds that are essential for plant survival (Lima et al., 2019).



**Figure 3.** Instantaneous water-use efficiency - WUEi in the passion fruit 'BRS Rubi do Cerrado' as a function of the recommendation nitrogen dose (ND) at 70 DAS, Pombal, PB, 2020.

According to the summary of the analysis of variance (Table 3), there was a significant effect ( $p < 0.01$ ) of the interaction between the SL × ND factors on the number of leaves, stem diameter, and electrolyte leaching in  $p < 0.05$  to plant height. On the other hand, the salinity levels of the irrigation water significantly influenced the number of leaves, leaf area, stem diameter, relative water content, and electrolyte leaching. The nitrogen doses were significant ( $p < 0.05$ ) for the number of leaves

and electrolyte leaching of the purple passion fruit 'BRS Rubi do Cerrado'.

By analyzing the regression equations (Figure 4A), it is verified that there is a decreasing linear behavior of the number of leaves (NL) in the plants fertilized with 50, 100, and 125 mg of N kg<sup>-1</sup> of soil, whose decreases were, respectively, 9.20, 8.11, and 10.28% per unitary increment of the ECw, that is, an increase of 30.30, 26.61, and 33.53% in the NL of the plants subjected to the ECw of 3.5 dS m<sup>-1</sup>, in relation to those irrigated with 0.3 dS m<sup>-1</sup>. Regarding the nitrogen dose of 125 mg of N kg<sup>-1</sup> of soil, the model to which the data best fit was the quadratic model, verifying similar decreases to the remaining doses, in which the plants that received this N dose and were under irrigation with ECw of 0.3 dS m<sup>-1</sup> provided the highest NL (13.97 leaves), and from this point there was a reduction in the number of leaves. The reductions in the NL as a function of water salinity might be related to the water deficit induced by the osmotic effect, promoting stomatal closure and reduction in gas exchanges, consequently reducing water and nutrient absorption by the plants, which results in lower growth (Lima et al., 2015). Ribeiro et al. (2013) also observed a reduction in the NL of the yellow passion fruit with the increase in the electrical conductivity of irrigation water in field conditions.

**Table 3.** Summary of the analysis of variance for the number of leaves (NL), leaf area (LA), stem diameter (SD), plant height (PH), relative water content (RWC), and electrolyte leaching (EL) of the passion fruit cultivar 'BRS Rubi do Cerrado' subjected to different salinity levels (SL) of the irrigation water and doses of nitrogen (ND) at 70 DAS, Pombal, PB, 2020.

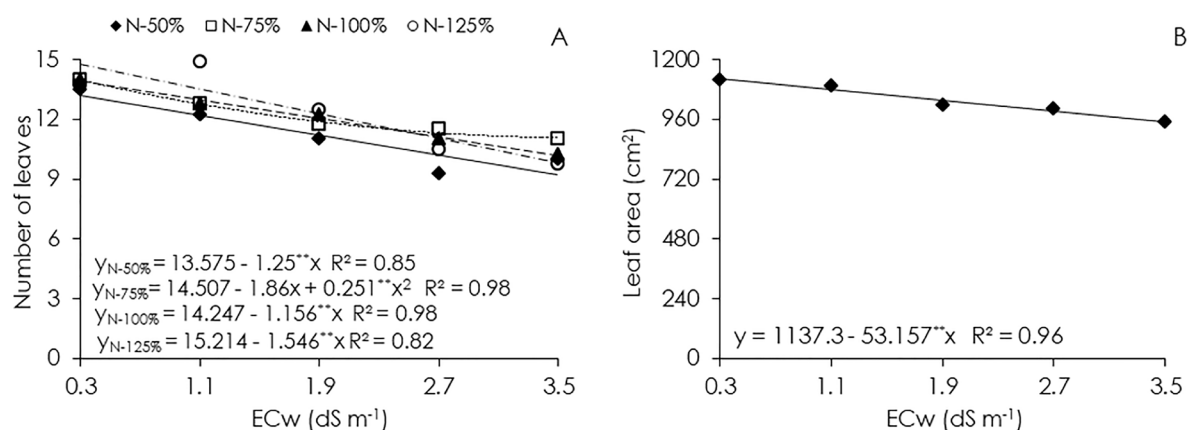
Source of variation	DF	Mean squares					
		NL	LA	SD	PH	RWC	EL
Saline levels (SL)	4	31.934**	74933.830*	2.479**	70.442 <sup>ns</sup>	167.633**	120.459**
Linear regression	1	114.751**	216490.292**	9.384**	146.274 <sup>ns</sup>	597.838 <sup>ns</sup>	248.951**
Quadratic regression	1	5.947*	29120.275 <sup>ns</sup>	0.280*	51.703 <sup>ns</sup>	9.937 <sup>ns</sup>	19.956**
N doses (ND)	3	4.003*	41274.898 <sup>ns</sup>	0.371 <sup>ns</sup>	192.903 <sup>ns</sup>	6.646 <sup>ns</sup>	32.929*
Linear regression	1	7.700*	44657.144 <sup>ns</sup>	0.930*	185.473 <sup>ns</sup>	0.391 <sup>ns</sup>	24.201 <sup>ns</sup>
Quadratic regression	1	2.278 <sup>ns</sup>	12392.684 <sup>ns</sup>	0.014 <sup>ns</sup>	385.125 <sup>ns</sup>	7.284 <sup>ns</sup>	72.219**
Interaction (SL x ND)	12	3.867**	58362.604 <sup>ns</sup>	0.827**	225.681*	14.492 <sup>ns</sup>	27.179**
Blocks	3	2.536 <sup>ns</sup>	89643.411*	0.108 <sup>ns</sup>	217.036 <sup>ns</sup>	59.056 <sup>ns</sup>	21.939 <sup>ns</sup>
Residual	57	1.304	27001.857	0.153	113.188	24.613	9.330
CV (%)		9.55	15.85	8.96	15.46	6.33	16.86
Mean		11.956	1036.483	4.368	68.835	78.347	18.117

<sup>ns, \*\*</sup>, \*respectively not-significant, significant at  $p < 0.01$ , and significant at  $p < 0.05$ ; DF - Degree of freedom; CV - Coefficient of variation

Regarding the leaf area (LA) of the purple passion fruit, it was observed that the increasing salinity levels caused reductions (Figure 4B), providing a linear decreasing effect, with reductions equivalent to 4.67% per unitary increase of the ECw at 70 DAS. The reduction in the LA with the increase in the electrical conductivity of irrigation water may be associated with the reduction in stomatal conductance observed in this study (Figure 1A), especially due to the osmotic effect. Our results are in accordance with those obtained by Khan et al. (2014) who associate the general decrease in the growth and

yield of crops under salt stress to the cumulative effect of the interruption of ionic homeostasis, water imbalance, and reduction in the photosynthetic capacity of the plants.

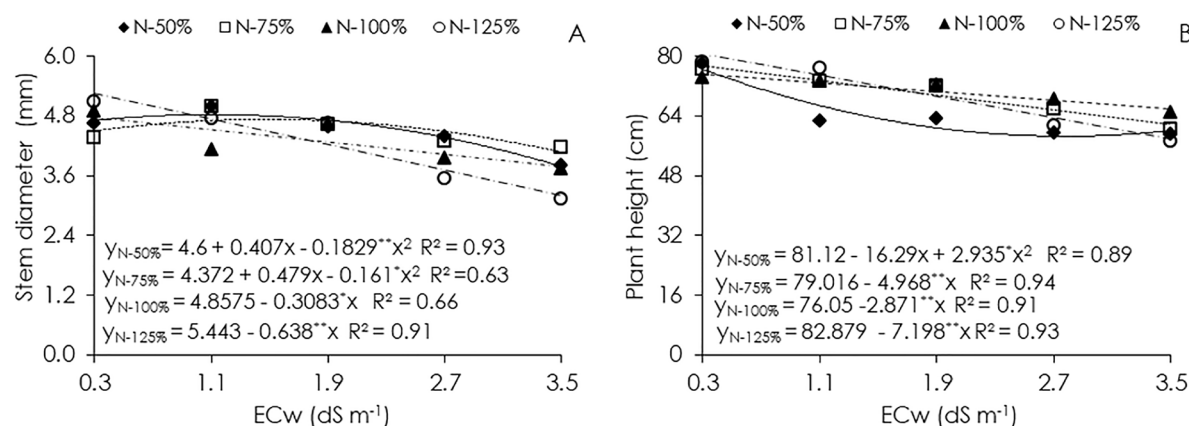
When analyzing the regression equations (Figure 5A), a linear decreasing behavior of the stem diameter (SD) is verified in the passion fruit plants fertilized 100 and 125 mg of N kg<sup>-1</sup> of soil, whose decreases were equivalent to 6.34 and 11.72% per unitary increment of the ECw, that is, a reduction of 20.88 and 38.37% in the SD of the plants subjected to the ECw of 3.5 dS m<sup>-1</sup> in relation to those



**Figure 4.** Number of leaves (A) as a function of the electrical conductivity of irrigation water – ECw and leaf area (B) of the passion fruit cultivar ‘BRS Rubi do Cerrado’ as a function of the electrical conductivity of irrigation water - ECw and doses of nitrogen fertilization - ND at 70 DAS, Pombal, PB, 2020.

irrigated with 0.3 dS m<sup>-1</sup>. Regarding the nitrogen doses of 50 and 75 mg of N kg<sup>-1</sup> of soil, the model to which the data best fit was the quadratic model, verifying that the plants that received these N doses and received irrigation with the ECw levels of 1.1 and 1.5 dS m<sup>-1</sup> provided the highest SD values, with 4.82 and 4.72 mm respectively (Figure 5A). It can be verified that the nitrogen doses were not sufficient to mitigate the salt stress imposed on the purple passion fruit plants; under these conditions, salt stress changes the hormonal balance and increases the production of reactive oxygen species, affecting cell expansion and division and vegetative and productive growth, besides being able to lead to plant senescence (Pang & Wang, 2008).

The plant height (PH) of the purple passion fruit was affected by the interaction between the studied factors (salinity levels x doses of nitrogen) at 70 DAS, and according to the regression equation (Figure 5B), linear decreases are verified in the PH of the plants fertilized with 75, 100, and 125 mg of N kg<sup>-1</sup> of soil, equivalent to 6.28, 3.77, and 8.68% per unitary increase of the ECw. As for the dose of 50 mg of N kg<sup>-1</sup> of soil, a quadratic effect is verified with higher growth in PH (76.49 cm) in the plants irrigated with 0.3 dS m<sup>-1</sup>, with significant reductions in PH from this level. Bezerra et al. (2016) also registered growth reductions in genotypes of the passion fruit cultivars ‘BRS Sol do Cerrado’ and ‘Redondo Amarelo’ subjected to irrigation with high-salinity water.

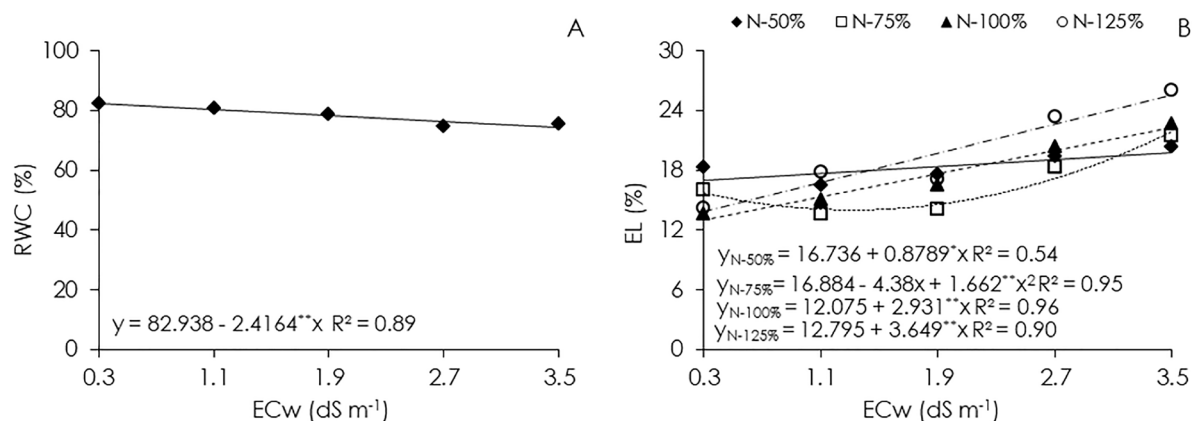


**Figure 5.** Stem diameter (A) and plant height (B) of the passion fruit ‘BRS Rubi do Cerrado’ as a function of the electrical conductivity of irrigation water - ECw and doses of nitrogen fertilization - ND at 70 DAS, Pombal, PB, 2020.

Figure 6 shows that the relative water content (RWC) of the purple passion fruit in the seedling production phase decreased with the increase in the salinity levels of the irrigation water. The decrease in the RWC per unitary increment of the ECw was equivalent to 2.91%, that is, a reduction of 9.40% in the plants irrigated with 3.5 dS m<sup>-1</sup> in relation to the treatment with the lowest salinity level (0.3

dS m<sup>-1</sup>). This occurred due to the difficulty of the plants in absorbing water, especially at the highest salt levels, interfering with physiological processes, as previously observed (Figure 2B) in the CO<sub>2</sub> assimilation rate of the passion fruit cultivar ‘BRS Rubi do Cerrado’ (Lobo et al., 2011).

It is verified (Table 3) that the interaction (SL x ND)



**Figure 6.** Relative water content – RWC (A) and electrolyte leaching – EL (B) of the passion fruit cultivar 'BRS Rubi do Cerrado' as a function of the electrical conductivity of irrigation water - ECw and doses of nitrogen fertilization - ND at 70 DAS, Pombal, PB, 2020.

promoted significant effects on electrolyte leaching (EL), and according to the regression equations (Figure 6B), a quadratic response of the plants fertilized with 75 mg of N kg<sup>-1</sup> of soil is verified, indicating that the highest value of EL (21.91%) was obtained with the ECw level of 3.5 dS m<sup>-1</sup>. Regarding the plants subjected to the doses of 50, 100, and 125 mg of N kg<sup>-1</sup> of soil (Figure 6B), there was a linear and growing effect on the EL equivalent to 5.25, 24.27, and 28.51% respectively, per unitary increase of the ECw, that is, an increase of 14.19, 41.99, and 45.67% in the EL when the plants were subjected to the ECw of 3.5 dS m<sup>-1</sup>, in relation to the lowest salinity level (0.3 dS m<sup>-1</sup>). The results corroborate those obtained by Pinheiro et al. (2019), when verifying that the increase in the salinity of the irrigation water promotes a rupture in the cell membrane, causing electrolyte leaching.

## Conclusions

The irrigation water salinity above 0.3 dS m<sup>-1</sup> compromised the leaf area and the relative water content of the purple passion fruit 'BRS Rubi do Cerrado'.

High nitrogen doses potentialized the deleterious effects of irrigation water salinity on stomatal conductance, transpiration, internal CO<sub>2</sub> concentration, CO<sub>2</sub> assimilation rate, number of leaves, stem diameter, and height of the purple passion fruit plants.

When using water with salinity levels up to 1.3 dS m<sup>-1</sup>, the dose of 125 mg of N kg<sup>-1</sup> of soil is recommendation for providing increments in the CO<sub>2</sub> assimilation rate of the purple passion fruit 'BRS Rubi do Cerrado', at 70 DAS.

Water salinity increases electrolyte leaching, regardless of the nitrogen doses.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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