

Growth, foliar gas exchange and biomass accumulation in passion fruit seedlings irrigated with saline waters and due to the applications of salt stress attenuators

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Abstract

The use of substances attenuating the harmful effects of salts on plants is an important strategy for the use of salt water in agriculture. The objective of this work was to evaluate the growth, gas exchange and biomass accumulation of passion fruit seedlings (*Passiflora edulis Sims*) irrigated with salt water due to the application of salt stress attenuators. The experiment was carried out in a randomized complete block design, in a 5 x 3 x 2 factorial scheme, with three replications, referring to the electrical conductivities of the irrigation water (1.5, 2.5, 3.5, 4.5 and 5.5 dS m⁻¹), three doses of humic acids (0, 10 and 20 mL) and two doses of citric acid (0 and 10 mL) as attenuators, respectively. Increased salinity of irrigation water reduced growth, accumulation of dry shoot biomass and gas exchange (stomatal conductance, transpiration, net photosynthesis, CO₂ internal concentration) of passion fruit sourced seedlings. Citric acid did not attenuate the negative effects of the salts and negatively interfered in the growth and accumulation of dry biomass of the shoots of passion fruit, mainly to the seedlings irrigated with water of low salinity. Humic substances at a dose of 20 mL promoted higher growth of passion fruit sprouts but were not sufficient to attenuate the negative effects of irrigation water salts. Therefore, citric acid and humic substances, under the conditions of the study, are not an option to use saline water in the production of passion fruit seedlings.

Keywords: Citric acid, humic acid, *Passiflora edulis Sims*, salinity in irrigation water.

Introduction

The passion fruit (*Passiflora edulis Sims*) is a tropical fruit with good adaptation for the Northeast region of Brazil and with great economic relevance for this region (Wanderley et al., 2018). However, the rainfall irregularity associated with water scarcity in this region are obstacles to fruit production (Silva et al., 2016), making irrigation necessary. Although passion fruit is of great importance for the Northeast, the salinity of the soil and the available water sources make it difficult to produce seedlings and establish a crop under conventional management (Bezerra et al., 2016).

The harmful effects of salinity have already been observed in the passion fruit culture (Bezerra et al., 2016; Medeiros et al., 2016; Ribeiro et al., 2016; Wanderley et al., 2018) and in other fruit such as papaya (*Carica papaya L.*) (Diniz et al., 2018) and cashew (*Anacardium occidentale L.*) (Torres et al., 2014). These effects may be mitigated by the use of organic inputs applied to the soil, such as humic substances. This highlights the need for research that results in viable technologies for

producers and may minimize the deleterious effects of salts on plants, since the use of saline water in agriculture is almost mandatory in semiarid regions.

Currently, many producers have been investing in the simultaneous fertilization of mineral and organic fertilizers, highlighting the use of humic substances in order to improve soils and mitigate the harmful effects of salinity. Prazeres et al. (2014), evaluating two organic sources (liquid cattle manure and Humitec®), two electrical conductivities of irrigation water (1.5 and 4.0 dS m⁻¹), and five doses of organic sources (0, 5, 10, 15 and 20%) in passion fruit, concluded that the application of organic inputs provided a growth of plants irrigated with saline water, similar to plants irrigated with water with less salinity.

Elhamid et al. (2014) when evaluating the attenuation of the adverse effects of salt stress in wheat (*Triticum sativum L.*) cultivars by foliar treatment with antioxidants, concluded that treatment with a compound of ascorbic acid and citric acid

may partially attenuate the harmful effect of salinity, especially at lower conductivities.

In this scenario, the objective was to evaluate the behavior of passion fruit seedlings, cultivar BRS Rubi do Cerrado, submitted to irrigation with water of different electrical conductivities due to the application of substances potentially attenuating salt stress (humic substances and citric acid), in an attempt to contribute with alternatives that may mitigate the damage caused by salinity in growth, physiology and biomass accumulation of passion fruit seedlings.

Results and Discussion

Effect of saline water on plant height

The interaction between the three factors (electrical conductivity x humic substances x citric acid) had no significant effect on the variables analyzed. The interaction of saline water x humic substances promoted a significant effect only for the number of leaves, saline water x citric acid for liquid photosynthesis and stomatal conductance and humic substances x citric acid for the stem diameter and dry biomass of the aerial part. As for the isolated factors, it was observed that irrigation with saline water significantly influenced the variables studied, except for the dry root biomass, which did not present a significant difference. The doses of humic substances and citric acid influenced only the number of leaves and the dry biomass of the aerial part.

The average height of the passion fruit seedlings was 7.85 cm; however, the highest height was obtained with the irrigation with water of 1.5 dS m⁻¹, obtaining in this 9.22 cm, whereas seedlings irrigated with water of 5.5 dS m⁻¹ had the lowest height (6.58 cm), representing a reduction of 40.33%, when compared to seedlings irrigated with water of 1.5 dS m⁻¹ (Figure 1A).

The average stem diameter of the passion fruit seedlings was 2.28 mm, however, when the seedlings were irrigated with 1.5 dS m⁻¹ water, the stem diameter was 2.52 mm; it was found that by increasing the electrical conductivity in the irrigation water the stem diameter of the passion fruit seedlings reduced to 2.00 mm, corresponding to a 20.6% reduction in the diameter of seedlings irrigated with water of 5.5 dS m⁻¹ in relation to those irrigated with water of 1.5 dS m⁻¹ (Figure 1B).

These results corroborate those obtained by Ribeiro et al. (2013), who obtained similar results in plant height and stem diameter of passion fruit when evaluating the effects of irrigation water salinity on two different substrates (cattle manure plus sand and only cow manure). According to Oliveira et al. (2015), the electrical conductivity in irrigation water above 1.5 dS m⁻¹ may promote phytotoxic effect on passion fruit plants, triggering a series of ionic and hormonal changes compromising the growth of plants (Sá et al., 2013).

Stem diameter

The association of citric acid and humic substances reduced the stem diameter of passion fruit seedlings; however, seedlings that received only humic substances without citric acid obtained a larger stem diameter, especially at a dose of 20 mL of humic substances, in which the stem diameter was 2.5 mm, that is, an increase of 15% in relation to seedlings without any of the products (Figure 2).

The increase in the stem diameter of passion fruit seedlings with the application of the highest doses of humic substances is due to the supply of nutrients present in humic substances, which promote changes in growth, formation pattern and

differentiation of plant organs (Canellas & Olivares, 2014). This stimulating action is attributed, in general, to a direct effect of plant hormones or even on hormonal behavior of plants (Chen et al., 1990; Boyhan et al., 2001), in particular the hormone auxin, which may be stimulated in the presence of humic acids, resulting in growth of the root system of plants (Baldotto et al., 2017) and consequently in the increase in stem diameter.

Number of leaves

The increase in electrical conductivity in irrigation water from 1.5 dS m⁻¹ to 5.5 dS m⁻¹ reduced by 21% the number of leaves of the passion fruit seedlings, regardless of the application of humic substances (Figure 3).

Under conditions of salt stress, it is common for morphological and anatomical changes to occur in plants, which reflect on the reduction of perspiration as an alternative to maintain low absorption of saline water; one of these adaptations is the reduction in the number of leaves (Silva et al., 2012). Similar results were found by Ribeiro et al. (2013), who observed a reduction in the number of leaves with an increase in the salinity of the water used in irrigating the passion fruit culture in two types of substrates (bovine manure plus sand and only bovine manure).

The passion fruit seedlings that received the highest doses of humic substances were the ones that had the largest number of leaves, which may be attributed to the effect of humic substances in attenuating the deleterious effects of salinity. Silva et al. (2014), when evaluating the effects of water salinity in the soil without and with liquid organic compounds (liquid bovine manure and Humitec®) on the production of two passion fruit genotypes, found that the increase in water salinity inhibited the leaf emission of seedlings in the soil without and with any of the organic inputs, but with supremacy of liquid bovine manure in relation to the control and the soil with Humitec®.

Dry biomass of the aerial part

The increase in electrical conductivity in irrigation water reduced the dry biomass of the aerial part of the passion fruit seedlings, so that the highest accumulation was observed in seedlings irrigated with water of 1.5 dS m⁻¹ (1.3 g) and the lowest dry biomass (0.7 g) with irrigation with water of 5.5 dS m⁻¹ (Figure 4A). According to Taiz & Zeiger (2013), saline stress decreases the availability of water and increases the concentration of salts that exert toxicity to plants, compromising cell division and consequently the root and aerial growth of seedlings.

The seedlings that received the dose of 20 mL of humic substances without the application of citric acid had the highest accumulation of dry biomass of the aerial part (1.3 g), while the seedlings that received citric acid presented the lowest accumulation of dry biomass in the aerial part regardless of the application of humic substances (Figure 4).

Effect of saline water and citric acid on photosynthetic activity

The rate of liquid photosynthesis (Figure 5A) and stomatal conductance (Figure 5B) were higher in the absence of citric acid when the seedlings received less saline water (1.5 dS m⁻¹). By increasing the electrical conductivity in irrigation water up to 5.5 dS m⁻¹, it was observed that the passion fruit seedlings reduced the rate of liquid photosynthesis and stomatal conductance regardless of the application of citric acid. These reductions are due to the restriction in the

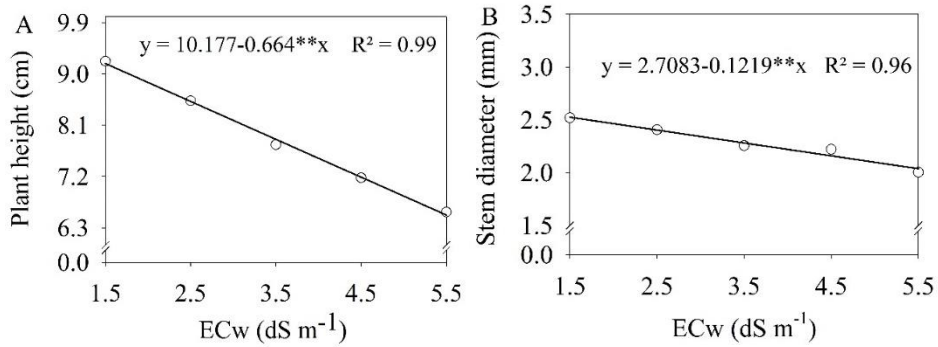


Figure 1. Plant height (A) and stem diameter (B) of passion fruit seedlings as a function of irrigation water salinity.

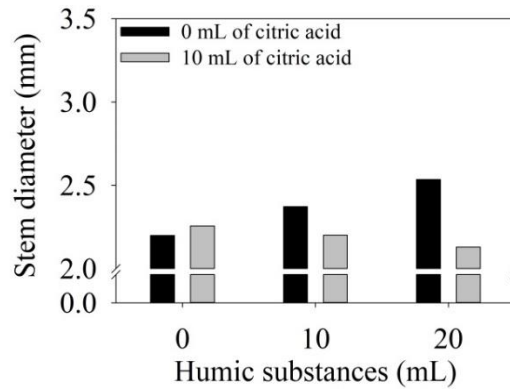


Figure 2. Stem diameter of passion fruit seedlings depending on the application of humic substances and citric acid. Averages followed by the same lower and upper case letters do not differ by Tukey's test at 5% probability for the application of humic substance and citric acid, respectively.

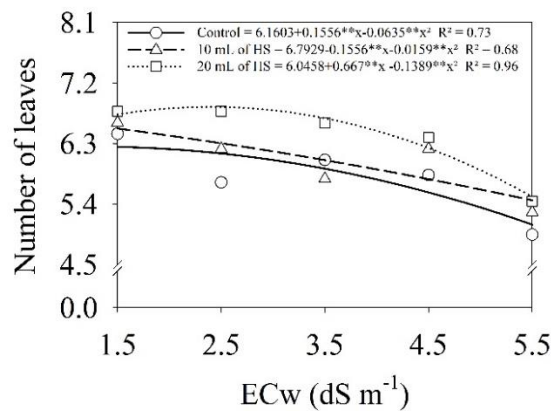


Figure 3. Number of leaves of passion fruit seedlings depending on the salinity of the irrigation water.

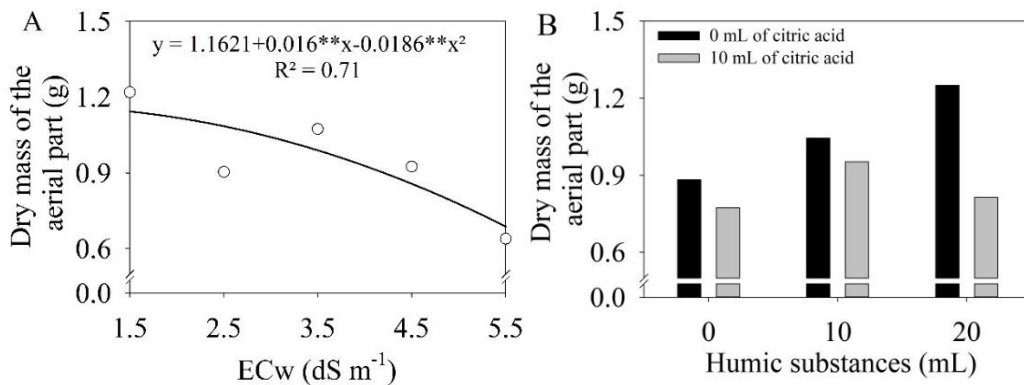


Figure 4. Dry biomass of the aerial part of passion fruit seedlings depending on the salinity of the irrigation water (A) and the application of citric acid (B). Averages followed by the same letters do not differ by Tukey's test at 5% probability.

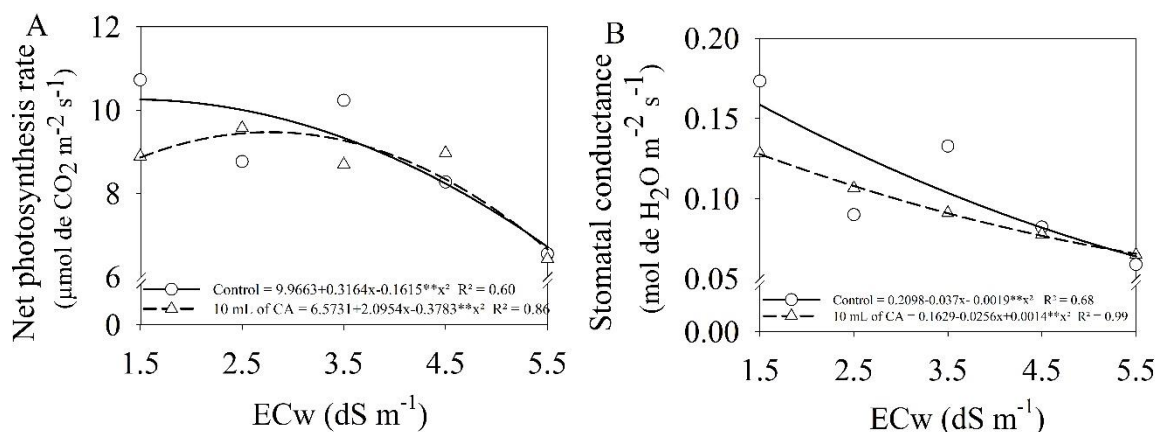


Figure 5. Net photosynthesis rate (A) and stomatal conductance (B) of passion fruit seedlings as a function of irrigation water salinity and citric acid (CA) application.

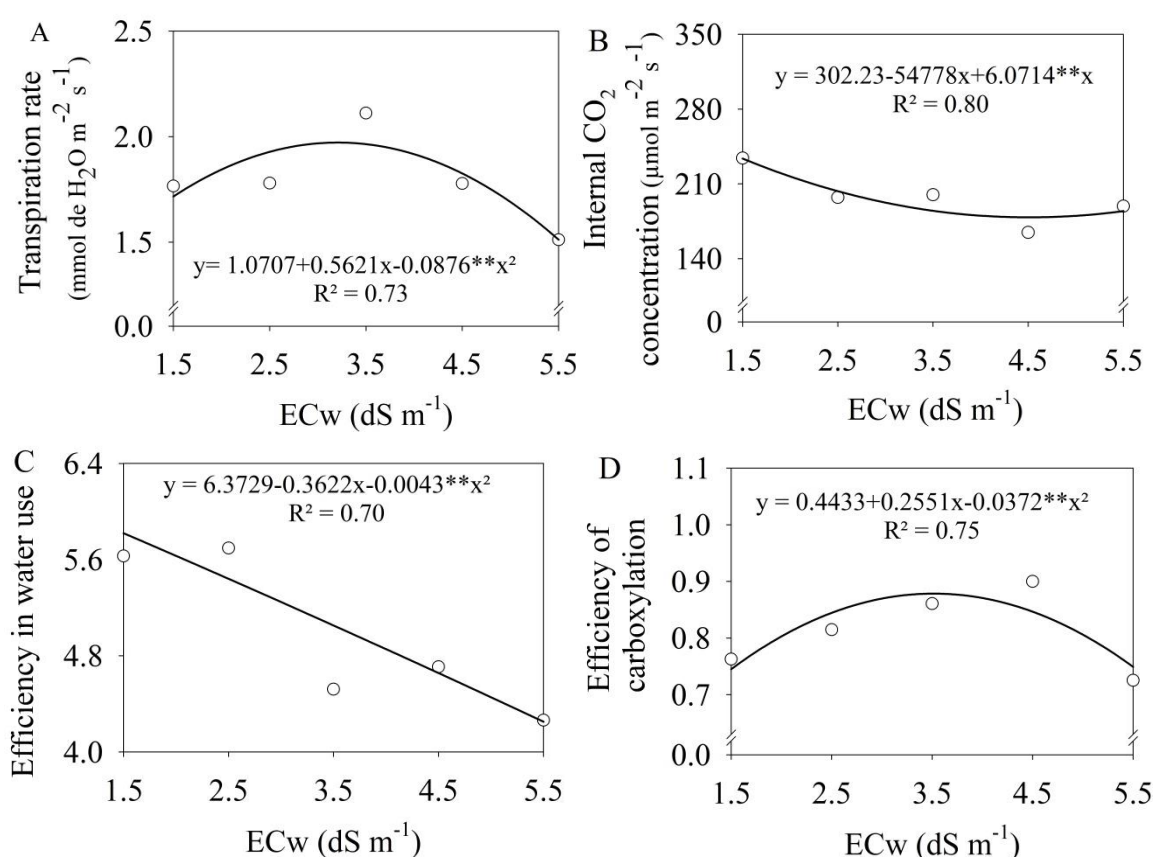


Figure 6. Transpiration (A), internal CO₂ concentration (B), water use efficiency (C) and instant carboxylation efficiency (D) of passion fruit seedlings as a function of irrigation water salinity.

absorption of potassium, due to the competition between sodium and potassium ions for the adsorption sites or a greater efflux of potassium from the roots. Potassium performs physiological functions, such as controlling cell turgidity, activating enzymes involved in respiration, water relations, photosynthesis and regulation of stoma opening and closing processes (Wang et al., 2013; Tatagiba et al., 2014).

In addition, the closure of stomata of the passion fruit seedlings may have occurred due to the stress conditions that irrigation with saline water provided as a strategy to reduce water loss through transpiration, a behavior also observed by Freire et al. (2014), who concluded that stomatal conductance in passion fruit was significantly impaired by the

increase in water salinity from 0.5 to 4.5 dS m⁻¹ (Wang et al., 2013; Jáklí et al., 2017).

In addition, the reduction in stomatal conductance occurs mainly due to the greater difficulty of seedlings in absorbing water from the soil; therefore, as a way to reduce water loss, there is stomatal closure, resulting in decreases in important processes for plants such as liquid photosynthesis and water use efficiency (Oliveira et al., 2017).

Leaf gas exchange

Transpiration (Figure 6A), which in general suffers from the depressive effects of excess salts in the root zone, presented a curve with a tendency to increase in seedlings irrigated with intermediate salinity water (3.5 dS m⁻¹) and then reduced

with the increase in the levels of electrical conductivity, obtaining the least transpiration in seedlings irrigated with water of 5.5 dS m⁻¹.

This result corroborates those obtained in the literature, since the excess of salts in the root zone has, in general, a depressive effect on the growth of plants, manifesting itself by a reduction in the rate of transpiration (Pedrotti et al., 2015). Saline stress increases the energy that needs to be released to absorb water from the soil and wears the plant. In addition, the rate of transpiration is associated with stomatal closure, a mechanism used by the plant to maintain its water status. As stomatal conductance presented a decreasing behavior, the tendency was that the same occurred with the rate of transpiration (Fernández Garcia et al., 2014).

The internal concentration of CO₂ (Figure 6B) decreased by approximately 20% with the increase in electrical conductivity in the irrigation water from 1.5 dS m⁻¹ to 5.5 dS m⁻¹. However, the reduction was even greater (about 30%) when the passion fruit seedlings were irrigated with water of 4.5 dS m⁻¹. It is likely that the low concentration of CO₂ in the seedlings irrigated with water of 4.5 dS m⁻¹ is related to the high photosynthetic rate in these seedlings, as the photosynthetic rate (Figure 5A) presented a more marked reduction when the seedlings were irrigated with water of 5.5 dS m⁻¹, which may justify the higher concentration of CO₂ in seedlings irrigated with this water, since the consumption of CO₂ was lower.

Efficiency in the use of water (Figure 6C), which is the relationship between liquid photosynthesis and transpiration, presented a very sharp decreasing curve, a fact that indicates low resistance of passion fruit, cultivar BRS Rubi do Cerrado, to salt stress conditions. The increase in water use efficiency is fundamental to reduce the waste of this resource, as this variable indicates the amount of carbon that the plant fixes, due to the amount of water that the plant loses in the transpiratory process (Taiz & Zeiger, 2013).

The instant efficiency of carboxylation (Figure 6D), which is the relationship between liquid photosynthesis and internal CO₂ concentration, presented a curve with an upward trend until treatment with salinity water of 3.5 dS m⁻¹ with subsequent reduction in seedlings under irrigation with water of 5.5 dS m⁻¹. Chaves (1991) mentions that this reduction in the instant efficiency of carboxylation may be attributed to changes in the photosynthetic capacity of the leaf mesophyll, as well as to stomatal and non-stomatal factors.

Materials and Methods

Experiment location

The experiment was conducted in a greenhouse during the period from May to August 2016 at the Federal University of Paraíba (UFPB), Campus Bananeiras – PB, Brazil (6° 46 S and 35° 38 W), with an altitude of 617 m and the climate, according to the Köppen classification, is As' (rainy tropical) hot and humid.

Experimental design and treatments

The experimental design adopted was in randomized blocks, in a factorial scheme of 5 x 3 x 2, referring to the electrical conductivities of irrigation water (EC_w – 1.5; 2.5; 3.5; 4.5 and 5.5 dS m⁻¹), three doses of humic acids (0, 10 and 20 ml) and two doses of citric acid (0 and 10 ml), with three repetitions. The experimental units were composed of six seedlings of passion fruit, totaling 360 seedlings, grown in polyethylene

bags with a capacity of 5 dm³. The polyethylene bags were filled with soil classified as Dystrophic Yellow Argisol (Embrapa, 2018), with the addition of washed sand in the proportion of 3: 1 (v/v).

Sowing

The seeds of passion fruit cv. BRS Rubi do Cerrado were obtained from fruits collected in the Gurugi II settlement, in Conde – PB, Brazil. The fruits had vigorous characteristics, with appropriate ripeness, firm consistency, free from insect attacks and other apparent physical defects. The seeds were extracted manually together with the mucilage and dried in the shade on newsprint, for two days, at room temperature. Subsequently, the seeds were subjected to breaking dormancy according to Melo et al. (1998), where it was brought to a constant temperature of 30 °C, for 15 minutes and then dried in the shade.

Sowing was done directly in the polyethylene bags in which five seeds were placed, and at twenty days after emergence the most vigorous seedling was selected and the other seedlings were thinned.

Irrigation

The irrigation of the passion fruit seedlings was standardized with 250 mL of water (according to each electrical conductivity – treatment) every two days, which allowed maintaining the soil moisture close to the field capacity. The different EC_w were obtained by diluting strongly saline water (15.8 dS m⁻¹) with non-saline water (0.6 dS m⁻¹) according to the methodology of Cavalcante et al. (2005). Irrigation with waters of different salinities was started after thinning the seedlings.

Treatment application

The source of the humic substances was a commercial product (Humitec[®]) liquid soluble in water, which contains 16.5% w/v (15% w/w) of total humic extract, 11.2% w/v (10% w/w) organic carbon, 13.2% w/v (12% w/w) humic acid, 3.3% w/v (3% w/w) fulvic acid, 9.0% w/v (8.0 w/w) of water-soluble nitrogen and 4.5% w/v (4% w/w) of potassium (K₂O) (Silva et al., 2014). Humic substances and citric acid were applied directly to the substrate 15 days before sowing and 30 days after sowing (DAS). The humic substances were diluted in water in the proportion of 100 g L⁻¹ and the citric acid in the proportion of 100 mmol L⁻¹ and subsequently were applied in homogeneous solution.

Variables analyzed

At 80 DAS, the growth variables were evaluated: seedling height, stem diameter, number of leaves, dry root biomass and aerial part; gas exchange: stomatal conductance (g_s, mol H₂O m⁻² s⁻¹), transpiration (E, mmol H₂O m⁻² s⁻¹), liquid photosynthesis (A, μmol CO₂ m⁻² s⁻¹), internal CO₂ concentration (C_i, μmol CO₂ m⁻² s⁻¹), water use efficiency (WUE – A/E, [(μmol m⁻² s⁻¹/mmol H₂O m⁻² s⁻¹)⁻¹]) and the instant efficiency of carboxylation (IcE – A/C_i, [(μmol m⁻² s⁻¹/μmol mol⁻¹)⁻¹]) in the period between 9:00 and 12:00 hours, using an IRGA infrared gas analyzer (ADC, model LCPro, Hoddesdon, UK), with 300 mL min⁻¹ air flow and 1200 μmol m⁻² s⁻¹ coupled light source (Konrad et al., 2005; Melo et al., 2009; Furtado et al., 2013).

Statistical analysis

The data were subjected to analysis of variance by the F test up to 5% probability, and when significant, the Tukey test for qualitative factors and regression analysis for the quantitative was performed with models adjusted up to 5% of significance using the statistical software R (R Core Team, 2018).

Conclusions

The increase in the salinity of the irrigation water reduced the growth, accumulation of biomass of the aerial part and gas exchange of the passion fruit seedlings. The doses of humic substances promoted greater growth of the passion fruit seedlings; however, their effectiveness was not evident, requiring studies with other doses and economic evaluations. Citric acid did not attenuate the negative effects of salts on passion fruit seedlings, being even harmful to seedlings irrigated with low salinity water. Citric acid and humic substances, under the conditions of the study, are not an option for the use of saline water in the production of passion fruit seedlings.

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