CELL DAMAGE AND BIOMASS OF YELLOW PASSION FRUIT UNDER WATER SALINITY AND NITROGEN FERTILIZATION

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ABSTRACT - The aim of this study was to evaluate the attenuating action of nitrogen doses on leaf cell membrane damage, dry biomass production and leaf area in the formation of yellow passion fruit seedlings irrigated with saline water. Treatments were arranged in a randomized block design, in split plots, corresponding to five levels of irrigation water salinity (plot) (ECw) (0.3; 1.0; 1.7; 2.4 and 3.1 dS m⁻¹) and five doses of nitrogen fertilization (subplot) (60; 80; 100; 120 and 140% of 300 mg of N dm⁻³), which were repeated in five blocks. Plants were grown in pots (Citropote®) with a volume of 3,780 mL, which were filled with a mixture of soil, aged bovine manure and sawmill residue (shaving) in a ratio of 2:1:0.5, respectively. Waters with different levels of salinity were applied from 40 to 85 days after sowing, when the plants were in transplanting conditions. At 85 days after sowing, the percentage of cell damage based on electrolyte leakage, variables of dry biomass, leaf area and specific leaf area were evaluated. Increment in irrigation water salinity reduces the biomass accumulation of yellow passion fruit seedlings; The increase in nitrogen dose did not mitigate the effect of salinity, which reduced cell membrane integrity, making the plant more sensitive.

Keywords: Passiflora edulis Sims. f. flavicarpa DEG. Salt stress. Leaf electrolyte leakage.

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DANO CELULAR E FITOMASSA DO MARACUJAZEIRO AMARELO SOB SALINIDADE DA ÁGUA E ADUBAÇÃO NITROGENADA

RESUMO - Objetivou-se estudar a ação atenuante de doses de nitrogênio sobre o dano à membrana celular das folhas, a produção de fitomassa seca e a área foliar na formação de mudas de maracujazeiro amarelo irrigadas com água salina. Para tanto, foi usado o delineamento experimental em blocos casualizados, com os tratamentos dispostos em parcelas subdivididas, estudou-se cinco níveis de salinidade da água de irrigação (parcela) (CEa) (0,3;1,0; 1,7; 2,4 e 3,1 dS m⁻¹) e cinco doses de adubação nitrogenada (subparcela) (60; 80; 100; 120 e 140%) de 300 mg de N dm⁻³), que foram repetidos em cinco blocos. As plantas foram cultivadas em citropotes com volume de 3,780 mL, que foram preenchidos com uma mistura de solo, esterco bovino curtido e resíduo de serraria (maravalha) na proporção de 2:1:0,5, respectivamente. A aplicação das águas com diferentes salinidades ocorreu no período de 40 a 85 dias após a semeadura, época em que as plantas estavam em condições de transplante. Aos 85 dias após a semeadura, estudaram-se o percentual de dano celular, por meio do extravasamento de eletrólitos, variáveis de fitomassa seca, a área foliar e área foliar específica. O incremento na salinidade da água de irrigação reduz o acúmulo de fitomassa das mudas de maracujazeiro amarelo; O aumento na dose de nitrogênio não mitigou o efeito da salinidade, que reduziu a integridade da membrana celular, proporcionando maior sensibilidade à planta.


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1Received for publication in 11/27/2019; accepted in 03/20/2020.

Paper extracted from the thesis of the first author.

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INTRODUCTION

Yellow passion fruit (*Passiflora edulis* Sims *f.* *flavicarpa* DEG.), also known as sour passion fruit, is a highly widespread species in Brazil, with production of 602,651 tons in an area of 42,731 ha, and the largest producers are the states of Bahia, Ceará, Santa Catarina, São Paulo, with Bahia being responsible for 26.9% of Brazilian passion fruit production (IBGE, 2017).

Thus, it is evident the potential of the Northeast region for the production of this crop, which is related to the favorable edaphoclimatic conditions, except for rainfall, which is lower than evapotranspiration, causing water deficit to plants (CAVALCANTE et al., 2002; FREIRE et al., 2014), making it necessary to use irrigation in order to ensure satisfactory levels of passion fruit yield.

Moreover, besides the low availability of water and the need to perform irrigation, in most cases the waters have high contents of dissolved salts (CAVALCANTE et al., 2002), which can induce physiological changes and compromise the development and production of the crop, which is considered sensitive to soil and water salinity.

The most striking effects of salinity on plants are changes in osmotic potential, ionic toxicity and imbalance in the absorption of nutrients, causing a generalized reduction in their growth (AHMED; MORITANI, 2010; SOUSA; BEZERRA; FARIAS, 2010).

According to Silva et al. (2014) and Lima et al. (2015), excess salts in irrigation water can cause changes in the permeability of cell membranes and in the physiological and biochemical functions of plants, leading to osmotic stress, which results in disorders in water relations, changes in the absorption and use of essential nutrients, as well as the accumulation of toxic ions (Na⁺ and/or Cl⁻) in chloroplasts, regardless of the nature of the salts.

On the other hand, as salinity can cause osmotic and ionic effects, the stimulus to the production of osmoprotective amino acids, carbohydrates and proteins is compromised, and proline is a stress-related metabolite that aids in osmoregulation and favors the increase in the tolerance to certain levels of water and salt stresses (SALAZAR et al., 2017). In this context, it is known that nitrogen (N) is an element that participates in the formation of several compounds considered indispensable, such as amino acids, proteins, nucleic acids and chlorophylls (TAIZ et al., 2017).

Thus, the proper use of N fertilization can contribute to the accumulation of organic solutes, as it acts as a constituent of the chlorophyll molecule, nucleic acids, amino acids and proteins (OLIVEIRA et al., 2010), increasing the osmotic adjustment capacity of plants to salinity, as observed by Bezerra et al. (2018), who studied guava plants under salt stress in the production stage and identified that 70% of the N fertilization recommendation is the most adequate level for the growth of plants under water salinity conditions.

In this context, the objective was to study the attenuating action of N doses on the damage to the leaf cell membrane, dry biomass production and leaf area in the formation of yellow passion fruit seedlings irrigated with saline water.

MATERIAL AND METHODS

The experiment was conducted from February to April 2015, in a greenhouse of the Center for Science and Agri-food Technology of the Federal University of Campina Grande (CCTA-UFCG), Campus of Pombal, PB, at the coordinates 6°48′16″ S and 37°49′15″ and 175 m altitude. According to Köppen’s classification, the predominant climate of the region is BSh, that is, hot and dry semiarid.

The experimental design was randomized blocks, with the treatments distributed in split plots, composed of five levels of irrigation water salinity in the plots (ECw) (0.3; 1.0; 1.7; 2.4 and 3.1 dS m⁻¹) and five doses of N fertilization in the subplots (60; 80; 100; 120 and 140% of the recommendation of 300 mg of N dm⁻³ (MALAVOLTA, 1980)), with five replicates, totaling 25 treatments and 125 experimental units, each with one plant.

Yellow passion fruit seeds were sown in commercial substrate arranged in polyethylene trays of 166 cells. At 30 days after sowing (DAS), the seedlings were transplanted to pots (Citropote®), with a capacity of 3,780 mL, containing the substrate composed of a mixture of soil, aged bovine manure and sawmill shavings in the proportion of 2:1:0.5, respectively. The soil used was NEOSSOLO FLUVICO (Entisol), specifically its horizon A, with the physicochemical characteristics shown in Table 1, collected in an experimental area of the CCTA/ UFCG. The soil and manure were sieved through 2-mm meshes and placed in the containers, leaving about 2.0 cm between the soil surface and the upper edge of the pots, in order to facilitate irrigation.
After transplanting, the seedlings received, for 10 days, irrigation with low-salinity water from the local supply system (0.3 dS m$^{-1}$), keeping the soil moisture close to the maximum retention capacity. After this period, the treatments began to be applied, lasting 45 days. Nitrogen fertilization was split into 4 portions with an interval of 5 days, applied in solution via irrigation water using urea as N source. Corrective fertilization followed recommendations proposed by Lima (2002).

The levels of water salinity were obtained by adding sodium chloride (NaCl), calcium chloride (CaCl$_2$.H$_2$O) and magnesium chloride (MgCl$_2$.6H$_2$O) salts in the equivalent proportion of 7:2:1, respectively, which is the predominant ratio of ions in sources of water used for irrigation, from the crystalline of northeastern Brazil (MEDEIROS et al., 1992).

The five levels of water salinity were prepared considering the relationship between ECw and salt concentration contained in Rhoades, Kandiah and Marshal (1992) (10*meq L$^{-1}$ = 1 dS m$^{-1}$ of ECw), valid for ECw from 0.1 to 5.0 dS m$^{-1}$ which encompasses the tested levels. In this process the salts were weighed, according to the salinity level, and water was added until the desired ECw level was reached and the values were checked with a portable conductivity meter, whose electrical conductivity was adjusted to the temperature of 25 ºC. After preparation the waters were stored in 60-L plastic containers.

Irrigation management was performed based on the daily water consumption obtained by the water balance method, through drainage lysimetry adapted as described in Mantovani, Bernardo and Palaretti (2009).

The lysimeters consisted of 3.780 mL pots, installed on gutters to collect the drained volume, at each salinity level, that is, different management as a function of the salinity levels studied. The volume applied (Va), daily, in the pots was obtained by the difference between the total volume applied to the pots on the previous day (V$\$_{t}$) and the volume drained (V$\$_{d}$) on the following day, dividing the result by the number of pots per gutter (n) and applying the leaching fraction of 20% (LF), as indicated in Equation 1.

\[ V_a = \frac{(V_{ta} - V_d)}{1 - 0.2} \]  

At 85 DAS, cell membrane damage (%D) was quantified through the electrolyte leakage technique (BAJI; LUTTS; KINET, 2001). For this, 8 leaf discs with diameter of 1.2 cm were collected transported to the laboratory, immediately washed with distilled water, dried on absorbent paper and placed in beakers containing 25 mL of distilled water at 25 ºC which remained at rest for 4 h. After this period a benchtop conductivity meter (MS Tecnopon® - mCA 150) was used to measure the initial electrical conductivity (C1), then the beakers with the discs were placed in an oven at temperature of 90 ºC for 2 hours. After this period, the final electrical conductivity was read (C2), and %D was calculated using Equation 2.

\[ %D = \left(\frac{C_1}{C_2}\right) * 100 \]  

Dry biomass was partitioned by separating the material and placing leaves, stems and roots in paper bags. After drying in an oven at 65 ºC, until obtaining constant weight, the mass was determined on a precision scale, obtaining the values of root dry mass (RDM), leaf dry mass (LDM), stem dry mass (SDM), shoot dry mass (ShDM) and total dry mass (FMST), and the data were presented in grams per plant (g plant$^{-1}$).

When the dry biomass of each plant was determined, 20 leaf discs of 5 cm in diameter were extracted to determine the leaf area (LA cm$^2$), through the ratio between the dry mass of a known area, i.e., dry mass of discs (DMD), area of discs (AD) and dry mass of leaves (DML), using Equation 3.

\[ LA = AD * \frac{DML + DMD}{DMD} \]  

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**Table 1. Physicochemical characteristics of the soil and manure used in the different treatments.**

<table>
<thead>
<tr>
<th>Physicochemical characteristics of the soil</th>
<th>pH (H$_2$O)</th>
<th>EC</th>
<th>P</th>
<th>K$^+$</th>
<th>Na$^+$</th>
<th>Ca$^{2+}$</th>
<th>Mg$^{2+}$</th>
<th>Al$^{3+}$</th>
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<td>1.59</td>
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<td>1.73</td>
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<td>7.62</td>
<td>1.23</td>
<td>80</td>
<td>2.15</td>
<td>4.4</td>
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<td>10.7</td>
<td>0</td>
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**Physicochemical characteristics of the manure**

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EC = electrical conductivity; SB = sum of bases; t = cations exchange; T = cation exchange capacity; V = percentage of saturation. OM = organic matter.
The data obtained were subjected to analysis of variance, F test, followed by regressions (linear and quadratic) for salinity levels at each N dose when interaction occurred, or for the factors, separately using the program Sisvar (FERREIRA, 2014).

RESULTS AND DISCUSSION

According to the summary of the analysis of variance (Table 2), the interaction between irrigation water salinity levels and N fertilization caused significant effect (P ≤ 0.05) on root dry mass (RDM), leaf dry mass (LDM), shoot dry mass (ShDM), leaf area (LA) and specific leaf area (SLA) at 85 DAS.

Water salinity significantly affected cell membrane damage (%D) as well as total dry mass (TDM) and stem dry mass (StDM), which was also significantly affected by N levels.

For the effect of the interaction between factors on most of the growth variables studied, which was also combined with the single effect of the factors, the plants exhibited different behaviors with the increase of water salinity and N levels. In a study on the effect of water salinity and N doses on the biomass formation of yellow passion fruit seedlings. Bezerra et al. (2014) observed that N fertilization attenuates the negative effects of the salinity of moderately saline irrigation water, although the inverse of this effect may occur.

Table 2. Summary of the analysis of variance for the percentage of cell membrane damage (%D). root dry mass (RDM), leaf dry mass (LDM), stem dry mass (StDM), shoot dry mass (ShDM), total dry mass (TDM), leaf area (LA) and specific leaf area (SLA) at 85 days after sowing (DAS), as a function of levels of irrigation water salinity and nitrogen doses.

![Figure 1](image)

Figure 1. Percentage of cell membrane damage (%D) at 85 days after sowing (DAS) in yellow passion fruit seedlings, as a function salinity levels in water.

Similar results were reported by Viudes and Santos (2014), when studying biochemical changes in Artemisia plants under salt stress caused by irrigation with NaCl solution (0, 100 and 200 mM), and by Karlidag, Yildirim and Turan (2009), who analyzed strawberry plants under salt stress. In both cases, there was also an increase in cell damage as salt concentration increased.

The accumulation of root dry mass (RDM) was significantly modified by the application of N doses in plants irrigated using water with salinity levels of 0.3, 1.7 and 2.4 dS m⁻¹ (Figure 2A), and increments in N dose reduced the values of RDM by 45.95, 44.73 and 47.41% at the respective levels of salinity. According to the regression analysis, the highest RDM value was 2.1 g, obtained in plants irrigated with 0.3 dS m⁻¹ water, when fertilized with the dose of 60% of the N recommendation, and the increase in fertilization to the dose of 140% caused a reduction of 0.96 g per plant.

![Figure 2](image_url)

**Figure 2.** Root dry mass (RDM) (g.plant⁻¹) (A) and leaf dry mass (LDM) (g.plant⁻¹) (B) at 85 days after sowing (DAS), in yellow passion fruit seedlings as a function of nitrogen doses for each level of irrigation water salinity.

The increase in water salinity up to 3.1 dS m⁻¹ caused reduction in RDM up to the estimated value of 0.829 g (Figure 2A) and, at this salinity level, the increment in N dose did not increase the formation of biomass. Moreover, the increment in N dose increased root biomass only under application of 0.3 dS m⁻¹ water, with the dose of 80% of the recommendation being the most adequate. This may be related to the recommendation of N fertilization taken as a reference, which may be underestimating the demand for N by plants, especially under the condition of highest salinity, as reported by Bezerra et al. (2018), so it is important to adjust fertilization.

According to Dias et al. (2012), N fertilization promotes growth and increments in yield, being able to reduce the effects of salinity on plants because NO₃⁻ reduces the absorption of Cl⁻. However, this did not occur in passion fruit plants until 85 DAS, since there was a reduction in growth with the increase in N dose for most salinity levels. Nonetheless, it is necessary to find an adequate dose according to the demand, which will vary with the applied level of salinity.

In addition, the linear reduction observed in RDM with the increase in N application may have occurred due to the acidity released during the process of ammonia nitrification by urea, in which there is release of hydrogen, with direct effect on soil pH (FAGERIA; BALIGAR; JONES, 2010) which, together with the salinity present in irrigation water, led to a negative effect on root dry mass (Figure 2A). This was also observed by Cavalcante et al. (2010), when studying water salinity levels and biofertilizer doses on the growth of passion fruit plants.

Regarding LDM (Figure 2B), it can be noted that the increase in N levels in plants irrigated with water of 1.0 and 3.1 dS m⁻¹ caused reductions in dry biomass formation on the order of 44.28% (2.16 g) and 42% (1.44 g), respectively, between the lowest (60%) and highest (140%) N levels applied. Soares et al. (2015) point out that the reduction in dry biomass is related to the photosynthetic capacity of plants, through the ionic interactions promoted by the excess salts contained in irrigation water. Thus,
salinity causes a reduction in the accumulation of photoassimilates, causing an extra expenditure of energy in the plant, mainly due to the reduction of osmotic potential, contributing to a reduction in the availability of water for plant growth (BÉZERRA et al., 2014; SALAZAR et al., 2017; BÉZERRA et al., 2018).

Irrigation water salinity reduced the accumulation of StDM (Figure 3A) by 10.90% for every 1 dS m\(^{-1}\) increase, leading to a reduction of 31.56% (1.49 g) in plants irrigated using water with ECw of 3.1 dS m\(^{-1}\), compared to plants irrigated with water of 0.3 dS m\(^{-1}\). This effect was noted because of the osmotic and ionic effects of salinity, which reduce cell division and elongation, especially in sensitive plants (SILVA et al., 2014; LIMA et al., 2015), since cell damage in these plants increased (Figure 1).

![Figure 3. Stem dry mass (StDM) (g plant\(^{-1}\)) as a function of irrigation water salinity levels (A) and nitrogen doses (B), in yellow passion fruit seedlings.](image)

The increase in N dose caused a decreasing linear effect on StDM at 85 DAS, which according to regression equations (Figure 3B) had a reduction equivalent to 0.32% for each 1% increment of N, i.e., a reduction of 34.61% (1.52 g) in plants fertilized with N dose of 140% compared to those that received the N dose of 60%.

According to the values obtained for ShDM (Figure 4A), there was a reduction due to the action of water salinity, and the highest mean value was 10.88 g, obtained in plants irrigated with water with ECw of 0.3 dS m\(^{-1}\), which is 34.97% (3.19 g) higher than the ShDM accumulation in plants that received water with ECw of 3.1 dS m\(^{-1}\).

The increase in N dose at salinity levels of 0.3, 1.0 and 3.1 dS m\(^{-1}\) caused reductions in ShDM, and these reductions were 0.29%, 0.42% and 0.38% per unit increase in N fertilization, respectively, resulting in 28.5% (3.14 g), 46.4% (4.77 g) and 40.35% (3.02 g) of mass losses when comparing the levels of 60% of N with those of 140% of N, respectively. This may have occurred due to the acidity released during the process of ammonia nitrification by urea, together with the salinity present in irrigation water, resulting in negative effect on ShDM due to the increase in N fertilization.

According to the growth in LA of passion fruit plants at 85 DAS (Figure 4B), the increase in N dose reduces the LA of plants irrigated using water of 0.3, 1.0 and 3.1 dS m\(^{-1}\), which is similar to the results observed for ShDM, as the values were linearly reduced by approximately 0.32%, 0.40% and 0.38%, respectively, with 1% increase in N fertilization, resulting in reductions of 32.68% (298.21 cm\(^2\)), 43.06% (515.76 cm\(^2\)) and 40.38% (340.24 cm\(^2\)) in leaf area, respectively, at the salinity levels of 0.3, 1.0 and 3.1 dS m\(^{-1}\), when the levels from 60% to 140% are compared.

The total dry mass (TDM) was reduced by the increments in water salinity and N doses (Figure 5A), with a 12.20% reduction in TDM per unit increase in ECw, which is equivalent to a 37.06% (3.99 g) reduction in the TDM of plants irrigated with water of 3.1 dS m\(^{-1}\) when compared to those under the lowest salinity level (0.3 dS m\(^{-1}\)). It is likely that this reduction in biomass is related to both the osmotic component and the ionic component, which are inseparable in salt stress, since there was effect of salinity on %D.
The increase in N level also reduced the TDM of passion fruit plants (Figure 5B), with a linear decrease in TDM on the order of 0.34% for every 1% increase in the N level applied from 60%, with a 34.46% (3.45 g) reduction in TDM between the values obtained in plants grown under the highest dose (140% N) and those subjected to the lowest dose (60% N).

The interaction (SxN) caused significant effects on the specific leaf area (SLA), and further analysis of the interaction indicated significant effects at the combinations between N doses and salinity levels of 1.0 and 3.1 dS m⁻¹ (Figure 6). N fertilization increased SLA values by 0.030% and 0.043% at the respective levels of salinity, for every 1% increase of N, i.e., a gain of 5.85 and 8.46 cm² g⁻¹ in SLA in plants that received the highest dose (140% N) compared to those under the lowest dose (60% N).
In general, the highest mean value of SLA (259.96 cm$^2$ g$^{-1}$) was obtained in plants fertilized with the N dose of 140% and subjected to irrigation using water of 2.4 dS m$^{-1}$. These results are similar to those observed by Nobre et al. (2014), who studied the SLA of castor bean plants cv. BRS Energia under levels of irrigation water salinity associated with N fertilization doses and highlight the increment in SLA with the increase in N level.

Thus, it is inferred that the increase in the N level applied contributed to cell growth. although this was not reflected in biomass formation, so it was not sufficient to suppress the effects of salinity on growth variables and cell damage.

CONCLUSIONS

Increments in irrigation water salinity reduce the biomass accumulation of yellow passion fruit seedlings and cause cell damage.

Increase in nitrogen dose did not mitigate the effect of salinity, which reduced the cell membrane integrity, making the plant more sensitive.

Increment in nitrogen dose increases the specific leaf area, but does not provide conditions to reduce the ionic effects of water salinity on passion fruit plants.

Nitrogen dose should be adjusted according to the salinity level applied, due to the reduction in plant growth.

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