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ABSTRACT

This study evaluated the growth and gas exchanges of guava, cv. 'Paluma' cultivated in salinized soil and subjected to different nitrogen (N) doses in a protected environment in the municipality of Campina Grande-PB. The experimental design was randomized blocks, in a 5 x 4 factorial arrangement with three replicates, and the treatments resulted from the combination of five salinity levels in the soil saturation extract - ECse (2.15, 3.15,4.15, 5.15 and 6.15 dS m^{-1}) and four N doses (70, 100, 130 and 160% of the recommended N dose). The dose referring to 100% of N corresponded to 541.1 mg of N dm^{-3} of soil. At 120 and 180 days after (DAT), plant growth was evaluated based on stem diameter (SD), leaf area (LA) and number of leaves (NL). At 210 DAT, the following variables of leaf gas exchanges were evaluated: stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), transpiration (*E*) and CO_2 assimilation rate (*A*). ECse above 2.15 dS m^{-1} reduced stem diameter, leaf area, number of leaves, stomatal conductance, internal CO_2 concentration, transpiration and CO_2 assimilation rate, in both evaluation periods. N dose above 70% of the recommendation (378.7 mg N dm^{-3} of soil) did not mitigate the deleterious effects caused by the salt stress on the growth and gas exchanges of guava plants.

Keywords: fertilization, physiology, *Psidium guajava* L., salt stress.

Interação entre salinidade do solo e nitrogênio no crescimento e trocas gasosas de goiabeira

RESUMO

Objetivou-se, neste trabalho, avaliar o crescimento e as trocas gasosas de goiabeira cv. 'Paluma', cultivada em solo salinizado e submetidas a diferentes doses de nitrogênio, em ambiente protegido no município de Campina Grande-PB. O delineamento experimental foi em blocos casualizados, em arranjo fatorial 5 x 4, com três repetições, cujos tratamentos resultaram da combinação de cinco níveis de salinidade no extrato de saturação do solo – CEes (2,15; 3,15; 4,15; 5,15 e 6,15 dS m^{-1}) e quatro doses de nitrogênio (70, 100, 130 e 160% de N, da dose recomendada). A dose referente a 100% de N correspondeu a 541,1 mg de N dm^{-3} de solo. Aos 120 e 180 dias após o transplantio (DAT) foi mensurado o crescimento das plantas em: diâmetro do caule (DC), área foliar (AF) e número de folhas (NF), e aos 210 dias após o transplantio foram realizadas as leituras das trocas gasosas foliares: condutância estomática (*gs*), concentração interna de CO_2 (*Ci*), transpiração (*E*) e taxa de assimilação de CO_2 (*A*). A CEes acima de 2,15 dS m^{-1} reduziu, o diâmetro de caule, a área foliar, o número de folhas, a condutância estomática, a concentração interna de CO_2, a transpiração e a taxa de assimilação de CO_2, em ambas épocas de avaliação. A dose de N acima de 70% da recomendação (378,7 mg N dm^{-3} de solo) não mitigou os efeitos deletérios ocasionados pelo estresse salino sobre o crescimento e as trocas gasosas das plantas de goiabeira.

Palavras-chave: adubação, estresse salino, fisiologia, *Psidium guajava* L.

1. INTRODUCTION

Guava (*Psidium guajava* L.) is a fruit crop widely cultivated in the Southeast and Northeast regions of Brazil, and is a highly profitable activity with great potential for expansion. The production of guava fruits in Brazil has substantially increased in the last years, from 281 thousand tons in 2001 to 359 thousand tons in 2014 in an area of 16 thousand hectares (IBGE, 2014).

Soil salinization is a critical factor for plant production in protected environments, due to the low tolerance to salinity of commercial plants (Eloi et al., 2011; Medeiros et al., 2012). In general, in a protected environment there is an accumulation of certain ionic species, such as the cations Na^+, Ca^{2+} and Mg^{2+} and the anions Cl^- and SO_4^{2-}, affecting plant water consumption, lowering the osmotic potential in the soil and decreasing the water potential of the plants, besides causing ionic toxicity due to excess of Cl^-, Na^+ and Ca^{2+}, among others.

The excess of sodium salts, besides causing damage to the soil's physical properties, results in generalized reduction of growth in the cultivated plants, leading to serious damage to agricultural activity (Cavalcante et al., 2010).

Among various processes affected by salinity, it is known that water absorption by plants and gas exchanges are negatively affected due to the reduction in the osmotic potential. It is assumed that this behavior can reduce growth due to the lower absorption of CO_2 from the atmosphere and, consequently, lead to reduction of photosynthesis (Praxedes et al., 2010). According to Távora et al. (2001), in the initial growth stage guava can be classified as sensitive to salinity, with threshold salinity in the soil saturation extract of 1.2 dS m^{-1}.

Fertilization management practices have been developed in various countries, in the attempt to mitigate the deleterious effects of the salts contained in both soil and irrigation water on the plants. These practices include conventional fertilization techniques, based on the use of fertilizers, which favor the absorption of nutrients by the plants under saline conditions (Silva et al., 2011a).

The existing recommendations for the application of nutrients in guava plants need scientific support, because the number of studies on the topic is small and the recommended doses of fertilizers are very different from those practiced by the fruit growers (Mendonça et al., 2012).

Barhoumi et al. (2010) highlighted that nitrogen (N) fertilization, besides promoting plant growth, can also reduce the effect of salinity on the plants. Such effect can be attributed to the functions of the N in the plants, since it performs a structural function, participating in various organic compounds that are vital for the plant, such as amino acids, proteins, proline, among others. Studies have demonstrated that the accumulation of these organic solutes increases the osmotic adjustment capacity of the plants to salinity and increases the resistance of the crops to water and salt stresses (Lacerda et al., 2003; Silva et al., 2008).

The application of nitrogen can minimize the adverse effects of salinity on the development of plants, because according to Del Amor et al. (2000) there is evidence of competition in nitrate and chloride uptake, so that an increase in nitrate concentration in the root zone may decrease chloride uptake by the plant. In this context, several studies indicate a positive effect of the interaction between water salinity and nitrogen fertilization, Lima et al. (2014) in castor beans, Guedes Filho et al. (2015) in sunflower crops. However, Silva et al. (2017), evaluating the effects of irrigation with saline waters (ECw: from 0.3 to 3.5 dS m^{-1}) on the formation of rootstocks, and Paluma and Souza et al. (2016), also studying the irrigation with saline waters and nitrogen doses on Crioula guava during the formation of rootstocks, did not find a beneficial effect of this interaction on the growth and quality of guava rootstocks.

Although there are studies related to evaluation of the interaction between salinity and nitrogen in the guava crop, such studies are limited to the evaluations during the formation of rootstock phase. Considering that there may be variation in the degree of tolerance of the crops in the function of development stages, salt concentration, edaphoclimatic conditions and fertilization management, it is important to conduct new studies to evaluate the mitigation effect of nitrogen on the growth and gas exchange of guava under saline stress in the post-grafting phase.

Thus, this study evaluated the growth and gas exchanges of ungrafted guava plants, cv. 'Paluma', as a function of soil salinity and increasing N doses.

2. MATERIAL AND METHODS

The study was carried out from October 2015 to May 2016 at the Center of Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), in a greenhouse located in the municipality of Campina Grande-PB (7º13'11" S; 35º53'31" W; 550 m).

The experimental design was in randomized blocks distributed in a 5 x 4 factorial scheme, with three replications. The treatments were composed of five levels of electrical conductivity of the saturation extract - ECse (2.15, 3.15, 4.15; 5.15 and 6.15 dS m^{-1}), and four doses of nitrogen fertilization (378.7, 541.1, 703.4 and 865.7 mg of N dm^{-3} of soil), corresponding to 70, 100, 130 and 160% of the recommendation of Souza et al. (2016). The values of electrical conductivity of the soil saturation extract were based on the threshold salinity of the crop (ECse = 1.2 dS m^{-1}).

The soil material was placed in lysimeters without drainage (110 kg per lysimeter). The soil was classified as eutrophic Regolithic Neosol, with loamy sand texture, collected in the 0-30 cm layer in the municipality of Esperança-PB, whose physical and chemical characteristics (Table 1) were determined according to methodologies described by Donagema et al. (2011).

Feralization at planting was manually performed by applying in each lysimeter 183.3 g of single superphosphate (single basal dose) and 27.5 g of potassium chloride, split, 1/3 of the recommended dose as basal and the other 2/3 divided into two equal applications at 30 and 60 days after transplanting. The treatment of N fertilization, using urea (45% N) as source, was split into 12 equal applications in intervals of 30 days from transplantation on. The amount of fertilizer applied in the treatments was calculated considering the N (100%) as equivalent dose.

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

Density ($g\ cm^{-3}$)	Total porosity (%)	Water content (%) 0.33 atm	15 atm	Saturation	Organic matter ($g\ kg^{-1}$)	P ($mg\ dm^{-3}$)	Exchange complex ($cmol_c\ dm^{-3}$) Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
1.14	58.24	19.42	7.57	36.66	29.3	3.98	9.07	2.78	1.64	0.23

Saturation extract									
pH_{se}	EC_{se} ($dS\ m^{-1}$)	Ca^{2+} ($mmol_c\ dm^{-3}$)	Mg^{2+}	K^{+}	Na^{+}	Cl^{-}	SO_4^{2-}	CO_3^{2-}	HCO_3^{-}
5.43	2.15	9.25	6.50	0.18	9.18	12.50	0.00	0.00	2.10

pH_{se} - pH of the soil saturation extract; EC_{se} - electrical conductivity of the soil saturation extract at 25°C.

After the soil was placed in the lysimeters, it was salinized by dissolving the salt applied via irrigation in ECse treatments of 3.15, 4.15, 5.15 and 6.15 dS m^{-1}, respectively adding 25.80, 51.61, 77.42 and 103.23 g of NaCl (without iodate). The respective doses of the salt were determined through a previous test and the amount was calculated by Equation 1, as cited by Rhoades et al. (2000):

$$C = 640 * (ECse - ECsei) * Ws \quad (1)$$

Where:

C – amount of salt, mg L^{-1}

ECse – pre-established value of electrical conductivity of the soil saturation extract, dS m^{-1}

ECsei – initial value of electrical conductivity of the soil saturation extract.

Ws – weight of soil in dry weight, kg

After salinization, the lysimeters were irrigated with rainwater (0.06 dS m^{-1}) to bring the soil to field capacity. Then, after rest, the ungrafted guava seedlings, cv. 'Paluma', were transplanted.

Irrigations were equally performed for all plants using rainwater every three days, increasing soil moisture to the mean tension of the field capacity. Thus, there was no leaching in any of the irrigations.

At 60 days after transplanting (DAT), the branches were selected regarding vigor and sanity, and thinning was performed to standardize the plants (leaving three 20-cm-long main branches per plant), responsible for forming the base of the crown, according to the recommendation of EMBRAPA (2010).

At 120 and 180 DAT, the following variables were evaluated: stem diameter - SD (mm), measured with a digital caliper 5 cm above the soil level; number of leaves - NL, by counting the leaves with fully expanded leaf blade; and leaf area - LA (cm^2), estimated using the equation proposed by Lima et al. (2014), based on the midrib length (L), according to Equation 2.

$$LA = (0.3205 * L^{2.0412}) \quad (2)$$

Where:

LA – leaf area, cm^2;

L – midrib length of the central leaf, and the final sum of the areas per leaf provides the total leaf area of the plant.

At 210 DAT, the following measurements were perfomed: internal CO_2 concentration (*Ci*), stomatal conductance (*gs*), CO_2 assimilation rate (*A*) and transpiration (*E*), in fully developed leaves, from 07:00 to 09:00 a.m., using the infrared gas analyzer IRGA ("LCPro+" - ADC BioScientific Ltda). The results were subjected to analysis of variance by F test at 0.05 and 0.01 probability levels and, for significant effect, linear and quadratic regression analyses were applied, using the program SISVAR (Ferreira, 2011). Stomatal conductance and leaf area required transformation of the original data to $\sqrt{x}$, while transpiration and CO_2 assimilation rate data were transformed to $\sqrt{x+1}$.

3. RESULTS AND DISCUSSION

According to Table 2, there was significant effect of the saline levels ($p<0.01$) on the variable SD at 120 DAT and ($p<0.05$) at 180 DAT, besides the variables LA and NL ($p<0.01$) in both evaluation periods. For the factor N doses (ND), there was significant effect of the doses ($p<0.05$) and ($p<0.01$) at 120 and 180 DAT, respectively, on all variables. Regarding the interaction between factors (S x ND), there was no significant effect on any of the studied variables.

Table 2. Summary of the analysis of variance for stem diameter (SD), leaf area (LA) and number of leaves (NL) of ungrafted guava plants, cv. 'Paluma', in salinized soil under nitrogen doses, at 120 and 180 days after transplanting (DAT).

Source of variation	DF	Mean square					
		SD		LA		NL	
		120 DAT	180 DAT	120 DAT[1]	180 DAT	120 DAT	180 DAT
Salinity (S)	4	11.23^{**}	5.02^{*}	10099819.95^{**}	20491924.61^{**}	3362.80^{**}	9445.47^{**}
Linear regression	1	37.85^{**}	19.02^{**}	30280711.60^{**}	69958524.82^{**}	10584.40^{**}	36960.30^{**}
Quadratic regression	1	3.49^{ns}	0.03^{ns}	6329989.99^{**}	2373935.65^{ns}	1243.14^{*}	46.09^{ns}
N doses (ND)	3	3.53^{*}	11.52^{**}	5425079.10^{*}	20906760.72^{**}	2132.37^{*}	13575.39^{**}
Linear regression	1	9.90^{**}	32.58^{**}	15615863.64^{**}	52279312.34^{**}	6274.61^{**}	32344.08^{**}
Quadratic regression	1	0.68^{ns}	1.98^{ns}	17032.24^{ns}	6892536.06^{ns}	112.06^{ns}	8378.01^{*}
Interaction (SxND)	12	1.32^{ns}	1.08^{ns}	413189.30^{ns}	2960533.01^{ns}	248.75^{ns}	678.63^{ns}
Blocks	2	2.29^{ns}	22.10^{**}	1882558.36^{ns}	45280843.61^{**}	315.11^{ns}	4138.40^{ns}
CV (%)	-	13.54	10.27	17.27	12.27	23.39	17.81

** and * Significant at 0.01and 0.05 probability levels by F test; ^{ns} Not significant by F test, [1]Statistical analysis performed after data transformation to $\sqrt{x}$.

The stem diameter of the guava cv. 'Paluma' decreased linearly as a function of the increment in ECse levels and, according to the regression equations (Figure 1A), SD was reduced as the ECse levels increased, with decreases of the order of 5.48 and 2.62% per unit increase in ECse, at 120 and 180 DAT, respectively. The inhibition of growth must have been caused mostly by the toxic effects of the salts absorbed by the plants and the low osmotic adjustment capacity of the crop, which delay cell expansion and division, promoting negative effects on the photosynthetic rate and compromising the physiological and biochemical processes of the plants (Gomes et al., 2011; Nunes et al., 2012), consequently causing decrease in SD, which may have occurred because of these factors.

For the N doses, according to the regression equation (Figure 1B), the SD was negatively influenced, with reductions on the order of 5.33 and 4.10% for every 30% increase in the N dose, at 120 and 180 DAT, respectively. These results agree with those obtained by Souza et al. (2016), who studied the effects of N doses on guava development and observed that the highest N doses also caused higher reduction in stem diameter.

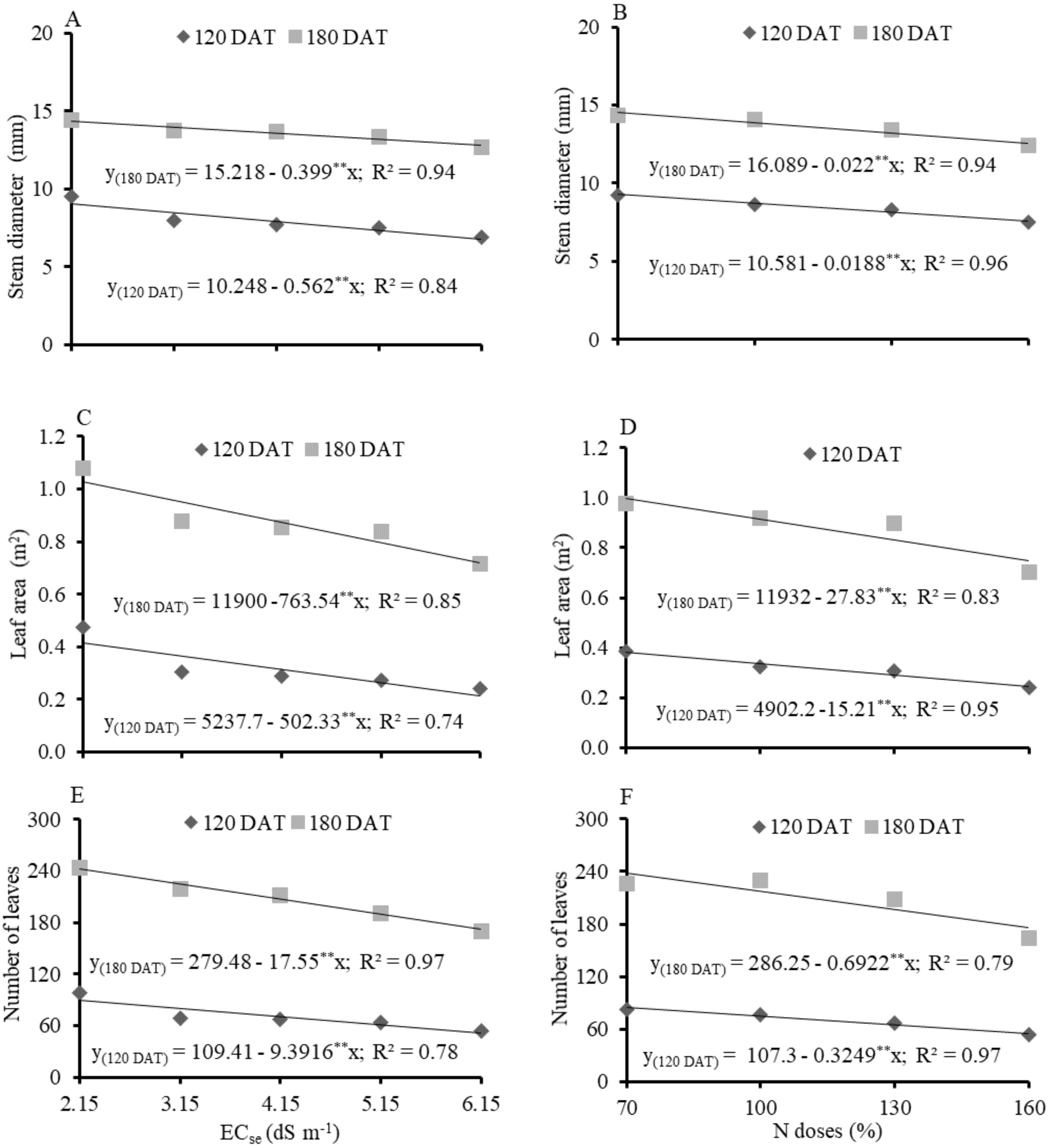

Figure 1. Stem diameter (A, B), leaf area (C, D) and number of leaves (E, F) of guava plants, cv. 'Paluma', as a function of soil salinity – ECse and nitrogen doses, at 120 and 180 days after transplanting (DAT).

As observed for stem diameter, leaf area also suffered linear reduction as a function of the increase in soil salinity, with reductions of 9.59 (120 DAT) and 6.41% (180 DAT) per unit increase in the ECse (dS m^{-1}) of the soil (Figure 1C). Guava plants cultivated in soil with ECse of 6.15 dS m^{-1} showed reductions of 48.32 and 29.77%, respectively, in relation to those subjected to 2.15 dS m^{-1}. It can be stated that the LA of guava plants at 120 and 180 DAT is sensitive to the increase in the salt stress of the soil. According to Alves et al. (2011), the reduction in leaf area, caused by the salt stress, is related to the effect of the osmotic potential of the soil solution, inhibiting the absorption of water and nutrients by the plants.

According to the LA as a function of the application of N doses (Figure 1D), there was a linear effect, with decreases of the order of 9.30 and 6.99% for every 30% increment in N dose, respectively, for 120 and 180 DAT. The highest reductions in LA are intensified with the increase in the N doses; however, in both periods, the dose of 70% (378.7 mg of N dm^{-3} of soil) was sufficient to promote the highest LA. Similar results were obtained by Souza et al. (2016), who worked with 'Crioula' guava seedlings and observed greater LA growth at the mean dose of 541 mg of N dm^{-3} of soil, corresponding to 70% of N of the dose applied on the guava rootstock in the evaluation periods.

The number of leaves (NL) was significantly reduced by soil salinity in the evaluations. There were linear decreases in NL as a function of the increase in ECse, equal to 8.58 and 6.27% per unit increase in ECse at 120 and 180 DAT, respectively (Figure 1E). Oliveira et al. (2011) cite that the reduction in NL is related to morphological and anatomical modifications in the plant, with the objective of maintaining water absorption under saline conditions and reducing transpiration, which may have occurred in the present study, causing reduction in NL with the increase in soil salinity.

Figure 1F shows the regression analysis for NL as a function of the N fertilization, which demonstrates a linear reduction with the increase in N doses, of the order of 9.08 and 7.25%, at 120 and 180 DAT, respectively, for every 30% increment in the N dose. The decrease in guava NL can be related to the lower plant growth due to the restriction in the process of absorption; consequently, there is a reduction in the flow of water and nutrients in the soil-plant-atmosphere direction, causing morphological and anatomical alterations in the plant. Similar results of NL were observed by Souza et al. (2016), who worked with guava, cv. 'Crioula', under N fertilization, and also obtained reduction in NL as the N doses increased.

According to the summary of the analysis of variance (Table 3), the factor saline levels significantly affected stomatal conductance, internal CO_2 concentration, transpiration and CO_2 assimilation rate ($p<0.01$), but there was no significant effect of the N doses or the interaction of S x ND on any of the studied variables.

Stomatal conductance (gs) in the cv. 'Paluma was reduced by the increment of soil ECse (Figure 2A). Plants subjected to ECse of 2.15 and 6.15 dS m^{-1} showed gs values of 0.20 and 0.12 mol of H_2O m^{-2} s^{-1}, respectively, corresponding to a relative reduction of 36.22% in *gs*. There was a relative decrease of 7.58% at 210 DAT, per unit increase in ECse. This fact was possibly due to the water regulation mechanism of the guava crop, when subjected to low water potentials in the soil because of the increase in salinity, compromising stomatal opening. Silva et al. (2013) also reported that plants close their stomata to reduce water losses through transpiration, resulting in a lower photosynthetic rate, which constitutes one of the causes of the reduced growth of the species under salt stress conditions (Travassos et al., 2011).

The internal CO_2 concentration (Ci) also decreased linearly as a function of the increment in ECse. According to the regression equation (Figure 2B), guava plants subjected to ECse of 2.15 and 6.15 dS m^{-1} significantly reduced the Ci, showing values of 267.64 and 218.92 µmol m^{-2} s^{-1}, respectively, with a reduction in Ci of 18.20%, in relation to plants developed in the treatments with higher ECse. The relative reduction for each increment in ECse was equal to 4.14%, at 210 DAT. This relative reduction in Ci can be attributed to the lower stomatal conductance, a common response of the plants to salt stress (Praxedes et al., 2010; Silva et al., 2011b).

Table 3. Summary of the analysis of variance for stomatal conductance (g_s), internal CO_2 concentration (C_i), transpiration (E) and CO_2 assimilation rate (A) in ungrafted guava plants, cv. 'Paluma', in saline soil under nitrogen doses, at 210 days after transplanting (DAT).

Source of variation	DF	Mean square			
		gs[1]	*Ci*	*E*[2]	*A*[2]
Salinity (S)	4	0.0118**	4838.233**	1.541**	13.903**
Linear regression	1	0.0403**	17812.033**	6.075**	53.734**
Quadratic regression	1	0.0038ns	1281.523ns	0.053ns	0.002ns
N doses (ND)	3	0.0028ns	48.016ns	0.200ns	1.134ns
Linear regression	1	0.0027ns	142.830ns	0.333ns	1.840ns
Quadratic regression	1	0.0001ns	0.816ns	0.266ns	0.541ns
Interaction (SxND)	12	0.0022ns	7.266ns	0.130ns	1.705ns
Blocks	2	0.0005ns	152.216ns	0.050ns	6.112**
CV (%)	-	18.41	7.04	14.26	9.66

**and *Significant at 0.01and 0.05 probability levels by F test; nsNot significant by F test; [1, 2]Statistical analysis performed after data transformation to $\sqrt{x}$ and $\sqrt{x+1}$, respectively.

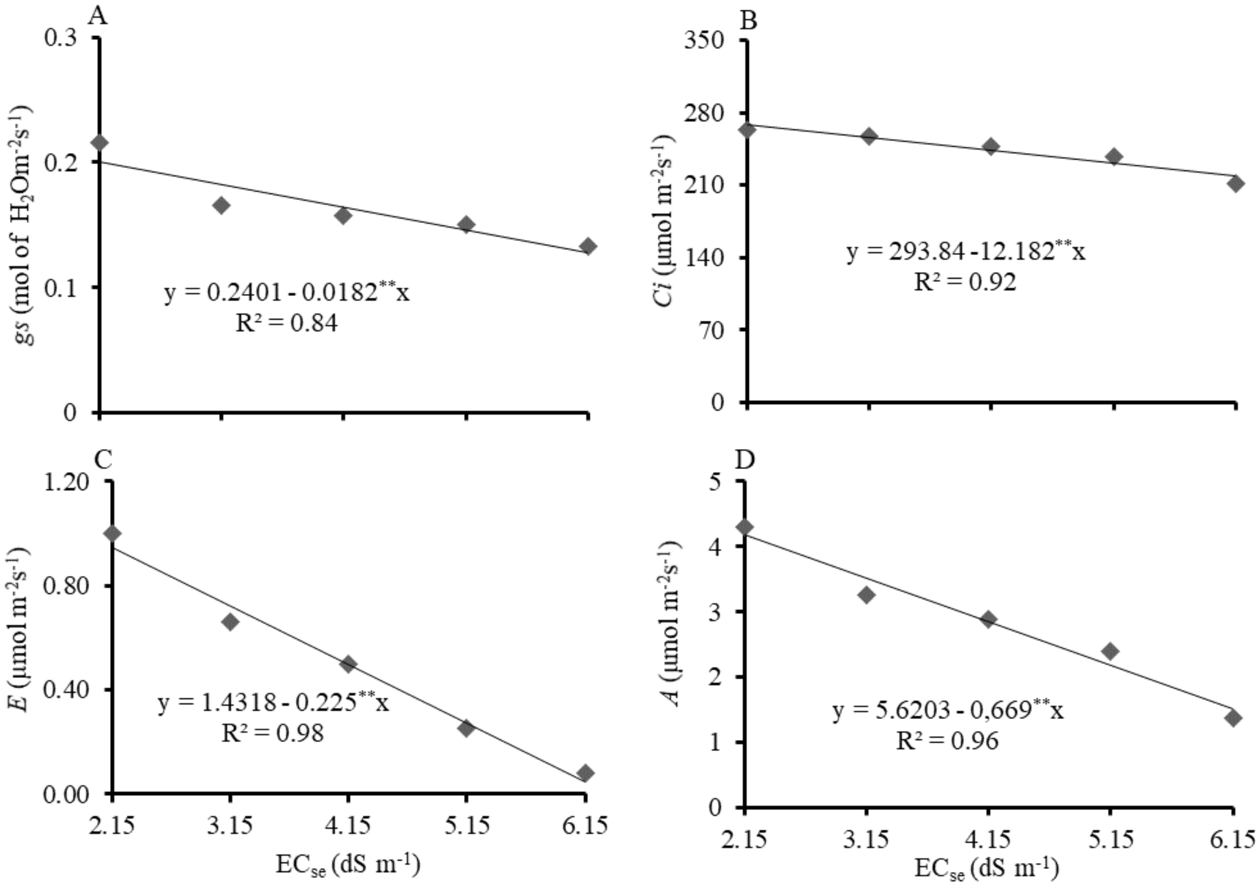

Figure 2. Stomatal conductance - *gs* (A), internal CO_2 concentration - *Ci* (B), leaf transpiration - *E* (C) and CO_2 assimilation rate - *A* (D) in guava, cv. 'Paluma', as a function of soil salinity - ECse, at 210 days after transplanting (DAT).

Leaf transpiration (E) of guava plants, cv. 'Paluma', subjected to ECse of 2.15 and 6.15 dS m^{-1} also decreased significantly with the increment in salinity, exhibiting values of

0.95 and 0.05 μmol m^{-2} s^{-1}, respectively, with a relative reduction of 15.71% at 210 DAT per unit increase in ECse, with reduction of 94.93% in E (Figure 2C). The reduction in plant transpiration observed with the increment in soil salinity occurred due to the osmotic effect of the salts around the roots and the possible accumulation of potentially toxic ions (Na+ and Cl-) in the leaf tissues. Consequently, the plants started to have greater control over the stomatal opening to avoid excessive water loss through transpiration (Souza et al., 2011). The reduction in the transpiration rate according to Pinto et al. (2008) is directly related to the decrease in stomatal conductance, and Machado et al. (2005) emphasize that if there is smaller stomatal opening, there is also a decline in transpiration, with a consequent increase in leaf temperature.

In addition, according to Figure 2D, the CO_2 assimilation rate (*A*) of the plants subjected to ECse of 2.15 and 6.15 dS m^{-1} decreased linearly at 210 DAT, with values of 4.18 and 1.50 μmol m^{-2} s^{-1}, respectively, and relative reduction of 11.90% per unit increase in ECse. The lower CO_2 assimilation rate can be a consequence of the stomatal closure. Amorim et al. (2010) observed that cashew plants under salt stress exhibit a lower CO_2 assimilation rate and hypothesized that it was caused by the reduction in stomatal opening, which was also found in the present study. Therefore, it can be inferred that the saline treatments caused stress on the plants, so that the salts may have stimulated stomatal closure. However, the prolonged exposure to the salts leads to alterations in the water status of the plants, inducing stomatal closure and, consequently, limitation in CO_2 entry. In addition, high concentrations of ions such as Na^+ and Cl^- have been pointed out as main causes of damages to the structure of enzymes and membranes, indirectly interfering with the CO_2 assimilation rate (Silva et al., 2011b).

4. CONCLUSIONS

The growth and gas exchanges of guava, cv. 'Paluma', are negatively affected by the increment of salinity in the soil saturation extract, and transpiration is the most compromised variable.

Nitrogen doses above 378.7 N dm^{-3} of soil promote reduction in guava growth and the effect is intensified according to the evaluation periods.

The interaction between the electrical conductivity of the soil saturation extract and nitrogen doses did not influence any of the evaluated variables of guava, cv. 'Paluma'.

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