RESEARCH ARTICLE

Emergence and morphophysiology of Sunki mandarin and other citrus genotypes seedlings under saline stress

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Abstract

The scarcity of good quality water is a limiting factor for irrigated agriculture, especially in arid and semiarid regions, where water sources generally contain high concentrations of ions. In such conditions, it is essential to cultivate genotypes with economic potential and tolerance to salinity. Considering the importance of citrus and the need to identify genetic materials that adapt to saline stress, this study evaluates the salinity tolerance of 10 genotypes with rootstock potential. For the irrigation water, five levels of electrical conductivity (ECw: 0.8, 1.6, 2.4, 3.2 and 4.0 dS/m at 25 °C) were used in a randomized block design with three replications, and seed germination and growth variables, as well as physiological plant parameters, were evaluated. The germination rate of the hybrid TSKC × CTARG – 019 was the best under salt conditions, as it was the genotype with the highest tolerance to salinity in the germination stage. Salinity reduced the growth of the citrus genotypes, with the possibility of using water with EC of up to 1.6 dS/m in the rootstock formation stage. Salt stress affected the photosynthetic rate of the genotypes 'San Diego' citrandarin and TSKC × CTSW – 018 by reducing the stomatal conductance, restricting the diffusion of CO_2 into the substomatal camera. The genotypes TSKC × CTARG – 019, TSKC × CTTR – 012 and TSKC × TRBK – 007 have the greatest potential for growth and photosynthetic apparatus efficiency when subjected to salinity.

Additional keywords: *Citrus* spp.; rootstock; salinity; photosynthesis; growth.

Abbreviations used: DAS (days after sowing); EP (emergence percentage); iC (internal CO_2 content); RGRNL (relative growth rate of the number of leaves); RGRPH (relative growth rate of the plant height); RGRSD (relative growth rate of the stem diameter); WUE (water-use efficiency).

Introduction

Citrus plants constitute a group of the most important fruit crops in Brazil, the world's leading producer of oranges and exporter of citrus juices (FAO, 2013), due to their nutritional and socioeconomic value. The Brazilian citrus industry continues to grow through market expansion and technological advances in the production chain.

Nonetheless, the productivity of citrus species in Brazil is low, reaching only 23 t/ha. This number decreases for the Northeast Region, which averages 13.6 t/ha (IBGE, 2014) due to weather irregularities (particularly water stress for periods of more than six months) that generally coincide with higher temperatures (Cruz *et al.*, 2003). Water stress problems are even more notorious in the semiarid part of this region, making it necessary to use irrigation to ensure good production. Considering the peculiarities of the region, inadequate management of irrigation and the quality of many water sources, coupled with high crop evapotranspiration contribute to soil salinization and reductions in plant productivity.

However, in many cases the adverse effects of salts in water or soil can affect stomatal opening in plants, which reduces transpiration rates and carbon content for photosynthesis. This is reflected in inhibition

and unevenness in plant growth, with a consequent reduction in production indexes (Hussain *et al.*, 2012). In citrus rootstock formation, variations of sensitivity to salts between species, genotypes and in the growth stages of the plants have been found (Brito *et al.*, 2008; Fernandes *et al.*, 2011; Syvertsen & Garcia-Sanchez, 2014).

Ferguson & Grattan (2005) mention that citrus species, used as scion, are sensitive to salt stress, with salinity threshold being between 1.2 and 1.5 dS/m. Sensitivity varies greatly for rootstock genotypes due to differences in the absorption and translocation of ions between roots and shoots (Grieve & Walker, 1983). 'Rangpur' lime (*Citrus limonia* Osbeck), for example, the main rootstock in the Brazilian citrus industry excludes chlorine and compartmentalizes sodium in the basal parts of the plant; Cleopatra mandarin (*C. reshni* hort. Ex. Tanaka) excludes both sodium and chlorine, while *Poncirus trifoliata* excludes only sodium, with chlorine being translocated to the leaves.

According to Ferguson & Grattan (2005) and Syvertsen & Garcia-Sanchez (2014), salinity may reduce citrus plant water absorption through osmotic pressure; show symptoms of plant toxicity due to excess ions such as sodium and chloride; and change the absorption of nutrients, resulting from ionic competition. Levy & Syvertsen (2004) report that citrus species in general constitute one of the few examples where Cl⁻ ions are more toxic to plants than Na⁺ ions.

A practice that can enable saline water usage in citrus production is the use of genotypes that are tolerant to this abiotic factor, as well as the consideration of the tolerance of the rootstock × scion combination (Syvertsen & Garcia-Sanchez, 2014). Studies with this focus have been developed in several countries (Forner-Giner *et al.*, 2011; Sykes, 2011; Gonzalez *et al.*, 2012; Grosser *et al.*, 2012) where citriculture is expanding and have identified promising genotypes to grow in saline conditions.

Thus, the careful selection of genotypes to form grafted seedlings can be critical to citriculture success in regions such as the Northeast of Brazil, where the use of Rangpur lime rootstocks prevails, regardless of weather and soil conditions in each location as well as the scion used (Mattos Junior *et al.*, 2005). Studies are needed to evaluate the cultivation potential of new rootstock materials that are tolerant to salt stress and drought. This work fits into research lines that study new hybrids that are tolerant to abiotic stress, with some promising results having already been obtained (Brito *et al.*, 2008, 2012; Fernandes *et al.*, 2011; Nascimento *et al.*, 2012; Silva *et al.*, 2014).

Much crossing between progenies that are tolerant to salt stress is underway in studies at Embrapa Cassava & Fruits. Here, obtained materials submitted to growth and physiological process assessment, aimed at identifying parameters related to possible mechanisms of tolerance, are possibly the fastest way to select these materials.

This study was carried out to evaluate if salt stress during germination and initial seedling formation could show significant salinity tolerance differences in citrus genotypes. This hypothesis was evaluated using ten citrus genotypes that had different development indexes when cultivated under saline conditions.

Material and methods

The experiment was conducted in plastic containers under greenhouse conditions at the Center of Agrifood Science and Technology (CCTA) of the Federal University of Campina Grande, Pombal Campus, PB, Brazil. The location has an altitude of 174 m and the coordinates are 6º48'16'' S latitude and 37º49'15'' W longitude.

The work studied 10 citrus genotypes with rootstock potential: G1, Hybrid trifoliate - 083 (HTR - 083); G2, Volkamer lemon (*C. volkameriana* V. Ten. & Pasq.) × Rangpur lime - 010 (LVK × LCR - 010); G3, LVK × LCR - 030; G4, 'San Diego' citrandarin [*C. sunki* (Hayata) hort. ex Tanaka × *Poncirus trifoliata* (L.) Raf. Swingle]; G5, common Sunki mandarin (*C. sunki*) × Argentina citrange [*C. sinensis* (L.) Osbeck × *P. trifoliata*] - 019 (TSKC × CTARG - 019); G6, TSKC × CTARG - 036; G7, TSKC × Swingle citrumelo (*C. paradisi* Macfad. × *P. trifoliata*) - 018 (TSKC × CTSW - 018); G8, TSKC × Troyer citrange - 012 (TSKC × CTTR - 012); G9, TSKC × *P. trifoliata* Benecke - 007 (TSKC × TRBK - 007); and G10, Sunki of Florida mandarin × C13 citrange - 012 (TSKFL × CTC13 - 012). The genotypes were submitted to five levels of irrigation water electrical conductivity (ECw): 0.8, 1.6, 2.4, 3.2 and 4.0 dS/m at 25 °C.

A statistical randomized block design, in a factorial (10 × 5), was used with three replications, and the plot had four containers with four plants each. In the definition of the ECw levels of the irrigation water, the value of the salinity threshold of the sweet orange tree, 2.0 dS/m was considered, as found in Syvertsen & Garcia-Sanchez (2014). The ECw salinization levels of the local water supply system ensured an equivalent ratio of 7:2:1 between Na:Ca:Mg for the salts NaCl, CaCl₂.2H₂O and MgCl₂.6H₂O, respectively.

The 10 citrus genotypes were obtained from the Citrus Breeding Program of Embrapa Cassava & Fruits, which provided the seeds. Irrigation with the salinity levels was done throughout the study period,

starting at sowing. Plants were grown in recipients of black polyethylene (12 cm in diameter and 30 cm in height, 3400 mL of substrate capacity. The properly selected seeds were treated with thiuram disulfide (4 g/kg seed) and seeded in a prepared substrate that used a portion of the A horizon of a Fluvisol, a portion of the A horizon of a Luvisol and two parts of a commercial substrate (composed of vermiculite, pine bark and humus).

The chemical properties of the substrate used were: pH=6.42; EC_{se}=0.62 d/m; Ca^{2+}=9.2 $cmol_c$/kg, Mg^{2+}=7.5 $cmol_c$/kg; Na^+=0.34 $cmol_c$/kg; K^+=0.45 $cmol_c$/kg; H + Al=1.16 $cmol_c$/kg and CEC=17.15 $cmol_c$/kg; V=91.98%, P=3.0 mg/dm^3 and OM=52 g/kg (Ca^{2+} and Mg^{2+} extracted with KCl 1 mol/L at pH 7.0; Na^+ and K^+ extracted with NH_4OAc 1 mol/L at pH 7.0; pH_{ps}-pH of satured paste; EC_{se}=conductivity in the saturation extract; OM=organic matter, determined by wet digestion Walkley-Black method; CEC=cation exchange capacity; V=base saturation value). The values were determined using methodologies recommended by EMBRAPA (2009). Four seeds were sown per container. After germination, only one plant with the greatest vigor per container was allowed to develop, with non-uniform plants (of zygotic origin) being removed. This was done in relation to the standard of each genotype, according to the methodology described in Brito (2010).

Plant irrigation was carried out daily with an amount of water that corresponded to the average evaporation in the control treatment (0.8 dS/m). This was done by weighing the containers and adding a leaching fraction (LF) of 20%. The applied volume (Va) per bag corresponds to the difference between the average mass of the containers under maximum water retention (Mwr), determined early in the morning (after drainage), and the average mass of the containers at the time of the next irrigation, in the evening (Mni), leading to Eq. [1]:

$$Va = \frac{Mwr - Mni}{(1 - LF)} \qquad [1]$$

Fertilization was performed weekly, alternating urea + KCl and MAP (monopotassium phosphate) + KCl, diluted in irrigation water, which corresponds to the respective levels of ECw, with a ratio of 2 g/L urea or MAP and 1 g/L KCl, applying 10 mL per plant, as recommended by Mattos et al. (2005). Micronutrient applications were sprayed at intervals of 15 days onto the leaves (Quaggio et al., 2005), wetting the entire plant, at a concentration of 1% of a commercial product (Albatroz) that had the following composition: 10% N, 52% P_2O_5, 10% K_2O, 0.1% Ca, 0.02% Zn, 0.02% B, 0.15% Fe, 0.1% Mn, 0.02% Cu and 0.005% Mo.

To assess the effect of the treatments on seedling germination and plant growth, the emergence percentage (EP) was evaluated. Germination percentages were evaluated daily from day 9 to day 25 after sowing (DAS), with seeds being considered germinated when the epicotyl emerged on the surface of the container up to 25 DAS.

The numbers of leaves, plant height (cm) and stem diameter (mm) were evaluated at 60 and 150 DAS. When counting the leaves, those with a length superior to 3 cm and with coloring, indicating that they are photosynthetically active, were considered. For plant height, the length of shoots from ground level to the apical meristem was considered; stem diameter was measured at 2 cm from the neck using a digital caliper.

With the data obtained through growth analysis, at 60 and 150 DAS, the relative growth rate (RGR) was determined through the number of leaves (RGRNL), plant height (RGRPH) and stem diameter (RGRSD). This rate, obtained using Eq. [2], measures the increase as a function of the pre-existing material, adapting procedures used in Hunt et al. (2002) for plant height and diameter, where: A_2 = plant growth at time t_2; A_1 = plant growth at time t_1; t_2-t_1 = time difference between samples; ln = natural logarithm:

$$RGR = \frac{(\ln A_2 - \ln A_1)}{t_2 - t_1} \qquad [2]$$

In the last evaluation at 150 DAS, gas exchange was determined through internal CO_2 concentration (iC), stomatal conductance (gs), transpiration (E) and CO_2 assimilation rate (A) readings. Based on these data, intrinsic carboxylation efficiency (CE) (A/iC) and instantaneous water-use efficiency (WUE) (A/E) (Magalhães Filho et al., 2008) were estimated. These measurements were performed with a determiner of plant gas exchange, which contained an infrared gas analyzer (LCpro-SD model, ADC Bioscientific, UK). The readings of these physiological parameters were carried out on the third leaf, counting from the apical bud, since it was fully expanded.

The data were evaluated through an F-test, using Sisvar 5.1 software. When values were significant, the Scott-Knott test ($p<0.05$) was performed for genotypes factor and regression analysis was used to find the water salinity factor (Ferreira, 2011).

Results

Emergence percentage (EP)

The interaction between genotypes and irrigation water salinity levels was significant during seed

emergence (EP), showing saline stress tolerance variation between genotypes.

Considering different saline levels, EP varied only for the genotypes LVK × LCR - 010, TSKC × CTTR - 012, TSKC × TRBK - 007 and TSKFL × CTC13 - 012 (Fig. 1). It is noted that the highest values (52.75%, 39.82%, 57.32% and 33.71%) were observed when seeds were submitted to water salinity levels 3.1, 2.4, 4.0 and 2.6 dS/m, respectively, with TSKC × TRBK - 007 standing out due to higher EPs, independent of saline level (Fig. 1). Emergence of the genotypes HTR - 083, LVK × LCR - 030, 'San

Diego' citrandarin, TSKC × CTARG - 019, TSKC × CTARG - 036 and TSKC × CTSW - 018 was not affected by salinity. The lowest EP values were recorded for these genotypes, a mean of 20.68%, indicative of the higher sensitivity of these particular genes to salt stress.

Growth variables: Number of leaves

The number of leaves of the genotypes varied and was influenced by the levels of ECw in both assessments (60 and 150 DAS), but without an interactive effect
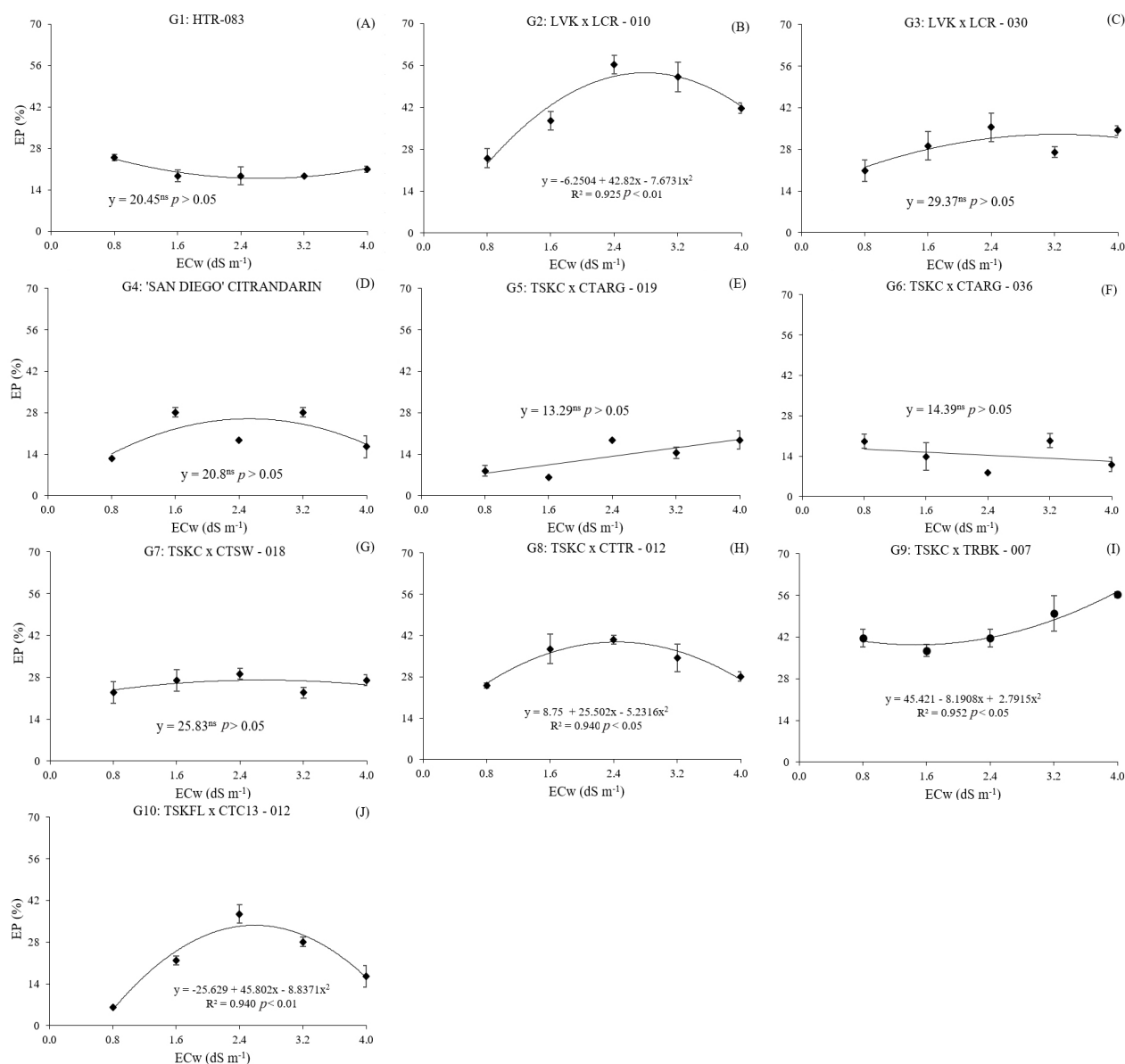
Figure 1. Effect of saline waters (dS/m) on the emergence percentage (EP) of citrus genotypes. HTR-Hybrid trifoliate; LVK-Volkamer lemon (*Citrus volkameriana* V. Ten. & Pasq.); LCR-Rangpur lime (*C. limonia* Osbeck); 'San Diego' citrandarin [*C. sunki* (Hayata) hort. ex Tanaka × *Poncirus trifoliata* (L.) Raf. Swingle]; TSKC-common Sunki mandarin (*C. sunki*); CTARG-Argentina citrange [*C. sinensis* (L.) Osbeck × *P. trifoliata*]; CTSW-Swingle citrumelo (*C. paradisi* Macfad. × *P. trifoliata*); CTTR-Troyer citrange; TRBK-*Poncirus trifoliata* Benecke; TSKFL-Florida Sunki mandarin; CTC13-C13 citrange.

between these factors (Fig. 2). With regards to higher leaf emission, in both seasons, the genotypes TSKC × TRBK - 007, TSKC × CTTR - 012 and TSKC × CTARG - 019 stood out, a fact which puts them among those with the greatest growth potential (Fig. 2).

At 60 DAS, the hybrid TSKC × CTARG - 036 had the lowest number of leaves, with 6.3 leaves per plant, though it recovered in the second evaluation (150 DAS) when 17.06 leaves were counted per plant. This is an improvement of 63.07% and demonstrates that these plants had potential to grow. Conversely, the genotype LVK × LCR - 010 and LVK × LCR - 030 in second evaluation had the lowest number of leaves, 12.56 and 17.04 leaves per plant, due to its low growth rate (Fig. 2).

With regard to the salinity of water irrigation, its effect on the number of leaves did not depend on the genetic material tested as rootstocks, the ECw levels reduced it linearly, with a decrease of 2.6 leaves per unit increase of salinity (Fig. 3A).
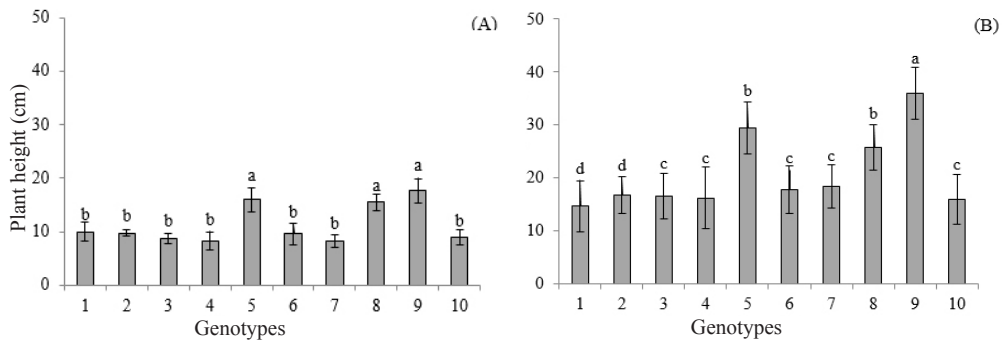
Plant height

Studying the clustering of genotypes using the Scott-Knott test, for plant height at 60 and 150 DAS, as seen in Figs. 4A and 4B, it is observed that the rootstock TSKC × TRBK - 007 had the highest growth in plant height in all evaluation periods, with average values for plant height between 17.64 and 55 cm, respectively.
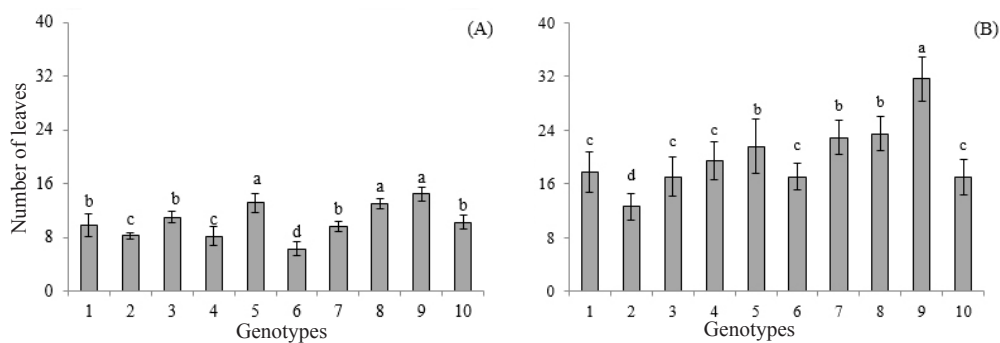
Figure 2. Mean values for the number of leaves of citrus genotypes at 60 (A) and 150 (B) DAS. Genotypes with the same letter do not differ by the Scott-Knott's test (p <0.05). Bars represent standard error of the mean (n = 15). Genotypes: see Figure 1.
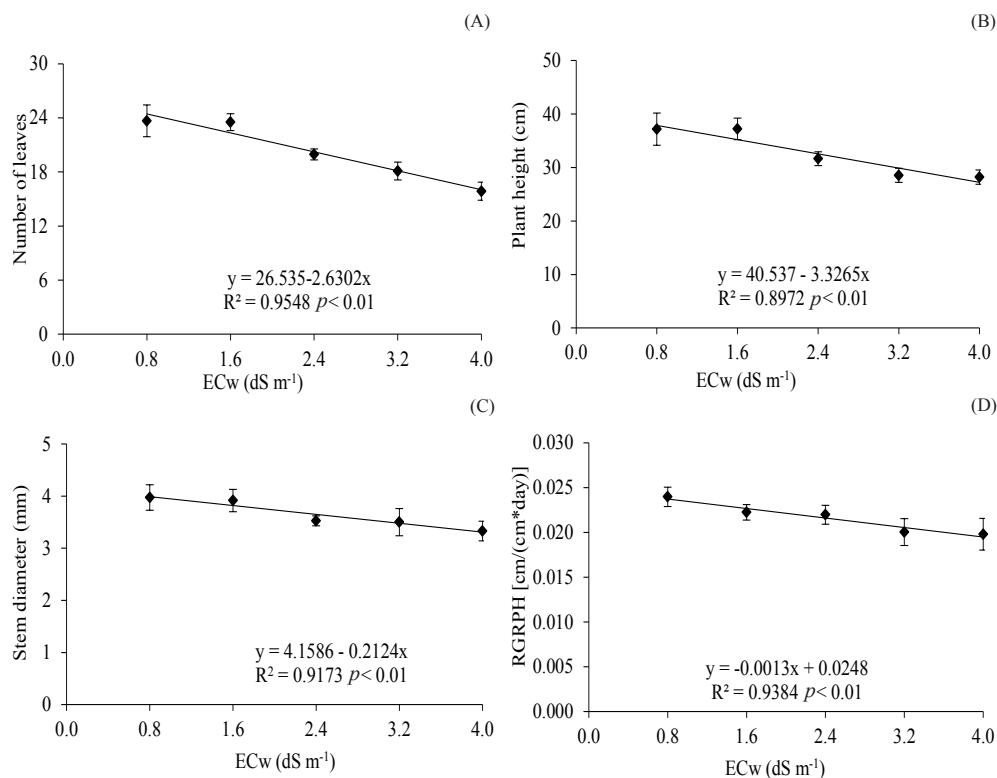
Figure 3. Number of leaves (A), plant height (B), stem diameter (C) and relative growth rate of plant height (RGRPH) (D) of citrus genotypes according to water salinity levels at 150 DAS.

Figure 4. Average of the plant height of citrus genotypes at 60 (A) and 150 (B) DAS. Genotypes with the same letter do not differ by the Scott-Knott's test, $p < 0.05$. The bars represent the standard error of the mean (n = 15). Genotypes: see Figure 1.

The genotypes TSKC × CTARG - 019 and TSKC × TRBK - 007 were classified in a second group, with an increase in mean height from 16.01 cm to 29.52 cm for TSKC × CTARG - 019 and from 17.64 cm to 35.94 cm for TSKC × TRBK - 007 (Fig. 4) being seen in the evaluation periods. It is noteworthy that over the evaluation periods, as evidenced by the number of leaves, 'San Diego' citrandarin was among the genotypes with the lowest height, approximately 8.20 cm at 60 DAS and 16.20 cm at 150 DAS. Moreover, increased water salinity linearly reduced average plant height in the order of 8.78% per unit increase of salinity, as shown in Fig. 3B.
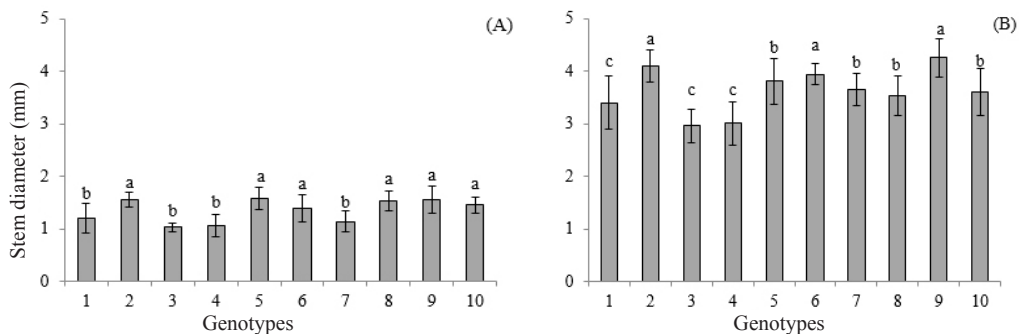
Stem diameter

According to the average stem diameter of rootstocks at 60 DAS (Fig. 5A), the formation of two groups of rootstocks can be seen. The highest averages were obtained for genotypes LVK × LCR - 010, TSKC × CTARG - 019, TSKC × CTARG - 036, TSKC × CTTR - 012, TSKC × TRBK - 007 and TSKFL × CTC13 - 012, with means between 1.03 and 1.55 mm. Analyzing stem diameter at 150 DAS (Fig. 5B), the Scott–Knott test ($p < 0.05$) showed lower secondary growth in the

rootstocks LVK × LCR - 010, LVK × LCR - 030 and 'San Diego' citrandarin, with stem diameter values being between 2.96 and 3.40 mm; the best performing group included TSKC × TRBK - 007, already mentioned for the number of leaves and plant height, with 4.26 mm in diameter, as well as LVK × LCR - 010 (4.10 mm) and TSKC × CTARG - 036 (3.94 mm).

For stem diameter, a decreasing linear effect of salinity at 150 DAS (Fig. 3C) in the order of 5.32% was found, with an increase of 1 dS/m in water salinity used for irrigation, compared to the lowest level studied (0.8 dS/m).

Relative growth rates

Although there was no effect of the interaction on the number of leaves, when studying the relative growth rate of the number of leaves (RGRNL), this result could be noted (Fig. 6), whereas for the hybrid TSKC × TRBK - 007, where the highest average number of leaves was seen, there was the highest growth rate and the lowest reduction per unit increase in water salinity (4.5%). Using regression analysis, a linear pattern in the relative growth rate of stem diameter (RGRSD) was observed for TSKC × CTSW - 018, with a 3.22% decrease

Figure 5. Averages for stem diameter of citrus genotypes at 60 (A) and 150 (B) DAS. Genotypes with the same letter do not differ by the Scott-Knott's test ($p < 0.05$). Bars represent standard error of the mean (n = 15). Genotypes: see Figure 1.
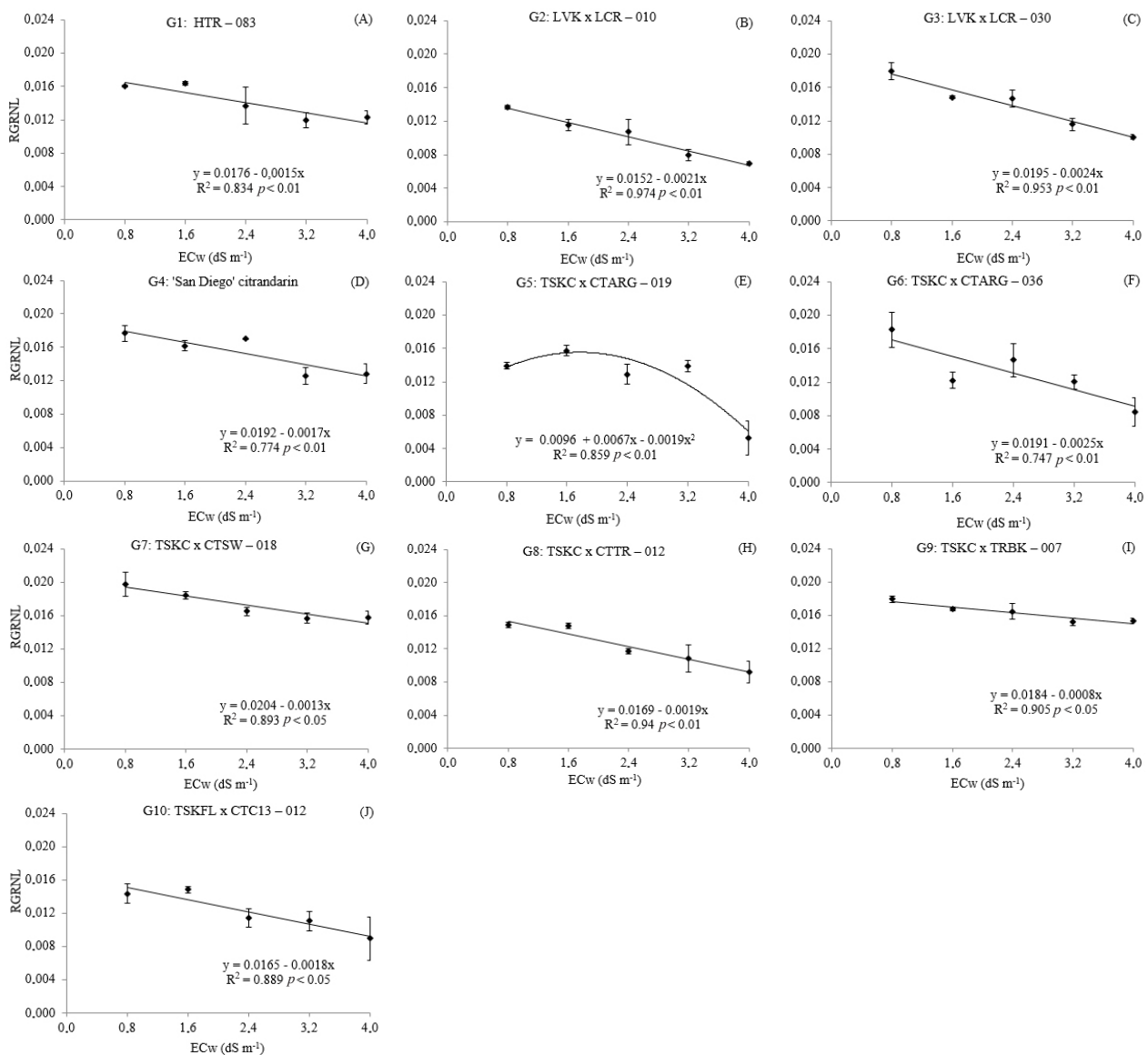
Figure 6. Effect of saline water (dS/m) on the relative growth rate of the number of leaves (RGRNL) of citrus genotypes. Genotypes: see Figure 1.

per unit increase in ECw, that is, a 12.90% reduction when comparing the data of 0.8 and 4.0 dS/m at 150 DAS (Fig. 7).

According to the regression equation (Fig. 3D), at 150 DAS the linear model indicates declines in the relative growth rate of plant height (RGRPH) of 5.24% per unit increase of ECw. This is a 20.96% reduction for plants irrigated with 4.0 dS/m water compared to the control (0.8 dS/m).

Physiological parameters

Fig. 8A presents data of the Scott-Knott test used to group means for internal CO_2 concentration on leaves of the citrus genotypes. The highest averages were obtained for LVK × LCR - 010, TSKFL × CTC13 - 012, TSKC × CTARG - 036, TSKC × CTARG - 019, HTR - 083 and

'San Diego' citrandarin, with approximately 248, 240, 239, 238, 235 and 232 μmol/mol of CO_2, respectively. The lowest averages were found for the genotypes TSKC × TRBK - 007, TSKC × CTTR - 012 and LVK × LCR - 030.

According to the data in Fig. 9, it appears that the transpiration rate of genotypes TSKC × CTARG - 036 and TSKC × CTTR - 012, when irrigated with saline water (4 dS/m), had, compared with plants irrigated with low salinity water (0.8 dS/m), reductions of 2.20 and 10.41% and of 1.91 and 2.12 mmol/(m²·s) to 1.78 and 1.41 mmol/(m²·s), respectively.

When the transpiration rate of citrus plants for the seedling hybrids HTR - 083, LVK × LCR - 010, LVK × LCR - 030, 'San Diego' citrandarin, TSKC × CTARG - 019, TSKC × CTSW - 018, TSKC × TRBK - 007 and TSKFL × CTC13 - 012 were compared, the values estimated through the
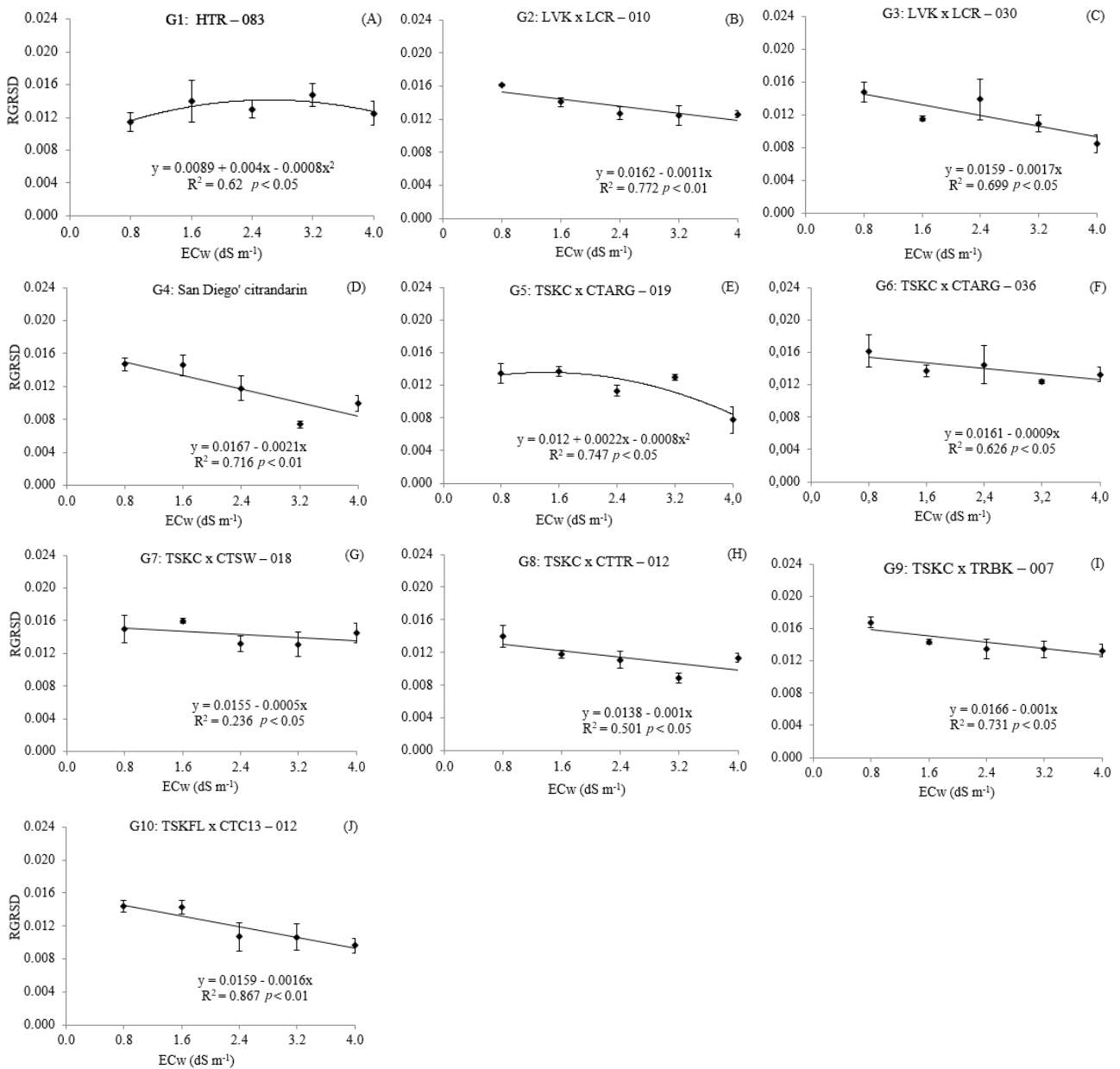
Figure 7. Effect of saline water (dS/m) on the relative growth rate of stem diameter (RGRSD) of citrus genotypes. Genotypes: see Figure 1.
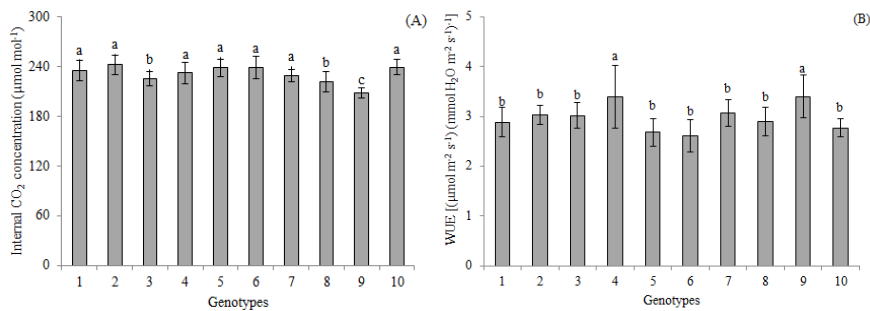
Figure 8. Averages relating to internal CO_2 concentration (A) and instantaneous water-use efficiency (WUE) (B) of citrus genotypes at 150 DAS. Genotypes with the same letter do not differ by the Scott-Knott's test; bars represent standard error of the mean (n=15). Genotypes: see Figure 1.
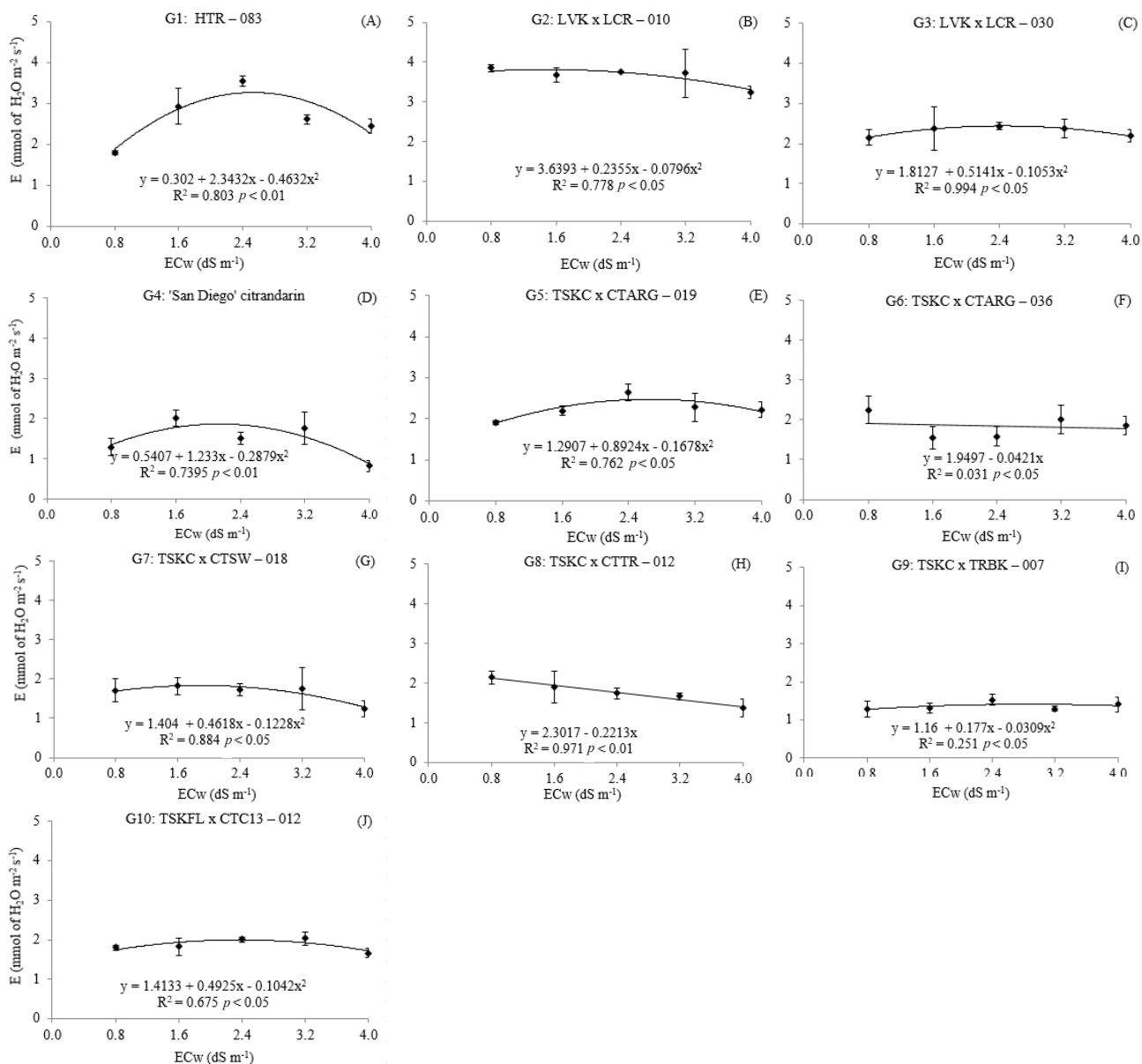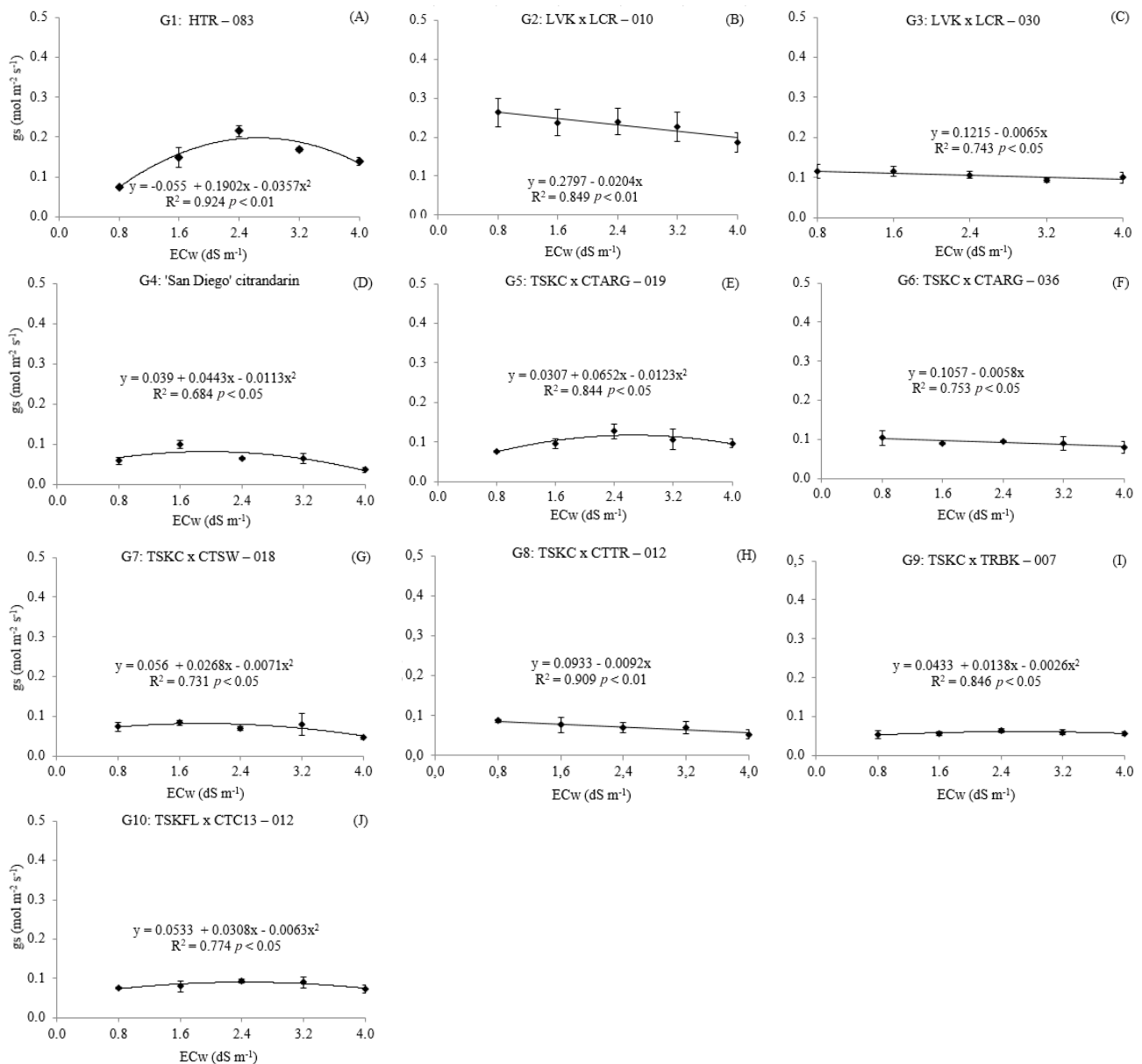
Figure 9. Effect of saline water (dS/m) on transpiration (E) of citrus genotypes at 150 DAS. Genotypes: see Figure 1.

regression equations (Fig. 9) for each genotype showed that LVK × LCR - 010 has the highest transpiration rates (*E*); in contrast, TSKFL × CTC13 - 012 showed, independent of water salinity levels, the lowest transpiration rates. These results show that this hybrid is very sensitive to variations in the EC of irrigation water, since salt levels (0.8 and 1.6 dS/m) below the salinity threshold for citrus were enough to cause significant negative effects on the gas exchange of this genotype.

As seen in Fig. 10, the stomatal conductance (*gs*) of the genotypes LVK × LCR - 010, LVK × LCR - 030, TSKC × CTARG - 036 and TSKC × CTTR - 012 had decreases of 7.74, 5.59, 5.74 and 10.70%, respectively. It was also observed that TSKC × CTARG - 036 had lower potential loss of gas exchange, even with increases

in water salinity. Yet for TSKC × TRBK - 007, where the highest increases in height and number of leaves were noted, regardless of the water salinity levels (dS/m), it presented the lowest rates of stomatal conductance.

With the reduction in transpiration rates and in the stomatal conductance of leaves, the assimilation rate was also compromised in plants irrigated with water of 4,0 dS/m. The genotypes LVK × LCR - 010 and LVK × LCR - 030 had the highest reductions in assimilation rates, 21.95% and 25.42%, respectively, when compared to the values obtained in plants irrigated with water of 0.8 dS/m (Fig. 11). However, no reduction in assimilation rates were observed in 'San Diego' citrandarin when water salinity increased, with mean values of 4.54 and 5.22 μmol/(m²·s) in plants irrigated with water of 0.8 and 4.0 dS/m, respectively, being obtained.
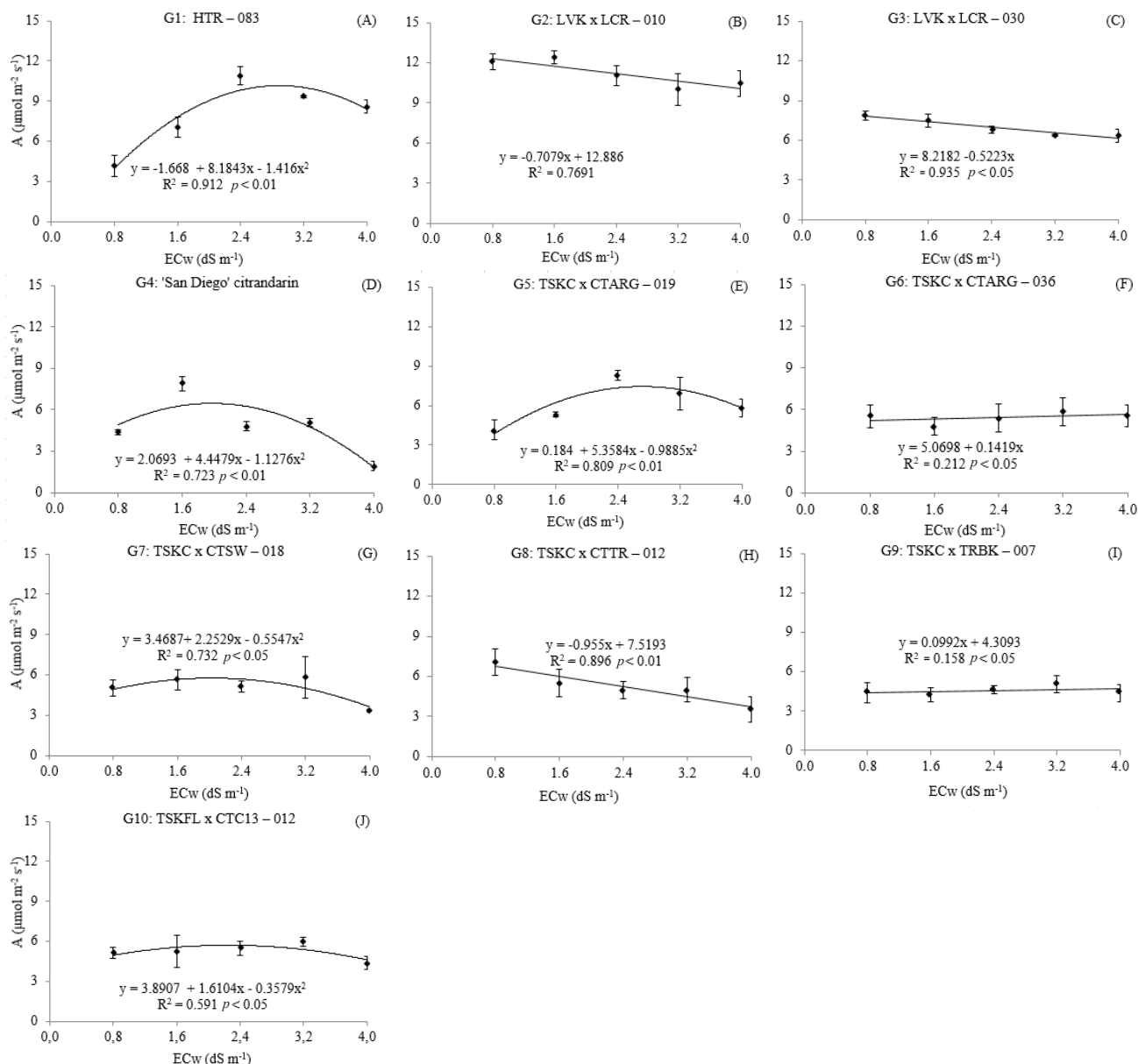
Figure 10. Effect of saline water (dS/m) on stomatal condutance (*gs*) of citrus genotypes at 150 DAS. Genotypes: see Figure 1.

As seen in Fig. 12, there was a decrease in intrinsic carboxylation efficiency (CE) when water salinity increased, for the genotypes LVK × LCR - 010, LVK × LCR - 030 and TSKC × CTTR - 012. Thus, water salinity did not affect internal CO_2 concentrations, but did restrict photosynthesis. The effect on the restriction in these genotypes, therefore, regarding the efficiency of carboxylation, may be related to the biochemical process of photosynthesis. Nonetheless, for TSKC × CTARG - 036 and TSKC × TRBK - 007, there was an increase of 6.56% and 1.89%, respectively, in CE per unit increase in water salinity. The CE results were quadratic for the genotypes HTR - 083, 'San Diego' citrandarin, TSKC × CTARG - 019, TSKC × CTSW - 018 and TSKFL × CTC13 - 012, with the highest

values being 0.0465, 0.0293, 0.0326, 0.0254 and 0.0235 [(μmol/(m²·s))/(μmol/mol)], respectively, with irrigation water electrical conductivity ranging from 2.0 to 3.0 dS/m (Fig. 12).

In relation to the instantaneous WUE of citrus genotypes, the best group was composed of TSKC × TRBK - 007, 'San Diego' citrandarin, TSKC × CTSW - 018, LVK × LCR - 010 and LVK × LCR - 030 (Fig. 8B), with 3.39, 3.38, 3.07, 3.03 and 3.02 μmol/(m²·s)/[(mol H_2O/(m²·s)]. The lowest instantaneous WUE rates were obtained for HTR - 083, TSKC × CTARG - 019, TSKC × CTARG - 036, TSKC × CTTR - 012 and TSKFL × CTC13 - 012. The reductions observed in WUE, notably in rootstocks TSKC × CTARG - 019 and TSKC × CTARG - 036, are reflections of

Figure 11. Effect of saline water (dS/m) on CO_2 assimilation rates (A) of citrus genotypes at 150 DAS. Genotypes: see Figure 1.

lower WUE under saline conditions, indicating a possible impairment of physiological activity (Fig. 8B).

Discussion

A positive effect of salinity on the EP was observed for the hybrids LVK × LCR - 010, TSKC × CTTR - 012, TSKC × TRBK - 007 and TSKFL × CTC13 - 012 until the salinity threshold highlighted in the results was reached, which is an indicator of priming (Fig. 1). It has been found in some studies that the emergence of citrus species depends on genetic genotype variation, as found by Siqueira *et al.* (2002) and Dias *et al.* (2012). They observed an increase in germination for

citrus rootstock varieties irrigated with saline waters, which may be relative to osmotic tolerant adjustments to salts during germination.

However, with increasing levels of salt, there was a reduction in seed EP for the genotypes HTR - 083, 'San Diego' citrandarin, TSKC × CTARG - 019 and TSKC × CTARG - 036. This reduction in germination with salinity increases may be related to the decrease in the osmotic potential of the soil solution, promoting delays in the mobilization of enzymes responsible for germination, and the toxic effects of ions in plant tissues, which can lead to ion toxicity and nutritional imbalances (Alaoui *et al.*, 2013).

A greater number of leaves was found in genetic genealogy material for trifoliate hybrids (Fig. 2). This
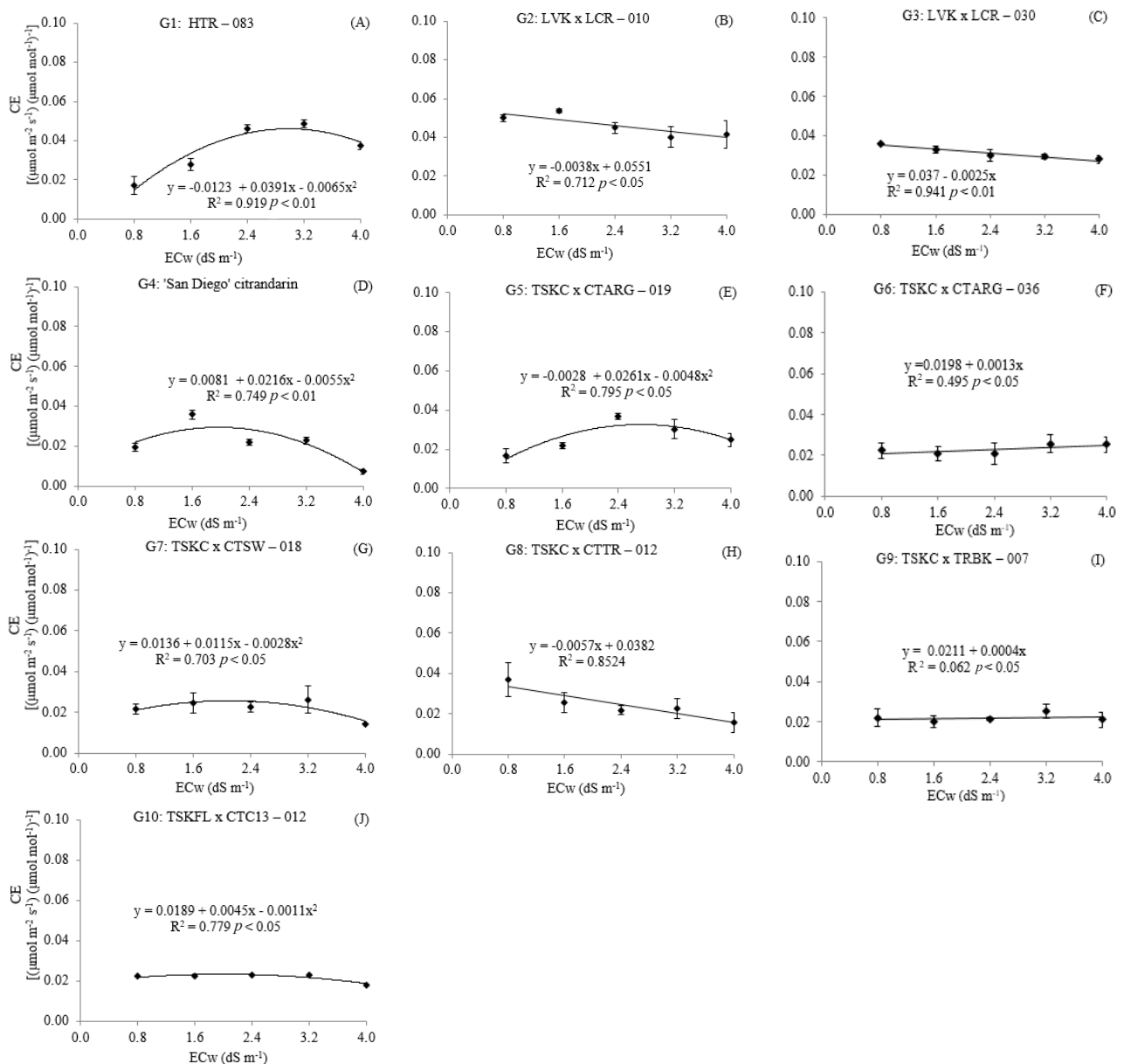
Figure 12. Effect of saline water (dS/m) on intrinsic carboxylation efficiency (CE) of citrus genotypes at 150 DAS. Genotypes: see Figure 1.

was also noted by Schmitz (1998), who associated this with hybrid vigor. Also working with new hybrid rootstocks, Brito *et al.* (2008) observed a higher emission of leaves in Sunki mandarin with Argentine citrange hybrids, which may be related to hybrid vigor due to crossing. This is a result similar to that obtained in this work, since TSKC × TRBK - 007 has the same genetic material as the female parent.

Increasing salt content in the water reduced the emission of leaves of the genotypes, which may be a plant survival strategy since reductions in the number of leaves means reductions in transpiration structures and water demand (Chaves *et al.*, 2009). Studies by Tozlu *et al.* (2000) corroborate these results, as citrus plants

subjected to salt stress reduced their foliar emission, with these effects being related to the excessive accumulation of specific ions in leaves. Applying this interpretation, a significant effect of the factors in this work would be expected; that is, the effects of salinity would vary for the genotypes, which did not happen. In this study, the reduction of percentages related to the number of the leaves of the genotypes were low, which may be seen as an adaptive mechanism to protect the branch apex and the physiologically active new leaves, as considered by Cruz *et al.* (2003).

Minor growth in plant height was observed for the rootstock TSKC × CTARG - 036 in all evaluation periods (Fig. 4). This genotype has some dwarf

characteristics, which is possibly an adaptation to the environment conditions (Stuchi & Girardi, 2011).

Plant height reduction under salinity (Fig. 3B) was also observed by Brito *et al.* (2014) when evaluating the scion-rootstock combination of citrus under similar conditions, with plant height being the most sensitive variable to saline stress. This reduction in growth may be associated with the inhibition of cell expansion caused by an increase in osmotic pressure in the soil solution. This results in cell plasmolysis, with consequences for cell division and elongation (Taiz & Zeiger, 2013).

In relation to stem diameter, we also did not notice significant effects from the interaction between factors, *i.e.*, its effects were isolated. Considering the genotypes at 60 DAS (Fig. 5A), it is possible to form two groups of rootstocks by stem diameter, with the highest averages being observed for the genotypes LVK × LCR - 010, TSKC × CTARG - 019, TSKC × CTARG - 036, TSKC × CTTR - 012, TSKC × TRBK - 007 and TSKFL × CTC13 - 012, noting, for these materials, averages between 1.03 and 1.55 mm. Analyzing stem diameter at 150 DAS (Fig. 5B), it was verified, through the Scott–Knott test ($p < 0.05$), that there was lower secondary growth in the rootstocks HTR - 083, LVK × LCR - 030 and 'San Diego' citrandarin, with stem diameter values between 2.96 and 3.40 mm; the best group consisted of TSKC × TRBK - 007, with 4.26 mm in diameter, already mentioned due to its number of leaves (Fig. 2) and plant height (Fig. 4), as well as LVK × LCR - 010 (4.10 mm) and TSKC × CTARG - 036 (about 3.94 mm in diameter).

Considering the growth variables (number of leaves, plant height and stem diameter), TSKC × CTARG - 019, TSKC × CTTR - 012, TSKC × TRBK - 007 and LVK × LCR - 010 rootstocks stood out in the two evaluations (60 and 150 DAS).

In relation to salinity levels, a decreasing linear effect on stem diameter at the 150 DAS (Fig. 3C) was found in the order of 5.32%, with an increase of 1 dS/m in the salinity of the water used for irrigation compared to the lowest studied level (0.8 dS/m). The average diameter values of the stem of the plants were low, between 3.5 and 4.2 mm (Fig. 3C), which are, nonetheless, superior to those achieved by Schmitz (1998), who, working with Trifoliata seedligs under different substrates, found an average diameter of 1.97 mm.

The relative growth rate of the number of leaves, stem diameter and plant height were reduced by increasing the salt content in the irrigation water (Figs. 8, 9 and 10). This may be associated with excess salts in the root zone, which generally have a deleterious effect on plants. This is manifested by a reduction in water absorption, in the rate of photosynthesis and, thus, in growth. Moreover, the toxic effects of salts absorbed by plants, mainly Na^+ and Cl^-, and the low osmotic adjustment capacity of the culture, may also have impaired physiological activities, resulting in a loss in the growth rate of the cultures (Medeiros *et al.*, 2007).

The internal CO_2 content (iC) varied at 150 DAS, following the inverse tendency observed in other variables; *i.e.*, increases of iC and reductions of assimilation rates were observed for the genotypes LVK × LCR - 010 and 'San Diego' citrandarin when salt content was increased in the water irrigation, reducing the CO_2 influx for organic synthesis (Fig. 8). Studies report that despite the fact that decreases in stomatal conductance concomitantly reduce CO_2 concentrations, in the substomatal camera they are maintained or even increase in stressed plants (Garcia-Sanchez *et al.*, 2007). This indicates that carbon is not metabolized by the photosynthetic apparatus, maybe due to the salt stress condition to which these plants were exposed during the rootstock formation phase.

The iC values in citrus plants is between 200 and 250 μmol/mol (Mattos Junior *et al.*, 2005), indicating, in fact, that citrus plants did not have their photosynthetic apparatus damaged by the application of salts in irrigation water, as noted by Brito (2010) for citrus plants under salt stress in field conditions. This is explained by a direct relationship between gas exchange and reductions in water availability, reflecting stomatal closure, as well as reductions in transpiration and assimilation rates (Bosco *et al.*, 2009), as identified in this work (Figs. 11B, 12, 13 and 14). Decreases in stomatal conductance observed in this study were also noted by Fernandes *et al.* (2011) and Brito *et al.* (2012) for citrus plants irrigated with saline water (Fig. 10).

The high growth and great salt tolerance of the hybrid TSKC × TRBK - 007 is relative as well to its best instantaneous WUE, obtained by the relationship between *A* and *E* (Rosales *et al.*, 2012). It is possible to realize that the hybrid TSKC × TRBK - 007 is more capable of using CO_2, even with the limitation of CO_2 diffusion through the stomata, which is probably due to higher efficiency for CO_2 fixation in mesophyll (Fig. 8B). Brito *et al.* (2014) informs that tolerances of genotypes to salt water can vary. This is also true for plant development stages.

The assimilation rate of citrus genotypes under saline water was similar to results obtained by Brito *et al.* (2012), who, studying the physiological behavior of combinations of citrus scions/rootstocks under water stress, observed values between 4 and 10 μmol/(m²·s). It is supposed that 'San Diego' citrandarin was able to increase its WUE under saline conditions, since reductions in water consumption means a reduction in the absorption of specific ions, thus avoiding toxic effects on the plants (Flowers & Flowers, 2005), which

can be related to the salt exclusion mechanism of roots (Fig. 11).

For intrinsic carboxylation efficiency at 150 DAS, the best results were obtained with the hybrid TSKC × TRBK - 007 (Fig. 12), which is probably because of the lower iC values in the plants. Thus, a succession of gas exchange changes may be explicitly related to its higher growth in the number of leaves and stem diameter. This may be justified by the gene expression variation of each genotype.

The germination speed index of the hybrid TSKC × CTARG - 019 was the best under saline conditions as it is a genotype with more tolerance to saline stress. Salinity reduces the growth of citrus genotypes during the rootstock formation phase, although this varies for each type. The genotypes TSKC × CTARG - 019, TSKC × CTTR - 012, and TSKC × TRBK - 007 have higher growth potential up to 150 DAS regardless of saline level. Salinity decreases the growth of most citrus genotypes, notwithstanding, one can use water up to 1.6 dS/m, which allows for suitable growth. Salt stress affects the photosynthetic rate of the genotypes 'San Diego' citrandarin and TSKC × CTTR - 012 through stomatal closure, thereby restricting the diffusion of CO_2. The genotype TSKC × TRBK - 007 has the best photosynthetic efficiency when subjected to different saline levels.

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