

## Salt balance in substrate and growth of 'Tahiti' acid lime grafted onto Sunki mandarin hybrids under salinity stress

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### Abstract

The salinity of water or soil may cause disturbance in growth and production of crops, particularly in citrus, which is considered sensitive to this abiotic factor. For this reason, it is important to choose tolerant materials of rootstocks and scion-rootstock combinations, and to evaluate the salt balance in soils. The effect of saline water irrigation on plant formation of 'Tahiti' acid lime [*C. latifolia* (Yu. Tanaka) Tanaka] seedlings grafted onto six rootstock genotypes of hybrid Sunki mandarin [*C. sunki* (Hayata) hort. ex Tanaka] was evaluated. Plants were evaluated for salt tolerance and the mineral balance in the plant cultivation substrate 300 days after seeding. The study consisted of five levels of saline water (0.8, 1.6, 2.4, 3.2 and 4.0 dS m<sup>-1</sup>) that were used for the irrigation of six different rootstock genotypes; on each the 'Tahiti' acid lime was grafted as scion. The rootstocks were five hybrids of 'Sunki' mandarin and one 'Santa Cruz Rangpur' lime plant, as control. The design consisted of randomized blocks with three replications and four plants per plot. The water salinity level treatments were applied starting 60 days after seeding (DAS) and continued until 300 DAS. The chemical contents of the soil and the total plant dry matter for the scion-rootstock combinations were determined. The increase in electrical conductivity of the irrigation water resulted in the accumulation of minerals in the substrate. The common hybrid of 'Sunki' mandarin (TSKC) × (Rangpur lime × *Poncirus trifoliata*) – 040 is the most tolerant to the salinity of irrigation water. The TSKC × 'Troyer' citrange – 012 hybrid is the most sensitive. Water with an electrical conductivity greater than 2.4 dS m<sup>-1</sup> is not recommended for use with 'Tahiti' acid lime grafted onto rootstocks.

**Keywords:** *Citrus* spp., hybrids, *Poncirus trifoliata*, salt tolerance, screening

### Introduction

Brazilian citrus production reached a total value of 5.7 billion Brazilian Reals (BRL) in 2012 and consists of sweet oranges [*Citrus sinensis* (L.) Osbeck], mandarins (various species) and acid limes, particularly 'Tahiti' acid lime [*C. latifolia* (Yu. Tanaka) Tanaka], and lemons [*C. limon* (L.) Burm. f.]. The production is distributed throughout Brazil in an area greater than 828,000 ha, in the State of São Paulo being the largest producer, with approximately 73% of the national production, and the states of Bahia and Sergipe in the Northeast accounting for approximately 10% of the total (IBGE, 2012). In the Brazilian Northeast, the cultivation of citrus fruits is of great socioeconomic relevance for the generation of jobs and income, especially in the states of Bahia, Sergipe and Paraíba. Nevertheless, production is generally low due to the lack of water that occurs in the hottest months of the year, making irrigation necessary during this period to obtain increased fruit production (Fernandes et al., 2011). Additionally, there are relatively high concentrations of salts in the water from the wells, dams and rivers of various locations in this region (Audry and Suassuna, 1995), which can negatively impact the growth, development, and yield of citrus plants because they are moderately sensitive to salinity (Levy and Syvertsen, 2004; Fernandes et al., 2011; Brito et al., 2014a). Under saline

conditions, citrus plants are affected by osmotic stress and by toxic ions, primarily those of chlorine (Cl), sodium (Na) and boron (Levy and Syvertsen, 2004; Dias and Blanco, 2010). In a recent review, Syvertsen and Garcia-Sanchez (2014) discussed the multiple additional stresses caused during salt stress, including nutritional and environmental stresses. Thus, the use of rootstocks that are tolerant to salinity enables the use of low-quality water and saline soils (Fernandes et al., 2011; Brito et al., 2014a). Another important aspect of citrus production relates to the interaction of soil ions with different rootstocks. Study of these interactions can enable the identification of nutrient absorption conditions and clarify the salt tolerance mechanisms of citrus plants. According to Murkute et al. (2005) and Syvertsen and Garcia-Sanchez (2014), evapotranspiration and the absorption of nutrients can vary as a result of saline stress. Thus, it is believed that the accumulation of salts differ among the substrates used to cultivate citrus seedlings. It is also necessary to consider that the production of citrus plants involves two genetically different genotypes, which are compatible individuals. The scion must be a variety with a high productive potential and the rootstock supports the scion and guarantees the scions good development (Mattos Junior et al., 2005). It has been observed in other fruit trees propagated by grafting that

salinity tolerance of citrus fruits must be evaluated considering the scion-rootstock combination (Brito et al., 2014a). The careful selection of both individuals is essential to the successful cultivation of citrus plants. Therefore, the study of salt balance in the cultivation environment combined with the study of plant growth under saline stress permits the identification of salt tolerance mechanisms. In turn, it permits to detect the genetic materials with the potential to adapt to saline stress. For this purpose, some studies have been performed to identify genotypes tolerant to salinity, aiming to increase the list of varieties that can be used in environments subjected to this abiotic stress (Brito et al., 2008; Fernandes et al., 2011; Brito et al., 2014b; Silva et al., 2014). However, these studies are restricted to the rootstock formation phase and require extension to the seedling formation phase. Therefore, the goals of the present study was to assess the growth of several combinations of 'Tahiti' acid lime scions grafted onto six types of Sunki mandarin rootstocks plants at different salinity levels. We also evaluated the salt balance in the substrate solution that was used for their cultivation.

## Results and Discussion

### *Salt balance in substrate*

The electric conductivity (EC) is the measure of salt concentration in the saturation extract of the soil ( $EC_{se}$ ). This enables an assessment of the degree of stress associated with a specific soil or substrate. This concentration varies with the soil type, particularly with its texture, and with the management practices such as addition of organic matters and type of water in use. This increase primarily occurs due to the leaching fraction and the peculiarities of the crop because the rise results in differences in the absorption of water and salts (Ayers and Westcot, 1985).

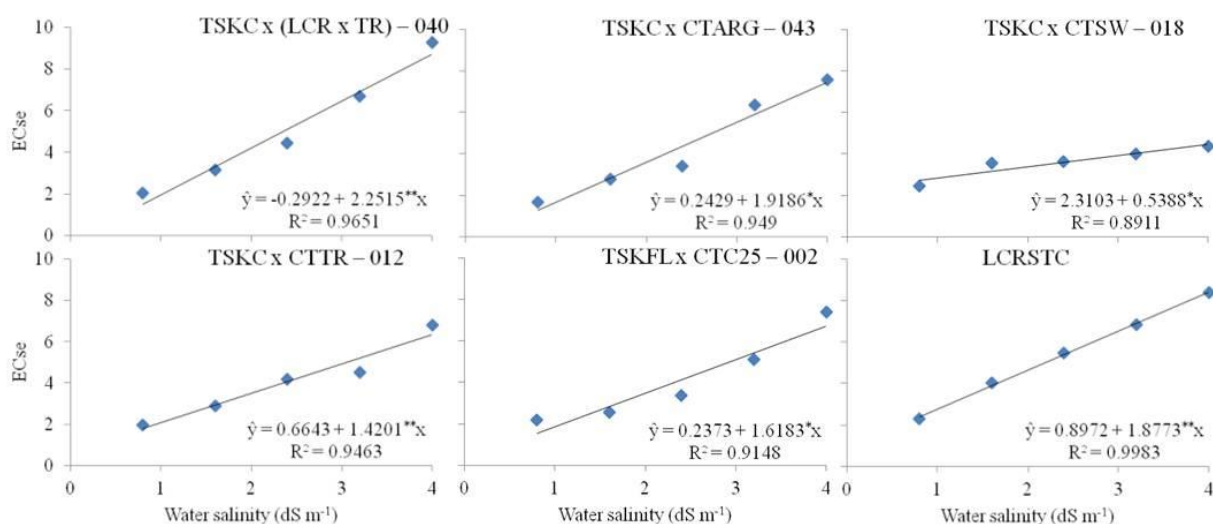
In the present study, the increase in the concentration of salts in the irrigation water resulted in a linear increase in the concentration of ions in the substrate (Fig 1). However, differences were observed in the salt concentration factor, determined as the slope of these lines, according to the genotype onto which 'Tahiti' acid lime was grafted, varying from 0.54 in TSKC × CTSW – 018 to 2.25 in TSKC × (LCR × TR) – 040. In the majority of the genotypes, the salt concentration factor for the substrate varied between 1.4 and 2.0  $dS\ m^{-1}$  with a unit increase in the salinity of the irrigation water. With these increases, the only irrigation water salinity level that did not cause salt accumulation in the soil above the salinity threshold was 0.8  $dS\ m^{-1}$  Singh et al. (2003). When the water with an EC of 1.6  $dS\ m^{-1}$  was applied, the concentration in the substrate was always greater than 2.5  $dS\ m^{-1}$ , which resulted in a stress level sufficient to reduce the growth and productivity of plants. This reduction is expected because the salinity threshold corresponds to the salt content that the crops tolerate. That is attributed to the salinity threshold, in which there is no decrease in the growth or the productivity of a specific species. Dias and Blanco (2010) reported the tolerance level of 1.7  $dS\ m^{-1}$  in sweet orange tree, whereas Singh et al. (2003) state that the salinity threshold for citrus fruits is 2.0  $dS\ m^{-1}$ . In the present study, when water with a salinity of 4  $dS\ m^{-1}$  was applied, the salinity of the saturation extract remained between 6 and 9  $dS\ m^{-1}$ . These values indicate severe salinity according to Ayers and Westcot (1985). Furthermore, the stress condition can vary among species, within varieties of the same species, and between the developmental phases of the same genotype. This has been identified in certain cultivars of citrus plants (Brito et al., 2008; Fernandes et al., 2011; Brito et al., 2014b;

Silva et al., 2014). In the present study, variation among citrus plant genotypes was also found among the progeny from crosses between the same parents. Although the  $EC_{se}$  allows inferences regarding the stress condition that a plant is experiencing, this condition varies in function of the ions present in the substrate. In particular, the Na, Ca and Mg concentrations and their relative proportions are of great importance because the Na ions are toxic to citrus plants. However, Ca and Mg are nutrients; thus, higher concentrations of these salts can be tolerated without producing harmful effects as long as they are not in the toxic range, although these nutrients can reduce the osmotic potential of the soil. Thus, it is crucial to study the sodium absorption ratio (SAR) because this ratio achieves more robust conclusions in studies of this nature and more frequently identifies stressful conditions related to higher EC values and higher relative Na concentrations. In the present study, the Na concentration in the substrate also increased linearly with the increase of the irrigation water in the EC (Fig 2). In the substrate of the containers used to cultivate the TSKC × CTSW – 018 hybrid, an increase in the Na concentration of the soil solution from 21.2 to 35.2  $cmol_c\ L^{-1}$  was observed. This genotype exhibited the lowest increase in the Na concentration with increase in the salinity of the water applied. An increase of approximately 20.4% per unit salinity observed when calculated over the salinity range tested and of 65.5% between the lowest and highest salinities. The largest increase in the Na concentration of the substrate was observed in the TSKC × (LCR × TR) – 040 hybrid, with an increase of 14.88  $cmol_c\ L^{-1}$  per unit increase in the water EC, which corresponds to an increase of 123.8% per unit, calculated over the range of tested salinities.

In all of the treatments, the Na concentration in the substrate was higher than 3  $mmol_c\ L^{-1}$ , a level considered to be moderately critical for crops by Ayers and Westcot (1985). According to Dias and Blanco (2010) and Syvertsen and Garcia-Sanchez (2014), imbalances and cytoplasmic damage can occur under these conditions, primarily at the edges and the apex of the leaf blade, from which the plant loses water by transpiration. The soil ions absorbed by the plant accumulate in the leaf areas, resulting in signs of toxicity. Even when the Na concentration is high, the concentration of other elements can also be elevated. So, the percentage of exchangeable Na and the SAR are not negatively affected. Prior to the application of the saline water treatments, the initial salt concentrations in the substrate were high, such as the Na concentration of 0.34  $cmol_c\ L^{-1}$  (Table 1). According to Ayers and Westcot (1985), the Na concentration in irrigation waters can vary from 0 to 40  $mmol_c\ L^{-1}$ . The waters used in the present study contained 5.6 and 28  $mmol_c\ L^{-1}$  of Na at the lowest and highest salinity level treatments applied, respectively. Thus, the Na concentrations in these waters were within the range described as high by the aforementioned authors. However, the concentration in the substrate can be much higher as a result of the adsorption capacity of clay micelles and also because of Na supplied by the substrate itself. There was an increase in the Ca concentration of the substrate with the increase in the irrigation water salinity (Fig 3) for all of the individuals used as rootstocks. This increase may be related to the use of  $CaCl_2$  salts in the preparation of the irrigation waters. Ca is an essential element for plants because Ca is part of the cellular structure. It also guarantees membrane integrity through its action as a cementing agent, regulates tissue permeability, stimulates nitrogen absorption and neutralizes organic acids (Taiz and Zeiger, 2009). Thus, the

**Table 1.** Sunki mandarin hybrids used as scion (grafted) in this experiment.

	Genotype	Abbreviation
1	Common ‘Sunki’ mandarin [ <i>C. sunki</i> (Hayata) hort. ex Tanaka] x [Rangpur lime ( <i>C. limonia</i> Osbeck) x <i>Poncirus trifoliata</i> (L.) Raf.] – 040	TSKC x (LCR x TR) – 040
2	Common ‘Sunki’ mandarin x ‘Argentina’ citrange ( <i>C. sinensis</i> x <i>P. trifoliata</i> ) – 043	TSKC x CTARG – 043
3	Common ‘Sunki’ mandarin x ‘Swingle’ citrumelo ( <i>C. paradisi</i> Macfad x <i>P. trifoliata</i> ) – 018	TSKC x CTSW – 018
4	Common ‘Sunki’ mandarin x ‘Troyer’ citrange – 012	TSKC x CTTR – 012
5	Florida ‘Sunki’ mandarin x citrange ‘C25’ – 002	TSKFL x CTC25 – 002
6	‘Santa Cruz Rangpur’ lime (Control)	LCRSTC (Control)



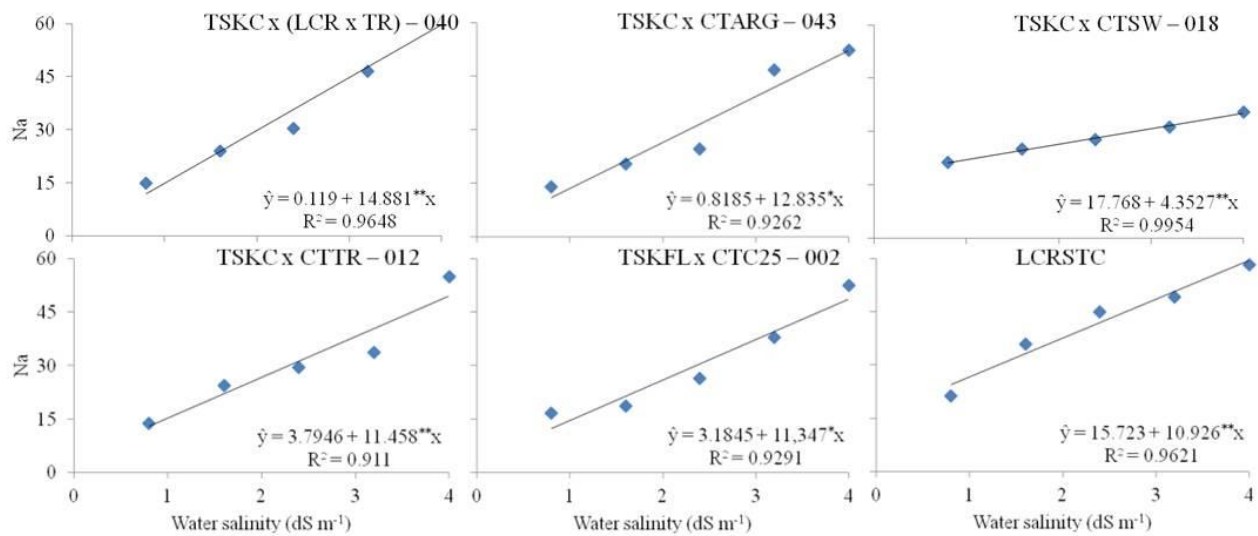
**Fig 1.** Electrical conductivity of the saturation extract ( $EC_{se}$ ) ( $dS\ m^{-1}$ ) measured 300 days after seeding and plotted according to the salinity of the applied water for each genotype grafted with ‘Tahiti’ acid lime [*Citrus latifolia* (Yu. Tanaka) Tanaka].

accumulation of Ca ions in the soil solution may favor absorption and decrease of toxic effects caused by Na or the osmotic effect resulting from salinity in general. The regression equations for the Ca concentrations in the soil solution, expressed according to the salinity of the applied water (Fig 3), shows that the highest Ca accumulation occurred in the TSKC x (LCR x TR) – 040 hybrid, with an increase of 98% per unit increase of salinity in the irrigation water over the Ca value obtained with the  $0.8\ dS\ m^{-1}$ , compared with the  $4\ dS\ m^{-1}$  salinity treatment. This increase corresponded to  $1.8$  to  $7.8\ cmol_c\ L^{-1}$  of Ca in the substrate used to cultivate the referenced genotype when the genotype was irrigated with water at a salinity of  $0.8$  to  $4.0\ dS\ m^{-1}$ , respectively. The highest Na concentrations were also found in the substrates used to cultivate the same genotype, as mentioned above. The lowest Ca accumulation was observed in TSKC x CTSW – 018, with a 23.4% per unit increase in the irrigation water salinity. This corresponds to a 74.9% increase in the concentration of this nutrient, comparing to the treatment with the highest level of salted water ( $4.0\ dS\ m^{-1}$ ) and lowest concentration of salts ( $0.8\ dS\ m^{-1}$ ). The Mg concentration in the substrate also increased with the increase in the salinity of the irrigation water (Fig 4), with the exception of the substrate used to cultivate TSKC x CTSW – 018, for which the effect was quadratic. The highest Mg concentration of  $2.1\ cmol_c\ L^{-1}$  was observed for this hybrid when water with a salinity of  $1.6\ dS\ m^{-1}$  was used for irrigation. The maximum Mg concentration ( $16.5\ cmol_c\ L^{-1}$ ) was recorded for TSKC x (LCR x TR) – 040 irrigated with water having a salinity of  $4.0\ dS\ m^{-1}$ . The greatest increase in the Mg concentration was observed in the substrate, in which TSKC x CTARG – 043 was cultivated, corresponding to a Mg increase of 114.4% per unit increase in the water salinity.

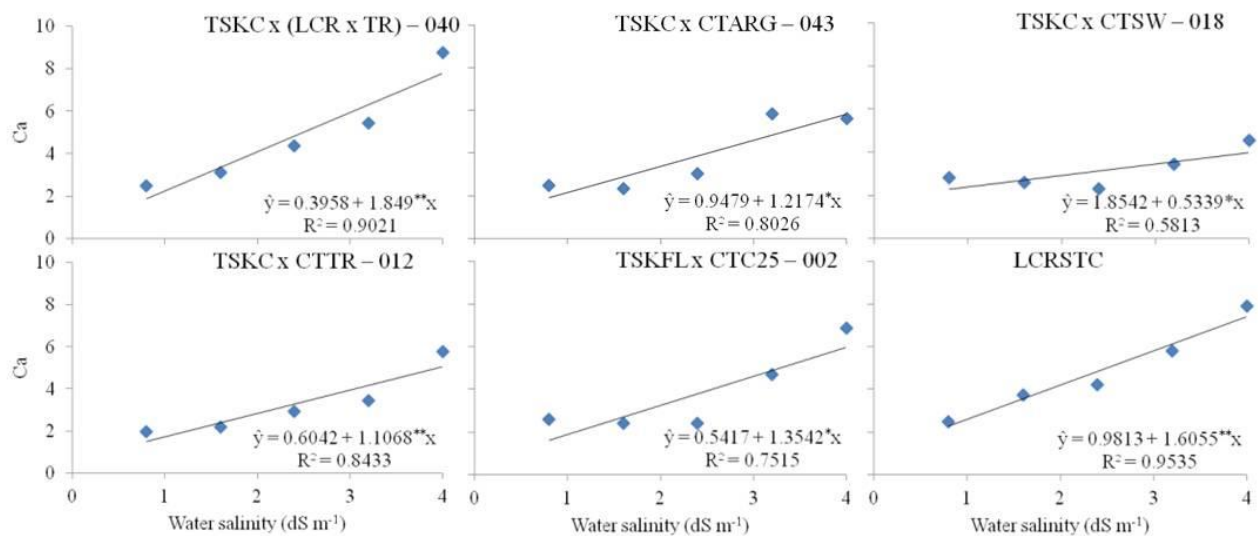
This represents a difference of 366.2% between the treatments with the lowest and highest salinity levels. The second largest increase was observed for the TSKC x (LCR x TR) – 040 hybrid, with values of 106.1% per unit increase in the EC of the irrigation water. Note that the greatest increases in the Na and Ca concentrations were observed in these genotypes. The increase in the Mg concentration; however, was lower than that recorded for sodium, which may affect the SAR. All of the substrates showed Mg concentrations greater than  $0.8\ cmol_c\ L^{-1}$ , which is considered high. Thus, this nutrient was available at unlimited levels. Moreover, Malavolta et al. (1997) reported that the Ca/Mg ratio should be between 3 and 5. The estimated value for this ratio was below 1.0 for all of the substrates, with a predominance of values between 0.4 and 0.6. Thus, although the irrigation water was prepared with only 10% Mg and 20% Ca, there was a greater accumulation of Mg than of Ca in the substrate, making the ratio lower than the optimal value. However, a greater Ca absorption by the plants is expected because Ca is the nutrient with the highest concentration in the leaves of citrus plants (Mattos Junior et al., 2005). This may have contributed to this result. Another important ratio is the sodium absorption ratio (SAR) that not only considers Na but also relates Ca and Mg. An increase in the SAR values was observed after increase in the concentration of salts in the irrigation water, generating values exceeding  $9\ [(cmol_c\ L^{-1})\ (cmol_c\ L^{-1})^{-0.5}]$  for all of the studied genotypes irrigated with water having a salinity of  $4\ dS\ m^{-1}$ . The values varied between  $7.0\ [(cmol_c\ L^{-1})\ (cmol_c\ L^{-1})^{-0.5}]$  for the TSKC x CTARG – 043 hybrid irrigated with water having a salinity of  $0.8\ dS\ m^{-1}$  to  $16.3\ [(cmol_c\ L^{-1})\ (cmol_c\ L^{-1})^{-0.5}]$  for ‘Santa Cruz’ Rangpur lime irrigated with water having a salinity of  $4.0\ dS\ m^{-1}$ . For all of the studied concentrations, there are

**Table 2.** Chemical attributes of the substrate used in the experiment before the begin the experiment.

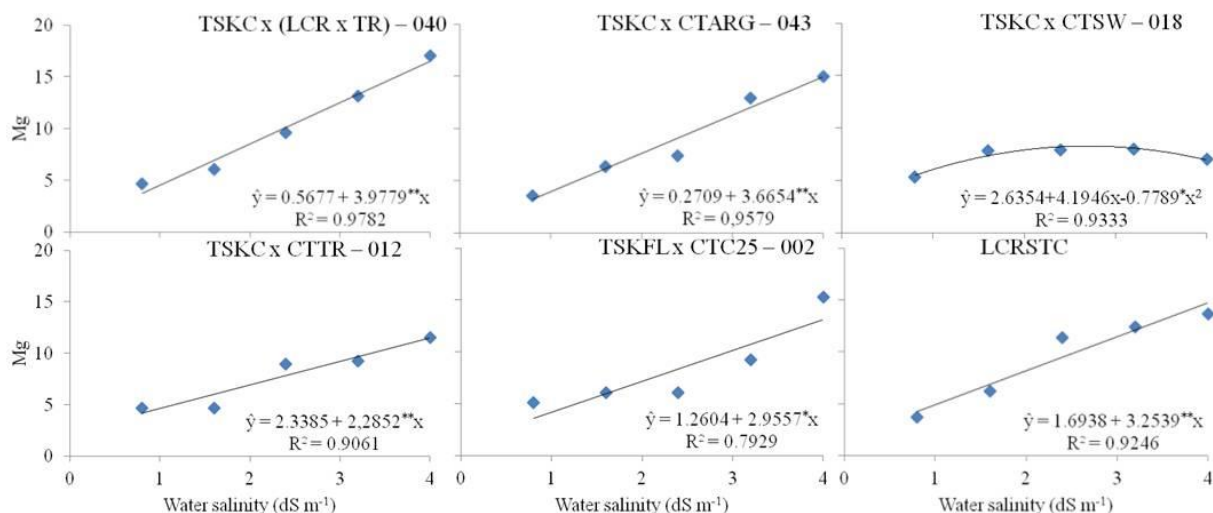
Attribute	Value
Saturation extract pH	6.42
Saturation extract electrical conductivity, dS m <sup>-1</sup>	0.62
Phosphorus, mg dm <sup>-3</sup>	3.00
Potassium, cmol <sub>c</sub> dm <sup>-3</sup>	0.45
Sodium, cmol <sub>c</sub> dm <sup>-3</sup>	0.34
Calcium, cmol <sub>c</sub> dm <sup>-3</sup>	9.20
Magnesium, cmol <sub>c</sub> dm <sup>-3</sup>	7.50
Aluminum, cmol <sub>c</sub> dm <sup>-3</sup>	0.00
Hydrogen + aluminum, cmol <sub>c</sub> dm <sup>-3</sup>	1.16
Sum of exchangeable bases, cmol <sub>c</sub> dm <sup>-3</sup>	17.15
Effective cation exchange capacity, cmol <sub>c</sub> dm <sup>-3</sup>	17.15
Cation exchange capacity at pH 7.0, cmol <sub>c</sub> dm <sup>-3</sup>	18.64
Base saturation index, %	91.98
Aluminum saturation index, %	0.00
Sodium saturation index, %	1.82
Organic matter, %	52.00



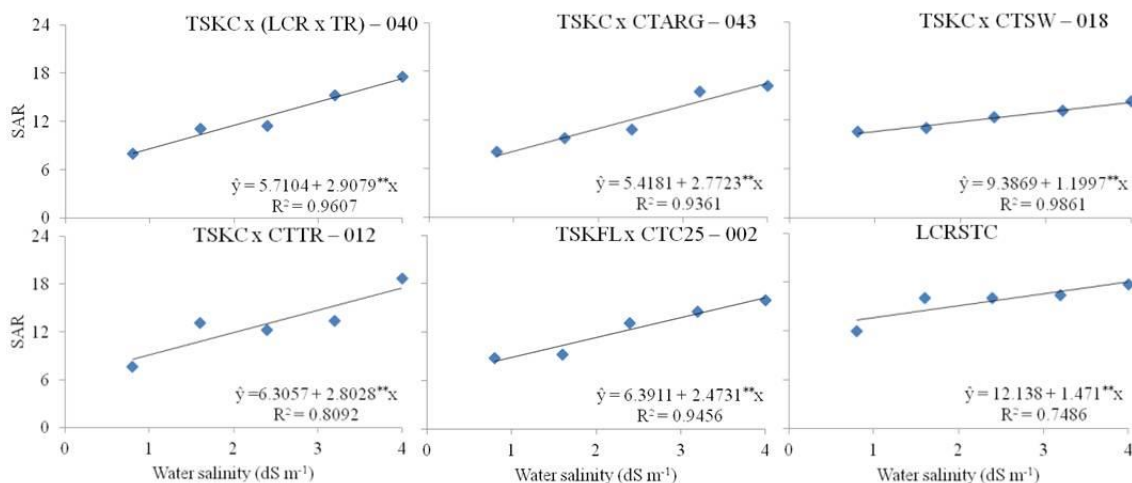
**Fig 2.** Sodium concentration (cmol<sub>c</sub> L<sup>-1</sup>) of the soil solution measured 300 days after seeding and plotted according to the salinity of the applied water for each genotype grafted with ‘Tahiti’ acid lime [*Citrus latifolia* (Yu. Tanaka) Tanaka].



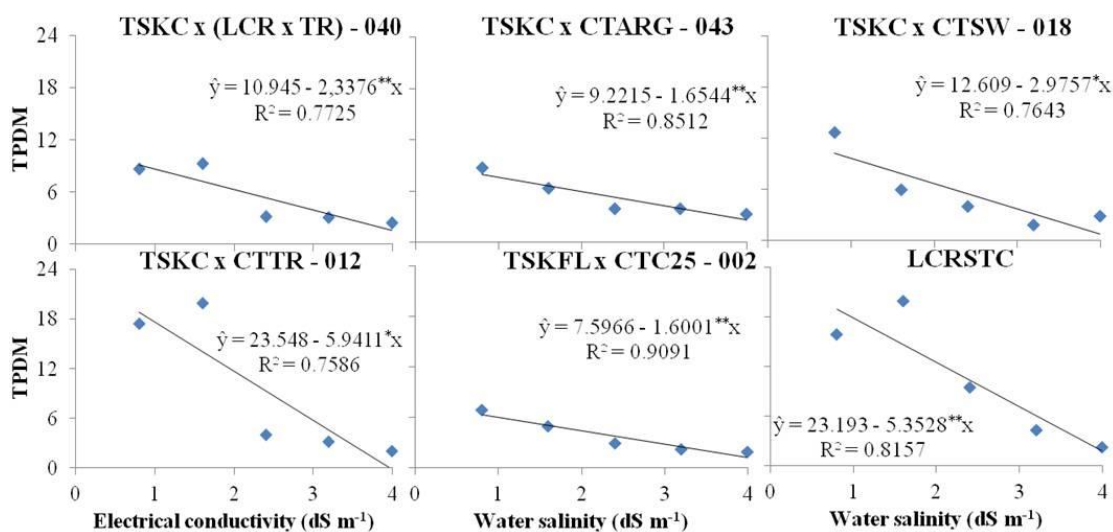
**Fig 3.** Calcium (Ca) concentration (cmol<sub>c</sub> L<sup>-1</sup>) of the soil solution measured 300 days after seeding and plotted according to the salinity of the applied water for each genotype grafted with ‘Tahiti’ acid lime [*Citrus latifolia* (Yu. Tanaka) Tanaka].



**Fig 4.** Magnesium (Mg) concentration ( $\text{cmol}_c \text{L}^{-1}$ ) of the soil solution measured 300 days after seeding and plotted according to the salinity of the applied water for each genotype grafted with ‘Tahiti’ acid lime [*Citrus latifolia* (Yu. Tanaka) Tanaka].



**Fig 5.** Sodium adsorption ratio (SAR) [ $(\text{cmol}_c \text{L}^{-1}) / (\text{cmol}_c \text{L}^{-1})^{0.5}$ ] for the soil solution measured 300 days after seeding and plotted according to the salinity of the water applied for each genotype grafted with ‘Tahiti’ acid lime [*Citrus latifolia* (Yu. Tanaka) Tanaka].



**Fig 6.** Total plant dry matter (TPDM) (g) measured 300 days after seeding and plotted according to the of the salinity of the applied water for each citrus plant genotype grafted with ‘Tahiti’ acid lime [*Citrus latifolia* (Yu. Tanaka) Tanaka].

risks of reduced soil permeability, as indicated in Ayers and Westcot (1985). However, there was no accumulation of water on the substrate surface or any drainage issues in any of the containers, which can be attributed to the organic matter content of the substrate (approximately 52%) according to the substrate analysis (Table 1). The analysis of the regression equations (Fig 5) shows an increasing linear trend in all of the studied genotypes, with the substrate of the TSKC × (LCR × TR) – 040 hybrid exhibiting the greatest increase in the SAR (50.9%) per unit increase in the water salinity calculated over the tested salinity range. This followed up by the TSKC × CTTR – 012 hybrid, with an increase of 44.4%. As discussed in the analysis of the Mg concentrations, a greater increase in the Na concentration relative to the accumulations of Ca and Mg was detected in the substrate, considering that an increase in the SAR occurred in the soil solution for the majority of the genotypes, as identified in TSKC × (LCR × TR) – 040, which showed the highest 'Na' concentration.

### ***Tolerance of scion-rootstocks to salinity***

For the TPDM, an increase in the irrigation water salinity resulted in a reduction of the dry matter production in all of the genotypes (Fig 6), in which a greater decrease was observed in TSKC × CTTR – 012, whereas a reduction of 31.6% in dry matter production per unit increase in the irrigation water salinity was recorded over the tested salinity range. For the TSKC × (LCR × TR) – 040 hybrid, there was a decrease of 25.7% per unit increase in the water salinity, coinciding with the higher accumulation of salts in the substrate. Thus, this genotype is promising for use as a rootstock in combination with 'Tahiti' acid lime, a scion variety considered to be sensitive to salinity (Brito et al., 2014a). Possibly, one of the salinity tolerance mechanisms of this hybrid is the exclusion of toxic ions during nutrient absorption, resulting in a higher accumulation of salts in the substrate. The combinations of 'Tahiti' acid lime grafted onto the TSKC × (LCR × TR) – 040, TSKC × CTSW – 018, TSKC × CTTR – 012 and TSKFL × CTC25 – 002 hybrids and onto LCRSTC are moderately tolerant to irrigation with water having a conductivity of 1.6 dS m<sup>-1</sup>, moderately sensitive to irrigation water with a conductivity of 2.4 dS m<sup>-1</sup> and sensitive to water with an EC greater than 3.2 dS m<sup>-1</sup>. This classification is based on tolerance criteria available in Fageria and Gheyi (1997), adapted for the plant matter production using the regression equations (Fig 6). In turn, the combination of 'Tahiti' acid lime grafted onto the TSKC × CTARG – 043 rootstock is tolerant to the use of irrigation water with an EC of 1.6 dS m<sup>-1</sup>, moderately tolerant to water with an EC of 2.4 dS m<sup>-1</sup>, moderately sensitive to water with an EC of 3.2 dS m<sup>-1</sup> and sensitive to waters with ECs greater than 4.0 dS m<sup>-1</sup>. The highest concentrations of Na and the highest values for the SAR and EC<sub>se</sub> were recorded in the substrates, in which the TSKC × CTARG – 043 and TSKC × (LCR × TR) – 040 genotypes were cultivated. Therefore, water with an EC higher than 2.4 dS m<sup>-1</sup> must not be used in the production of citrus seedlings because such water leads to growth reductions exceeding 40%, compromising plant quality.

## **Materials and Methods**

### ***Location and experimental design***

The experiment was developed in a protected environment (greenhouse with monitoring of temperature and relative humidity), at the Center for Agrifood Science and

Technology (CCTA) of the Federal University of Campina Grande (UFCG), located in Pombal, state of Paraíba (PB), Brazil, at a latitude of 6°47'20"S, a longitude of 37°48'01"W and an average elevation of 174 m. The studies assessed the salt balance and the growth of seedlings of 'Tahiti' acid lime grafted onto one of rootstocks describe in Table 1, being the genotypes were obtained from the Breeding Program for Citrus Fruits of Embrapa Cassava and Fruits (Embrapa Mandioca e Fruticultura - PMG Citros). The plants were irrigated with water at five salinity levels, namely, 0.8, 1.6, 2.4, 3.2 and 4.0 dS m<sup>-1</sup>, since 60 days after seeding (DAS) until 300 DAS.

Thus, it results in thirty treatments from a factorial arrangement (6 × 5), that was obtained by combining the six citrus genotypes used as rootstocks and the five salinity levels in a randomized block design with three replicates, with each plot consisting of four useful plants.

### ***Procedure to apply saline water***

The irrigation waters were prepared to provide a Na: Ca: Mg ratio of 7:2:1 from NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O and MgCl<sub>2</sub>.6H<sub>2</sub>O salts, respectively, due to reflect the predominant ions in water sources used for irrigation by small farms in Brazilian Northeast (Medeiros et al., 2003). The relationship between water electrical conductivity (EC<sub>w</sub>) and salt concentration, such as reported by Rhoades et al. (1992) (10 mmol \* L<sup>-1</sup> = 1 dS m<sup>-1</sup> EC<sub>w</sub>), is valid from 0.1 to 5.0 dS m<sup>-1</sup>, that fits in the levels tested, based on the existing water supply in place.

The experiment lasted 300 days, beginning in January and ending in October of 2012. The application of the salt treatments was initiated 60 days after seeding (DAS) in rootstock plants, that it was grafted at 180 DAS with 'Tahiti' lime acid, and continued to apply the saline water until experiment to be ended, within 300 days. Throughout the experimental phase (seeding, rootstock growth, grafting, and scion growth), plastic bags 12 cm in diameter and 20 cm in height were used to hold the substrate, resulting in an approximate substrate volume of 2 L.

### ***Procedures of plant growth***

During the initial phase, the seeding was conducted at the rate of four seeds per substrate bag, using seeds selected and treated with the fungicide thiuram disulfide (4 g kg<sup>-1</sup> of seeds). The bags were filled with the substrate containing a combination of vermiculite, pine bark and compost in a 1:1:1 ratio. The chemical characteristics of the substrate before of saline water application are listed in Table 1. After emergence, which stabilized at 30 DAS, only one seedling (a plant from one of the four seeds in container) was allowed to develop. The selection was performed by eliminating the non-uniform plants relative to the standard of each genotype to discard any individuals from sexual origin, seeking to maintain only those of asexual origin (nucellar) (Agrobyte 2006). Furthermore, the genotypes were classified by size, with the aim of standardizing the plants in the blocks to decrease possible differences caused by varying durations between seeding and emergence. The irrigation management for each genotype was performed based on the results from the weighing lysimeter for the lowest salinity level treatment studied, adding a leaching fraction (LF) of 20%. Thus, the plants from each genotype that were irrigated daily with water having an electrical conductivity (EC) of 0.8 dS m<sup>-1</sup> were weighed to obtain the actual weight that was used to calculate the consumption of the plants through the difference

with the weight that was obtained at the highest water retention level.

The necessary measures were taken to control weeds as well as to prevent and control pests and to meet the nutritional management standards recommended for the production of citrus seedlings (Mattos Junior et al., 2005; Agrobyte, 2006).

#### *Variables analyzed*

#### *Salt balance in substrate*

The electrical conductivity of the saturation extract ( $EC_{se}$ ); the levels of Na, calcium (Ca) and magnesium (Mg); and the sodium adsorption ratio (SAR) of the substrate, in which the plants were cultivated were determined using the methodology recommended by Embrapa (2009).

#### *Tolerance of scion-rootstocks to salinity*

At 300 days after seeding, the plants were collected, separated into shoots and roots and then dried in an air circulation oven at 65 °C, followed by weighing on an analytical balance to obtain the total plant dry matter (TPDM).

#### *Statistical analysis*

The data obtained were subjected to analysis of variance, and because there were significant differences among the genotypes and among the salinity levels, a regression analysis was conducted for each citrus genotype that was grafted with 'Tahiti' acid lime scion. These regressions examined the relationships between the applied water salinity, used as the independent variable, and each of the measured soil substrate parameters and the TPDM.

#### **Conclusions**

The concentration of ions in the substrate, in which seedlings are cultivated, varies with the genotype of rootstock grafted with 'Tahiti' acid lime scions. The TSKC × (LCR × TR) – 040 and TSKC × CTARG – 043 hybrids grafted with 'Tahiti' acid lime are the least sensitive of the six examined genotypes to water salinity, having tolerance mechanisms that prevent the excessive absorption of ions by the plants and affect the accumulation of Na in the substrate. The TSKC × CTTR – 012 hybrid is the most sensitive of the six examined genotypes to irrigation water salinity. Water with an EC greater than 2.4 dS m<sup>-1</sup> is not recommended for use with 'Tahiti' acid lime grafted on TSKC × (LCR × TR) – 040, TSKC × CTARG – 043, TSKC × CTSW – 018, TSKC × CTTR – 012 or TSKFL × CTC25 – 002 or on 'Santa Cruz' Rangpur lime because these salinity levels cause large reductions in plant matter.

#### **Conflict of Interest**

The authors declared no conflict of interest.

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