



# Screening of citrus scion-rootstock combinations for tolerance to water salinity during seedling formation

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**ABSTRACT.** Arid and semiarid regions are vulnerable to water deficits and salinity. Citrus plants are sensitive to saline stress and require the use of tolerant scion-rootstock combinations. Thus, this study aimed to evaluate and classify citrus scion-rootstock combinations with respect to their tolerance to salinity during seedling formation in a protected environment. An experiment was conducted in a randomized block design with a 5 x 12 x 2 factorial scheme corresponding to five levels of water salinity (0.8, 1.6, 2.4, 3.2, and 4.0 dS m<sup>-1</sup>) applied in 12 citrus rootstocks grafted with two scion varieties: 'Tahiti' acid lime and 'Star Ruby' grapefruit. The scion-rootstock combinations were evaluated for accumulated dry matter and survival index at 330 days after sowing the rootstocks. Salinity exerted different effects on the dry matter formation of scion-rootstock combinations. 'Star Ruby' was less sensitive to salinity, particularly when the rootstocks were the hybrids from 'Sunki of Florida' mandarin (TSKFL) with 'Troyer' citrange (CTTR) – 013 (TSKFL x CTTR – 013), common 'Sunki' mandarin (TSKC) with 'Argentina' citrange (CTARG) – 019 (TSKC x CTARG – 019), TSKC with 'Swingle' citrumelo (CTSW) – 031 (TSKC x CTSW – 031), and the trifoliolate hybrid (HTR) – 069, as well as the varieties Volkamer lemon and Santa Cruz Rangpur lime. When grafted with 'Tahiti' acid lime, the rootstocks displaying the less sensitive to salinity were TSKFL x CTTR – 013 and TSKC x CTARG – 019.

**Keywords:** *Citrus* spp.; *Poncirus* hybrids; survival index.

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## Introduction

Citrus are very important fruit crops worldwide, particularly in Brazil, because of the creation of jobs and income. According to FAO, the global production of sweet oranges [*Citrus sinensis* (L.) Osbeck] exceeds 73 million tonnes and Brazil is responsible for more than 23% of this production, which guarantees its position as a main producer and exporter, notably for concentrated orange juice (Food and Agriculture Organization of the United Nations (FAO, 2017). In 2018, Brazilian production was more than 16 million tonnes in an area larger than 684 thousand hectares: the Southeast and Northeast are the main producing regions, accounting for 81.5%, and 8.2% of production, respectively (Instituto Brasileiro de Geografia e Estatística [IBGE], 2019).

In Northeast Brazil, orange production is distributed in all states, with a mean yield of 13 ton ha<sup>-1</sup>, which is less than the national mean yield of 25 Mg ha<sup>-1</sup> (IBGE, 2019). This yield is inferior to the potential of the crop since the yield in some countries is more than 40 Mg ha<sup>-1</sup> (Food and Agriculture Organization of the United Nations [FAO], 2017). Nevertheless, the production of other citrus species, such as lemons and mandarins, which have a low yield, is approximately 25 Mg ha<sup>-1</sup> in Brazil and may be related to the use of low-yielding scion-rootstock combinations, in addition to the low level of use of technologies such as irrigation and fertigation (Simões et al., 2017; Teixeira, Reis, Leivas, Silva, & Struiving, 2017).

Furthermore, the use of irrigation in the Northeast region is limited by the quantity and quality of the water found in wells, which is normally brackish. This factor also compromises the production of citrus species, which are considered sensitive to soil and water salinity (Maas, 1993). The effects of salinity on citrus plants are related to osmotic disorders due to reduction in water availability and ionic effects, such as

the toxicity of specific ions, particularly chloride, and a nutritional imbalance (Habibi & Amiri, 2013; Syvertsen & Garcia-Sanchez, 2014; Brito et al., 2015).

However, the effect of salinity on citrus plants varies according to the genotype. For example, Fernandes et al. (2011) studied citrus genotypes subjected to salinity stress in the stage of rootstock formation and identified genotypes that were sensitive and tolerant to a water salinity of 4.0 dS m<sup>-1</sup> in this stage of nucellar seedling formation. On the other hand, Brito et al. (2014a) and Simpson et al. (2014) examined the effect of salinity on citrus rootstocks before and after grafting, and observed variability in their responses, highlighting the importance of considering the scion-rootstock combination in the selection of materials tolerant to salinity.

The generation of materials tolerant to these conditions is potentially associated with the use of parents with a history of tolerance. In this context, Hussain et al. (2015) highlight that the response of citrus plants to salt stress depends on the genetic origin and the potential genotypes derived from mandarins because they accumulate chloride and sodium ions in the leaves.

Sá et al. (2015) and Brito et al. (2016) also noted higher tolerance in citrus hybrids obtained by Embrapa Cassava & Fruits with 'Sunki' mandarin [*C. sunki* (Hayata) hort. ex Tanaka] as the female parent. During the rootstock formation stage, these authors verified that the hybrid between common 'Sunki' mandarin (TSKC) with 'Rangpur' lime (*C. x limonia* Osbeck) (LCR) and *Poncirus trifoliata* (L.) Raf. (TR), referred to as TSKC x (LCR x TR) - 040 by the authors, presented a particularly good salt tolerance, such as the hybrid between TSKC with 'Argentina' citrange (*C. x sinensis* x *P. trifoliata*) (CTARG) designated by TSKC x CTARG - 019. However, an evaluation of the tolerance of new hybrids produced in combination with different scions is important since the behavior may be different (Perez-Perez, Garcia-Sanchez, Robles, & Botía, 2015).

After considering these aspects, the optimization of citrus production, notably in Northeast Brazil and semiarid regions, will mainly depend on the generation of scion-rootstock combinations that are tolerant to salinity and effectively use the marginal quality water available in citrus orchards. Thus, this study aimed to classify citrus scion-rootstock combinations with respect to their tolerance to salinity during seedling formation in a protected environment.

## Material and methods

The experiment was conducted in a greenhouse of the Center of Technology and Natural Resources - CTRN of the Federal University of Campina Grande - UFCG, located in the municipality of Campina Grande, Paraíba State, Brazil, at the geographic coordinates of 7°15'18" S and 35°52'28" W, and an altitude of 550 m.

The experiment evaluated five levels of irrigation water salinity (EC<sub>w</sub>), 0.8, 1.6, 2.4, 3.2, and 4.0 dS m<sup>-1</sup>, based on the threshold salinity in the saturation extract described by Maas (1993) for 'Pera' sweet orange, which is equal to 1.4 dS m<sup>-1</sup>. These waters were prepared by dissolving NaCl, CaCl<sub>2</sub>, and MgCl<sub>2</sub> in tap water in equivalent proportion of 7:2:1, except for water with 0.8 dS m<sup>-1</sup>, which was prepared by dilution of tap water with rain water.

The tap water had an EC = 1.16 dS m<sup>-1</sup>, Ca<sup>++</sup> = 2.43 mmol<sub>c</sub> L<sup>-1</sup>, Mg<sup>++</sup> = 3.30 mmol<sub>c</sub> L<sup>-1</sup>, Na<sup>+</sup> = 6.73 mmol<sub>c</sub> L<sup>-1</sup>, K<sup>+</sup> = 0.20 mmol<sub>c</sub> L<sup>-1</sup>, Cl<sup>-</sup> = 8.78 mmol<sub>c</sub> L<sup>-1</sup>, HCO<sub>3</sub><sup>-</sup> = 2.26 mmol<sub>c</sub> L<sup>-1</sup>, CO<sub>3</sub><sup>-</sup> = 0.00 mmol<sub>c</sub> L<sup>-1</sup>, and SO<sub>4</sub><sup>-</sup> = 0.00 mmol<sub>c</sub> L<sup>-1</sup>.

The water with different salinity (EC<sub>w</sub>) levels was applied on the citrus plants from 60 days after sowing (DAS) the rootstocks to 330 DAS, a period that covered the stages of rootstock formation and grafting of the scion varieties. Notably, among the genotypes used as rootstocks, five were considered tolerant and six were considered moderately tolerant to salinity in an earlier study conducted by same group (Fernandes et al., 2011; Brito et al., 2014b), an experiment that focused the stage of rootstock (nucellar seedlings) formation, in which the 'Rangpur' lime (moderately sensitive) was the control (Table 1).

The seeds of the evaluated genotypes were provided by the Citrus Breeding Program of Embrapa Cassava & Fruits - CBP Embrapa. After rootstock formation, nucellar seedlings of these genotypes were grafted with two scion varieties at 180 DAS: Tahiti acid lime [*C. latifolia* (Yu. Tanaka) Tanaka] and 'Star Ruby' grapefruit (*C. paradisi* Macfad.). The buds of these varieties were also provided by the CBP Embrapa.

The experiment was conducted in randomized blocks with treatments arranged in a factorial scheme of 12 rootstocks x 5 water salinity levels x 2 scion varieties. The factors resulted in 120 treatments with three replicates, and nine plants were evaluated per plot.

**Table 1.** Screening of the tolerance of 12 citrus genotypes to salinity in the stage of rootstock (nucellar seedlings) formation.

Moderately Sensitive Genotype (MS)	Moderately Tolerant Genotypes (MT)
01. 'Santa Cruz Rangpur' lime	07. TSKFL x CTC13 - 025
Tolerant Genotypes (T)	08. 'Troyer' citrange
02. TSKC x CTSW - 064	09. LRF x (LCR x TR) - 005
03. TSKFL x CTC25 - 010	10. TSKC x (LCR x TR) - 029
04. HTR - 069	11. TSKC x CTARG - 019
05. TSKC x CTSW - 031	12. TSKFL x CTTR - 013
06. 'Volkamer' lemon	

'Santa Cruz Rangpur' lime (*Citrus limonia* Osbeck); TSKC: common 'Sunki' mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; CTSW: 'Swingle' citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.]; TSKFL: 'Sunki of Florida' mandarin; CTC25: 'C25' citrange [*C. xsinensis* (L.) Osbeck x *P. trifoliata*]; CTC13: 'C13' citrange; HTR - 069: trifoliolate hybrid of 'Pera' sweet orange (*C. xsinensis* L.) with 'Yuma' citrange; CTTR: 'Troyer' citrange, LRF: 'Florida Rough' lemon (*C. jambhiri* Lush.); TR: *P. trifoliata*; LCR: 'Rangpur' lime; LVK: 'Volkamer' lemon (*C. volkameriana* V. Ten. & Pasq.); CTARG: 'Argentina' citrange.

The seeds that had been previously selected and treated with fungicide (4 g of Thiram kg<sup>-1</sup> of seeds) were sown in tubes (three per tube) with a capacity of 288 mL in a commercial substrate containing a combination of 1:1:1 vermiculite, pine bark and humus. Only one seedling of nucellar origin was allowed to develop in each container (Kashyap, Banu, Shrivastava, & Ramchiary, 2018). The nucellar seedlings were identified based on the morphological characteristics, particularly the characteristics related to the leaf blade.

The nucellar seedlings produced in dibble tubes after germination and initial growth (five months) were transplanted to plastic bags with a height of 35 cm, top diameter of 22 cm and bottom diameter of 20 cm from 150 until 330 days after sowing. Grafting was performed after the transplantation and establishment of the rootstocks in the bags at approximately 180 days after sowing.

Irrigation was performed by applying same volume to all recipients, based on the mean evapotranspiration of the plants irrigated with water of the lowest EC<sub>w</sub> obtained by calculating the water balance after weighing of bags of the blocks and adding a leaching fraction of 20% (LF = 0.20) at a weekly frequency.

The prevention and control of pests and diseases, and the control of weeds, were performed according to the recommendations for the production of citrus rootstocks under nursery conditions (Arouca, Penna, Prado, & Rozane, 2011). Fertilization was performed weekly using urea, KCl, and MAP diluted in the irrigation water corresponding to the respective levels of EC<sub>w</sub>, according to the recommendations of Mattos Junior, Negri, Pio, & Pompeu Junior (2005). A commercial product containing micronutrients (B - 0,05%; Cu - 0.5%; Fe - 0.5%; Mn - 0.05%; Mo - 0.02%; and Zn - 0.5%) was also applied on the same days using 1 g dm<sup>-3</sup> of the product dissolved in irrigation water.

At 330 DAS, the index of survival of citrus plants under salt stress was determined using Equation 1:

$$\%Survival = \frac{n^s}{n^T_{pl}} \times 100 \quad (1)$$

where: %Survival is the survival index (% survival),  $n^s$  is the number of survivors after grafting and  $n^T_{pl}$  is the total number of grafted plants.

At 330 DAS, the surviving plants were collected and divided into roots, rootstock stem, stem on the grafting line, scion stem and branches and scion leaves. This material was dried to obtain the dry matter of roots (DMRoots), rootstock stem (DMRS), stem on the grafting line (DMGraft), scion stem and branches (DMScion) and scion leaves (DMLeaves), and then weighed with a precision of 0.01 g. The results were reported in 'g'. The dry matter of the aerial parts of the scion-rootstock combinations (DMAP) was obtained from the sum of DMRS, DMGraft, DMScion, and DMLeaves.

The obtained data were subjected to an analysis of variance using the F test, followed by the principal component analysis (PCA) to determine the main variables for genotype selection. The main variables were studied using a polynomial regression analysis (linear, quadratic or broken line models, if necessary) to determine the effect of the salinity of the irrigation water on each scion-rootstock combination. These variables were also utilized to determine the relative yield, which was used to generate the cluster of Euclidean distribution and group the genotypes according to their tolerance to salinity.

## Results

According to the analysis of variance (Table 2), a significant triple interaction affected the formation of dry matter of roots (DMRoots) and scion (DMScion). The interactions between rootstocks and salinity (Rtsk x Sal) and between rootstocks and scions (Rtsk x Scion) were significant for all variables, and the double interaction between salinity and scion (Sal x Scion) was significant for the dry matter of scion (DMScion) and dry matter of the aerial parts of the scion-rootstock combinations (DMAP).

Figure 1 shows the principal component analysis (PCA) of the variables contributing to dry matter formation, revealing a high correlation coefficient and greater representativeness of the results for the

growth of ‘Tahiti’ acid lime and ‘Star Ruby’ grapefruit grafted onto the rootstocks in the variables of dry matter of the aerial parts of the scion-rootstock combinations (DMAP) and dry matter of roots (DMRoots) (Figure 1A, B, C, and D).

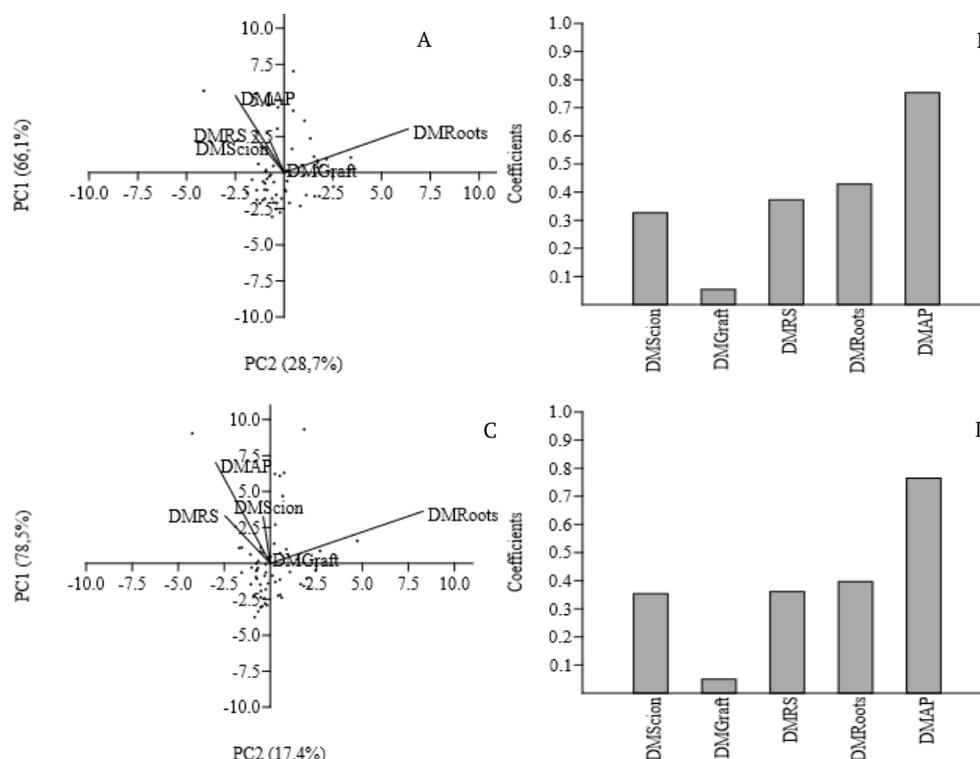
**Table 2.** Summary of the ANOVA for the variables dry matter of roots (DMRoots), dry matter of rootstock stem (DMRS), dry matter of stem on the grafting line (DMGraft), dry matter of scion (DMScion) and dry matter of scion-rootstock combinations (DMAP) in 12 citrus rootstocks (RS) and two scion varieties under five water salinity stress levels (Sal) at 330 days after sowing.

Variation source	DF	Mean square				
		DMRoots	DMRS	DMGraft	DMScion	DMAP
Salinity (Sal)	4	51.5765**	29.4657**	0.4529**	21.7944**	112.5791**
Rootstocks (Rtsk)	11	42.1520**	15.4788**	0.3547**	11.6992**	49.3261**
Scion	1	3.7089 <sup>ns</sup>	1.3177 <sup>ns</sup>	0.0138 <sup>ns</sup>	13.2755**	24.0985**
Sal x Rtsk	44	2.5635**	1.3092**	0.0288**	2.2024*	6.08736**
Sal x Scion	4	0.8872 <sup>ns</sup>	0.5614 <sup>ns</sup>	0.0229 <sup>ns</sup>	0.6292**	2.45120*
Rtsk x Scion	11	3.1535**	1.4476*	0.0210*	0.8138**	2.91343**
Rtsk x Scion x Sal	44	1.6555*	0.5013 <sup>ns</sup>	0.0091 <sup>ns</sup>	0.2279**	0.60608 <sup>ns</sup>
Block	2	3.1349 <sup>ns</sup>	6.8796**	0.0279 <sup>ns</sup>	0.0095 <sup>ns</sup>	7.57678**
CV (%)		20.33	21.92	22.72	21.37	18.67
Mean (g)		5.30	3.73	0.46	0.76	4.96

DF = degrees of freedom; CV = coefficient of variation; \*\*, \* and ns = significant at 1%, 5%, and non-significant according to the F-test, respectively.

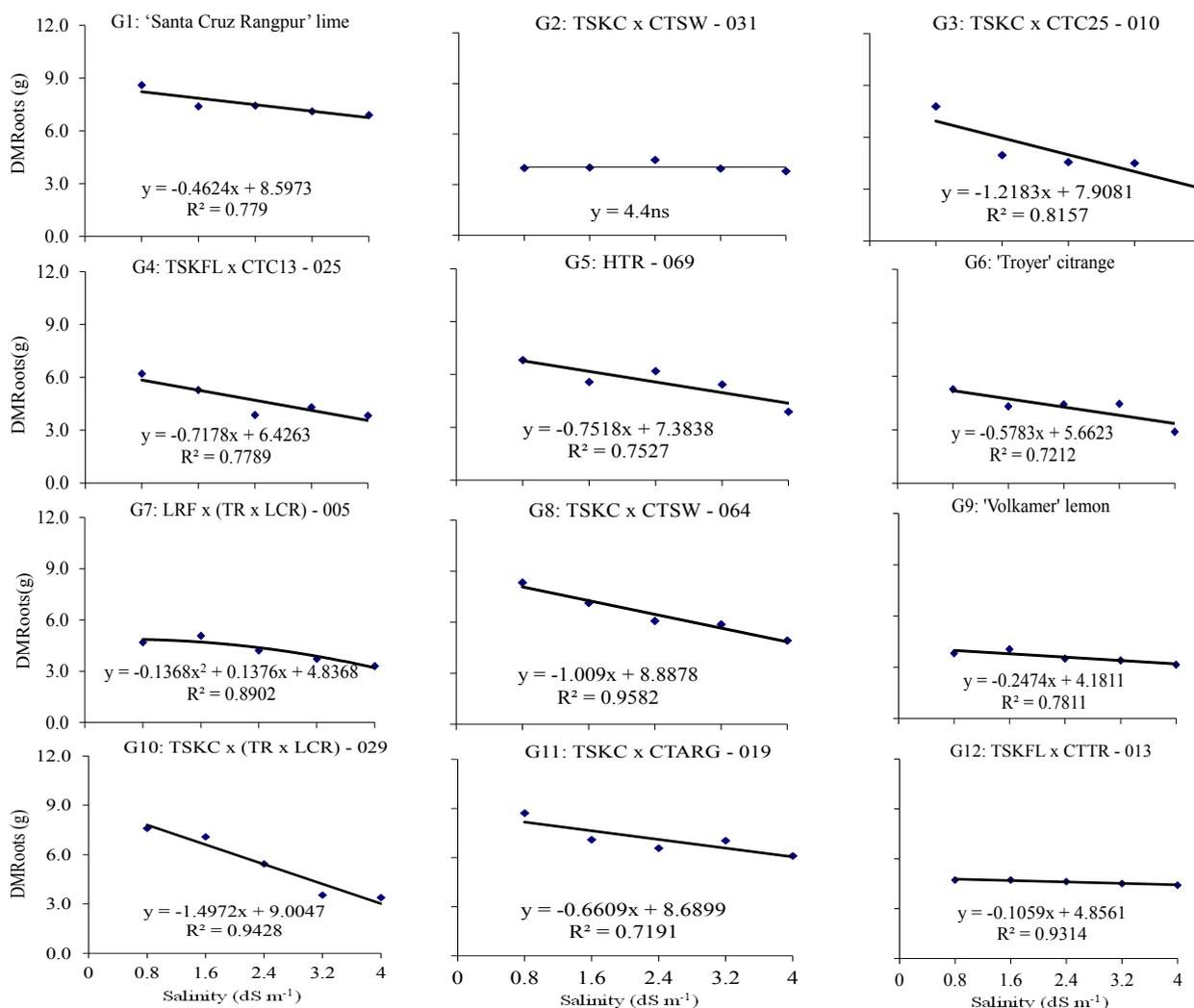
According to the PCA, the dry matter of scion-rootstock combinations was the main component and was responsible for distinguishing approximately 80% of the combinations between the genotypes grafted with both ‘Tahiti’ and ‘Star Ruby’. Meanwhile, the second component, the dry matter of roots, enabled the separation of approximately 40% of the genotypes used as rootstocks for both scions, and resulted in greater effect of salinity on scions than on roots.

Based on the effect of salinity on the formation of the dry matter of roots of the citrus rootstocks grafted with ‘Tahiti’ acid lime (Figure 2), greater decreases per unit increase in water salinity were observed in the combinations of this scion with the rootstocks TSKFL x CTC25 - 010, TSKFL x CTC13 - 005, TSKC x CTSW - 064, and TSKC x (LCR x TR) - 029, which showed respectively, reductions of 15.4, 11.2, 11.3, and 16.6% with per unit increase in ECw.



**Figure 1.** Principal component (PC) analyses (A and C) and correlation coefficients for PC1 (B and D) from variables dry matter of roots (DMRoots), dry matter of rootstock stem (DMRS), dry matter of the stem on the grafting line (DMGraft), dry matter of scion (DMScion), and dry matter of the aerial parts of the scion-rootstock combinations (DMAP) in 12 citrus rootstocks grafted with ‘Tahiti’ acid lime *Citrus latifolia* (Yu. Tanaka) Tanaka (A and B) and ‘Star Ruby’ grapefruit *C. paradisi* Macfad. (C and D) according to the irrigation water salinity at 330 days after sowing.

In the DMRoots of rootstocks grafted with ‘Tahiti’ acid lime (Figure 2), salinity exerted less of an effect on TSKC x CTSW - 031, TSKFL x CTTR - 013, ‘Santa Cruz Rangpur’ lime (LCRSTC), ‘Volkamer’ lemon (LVK), TSKC x CTARG – 019, and LRF x (LCR x TR) - 005, which showed reductions of less than 10%, namely, 0, 2.2, 5.4, 5.91, 7.6, and 9.8%, respectively, with per unit increase in the electrical conductivity of the irrigation water. HTR - 069 and ‘Troyer’ citrange exhibited an intermediate level of sensitivity among the rootstocks and a reduction of 10.2% in the root dry matter formation of these combinations as the saline levels increased.

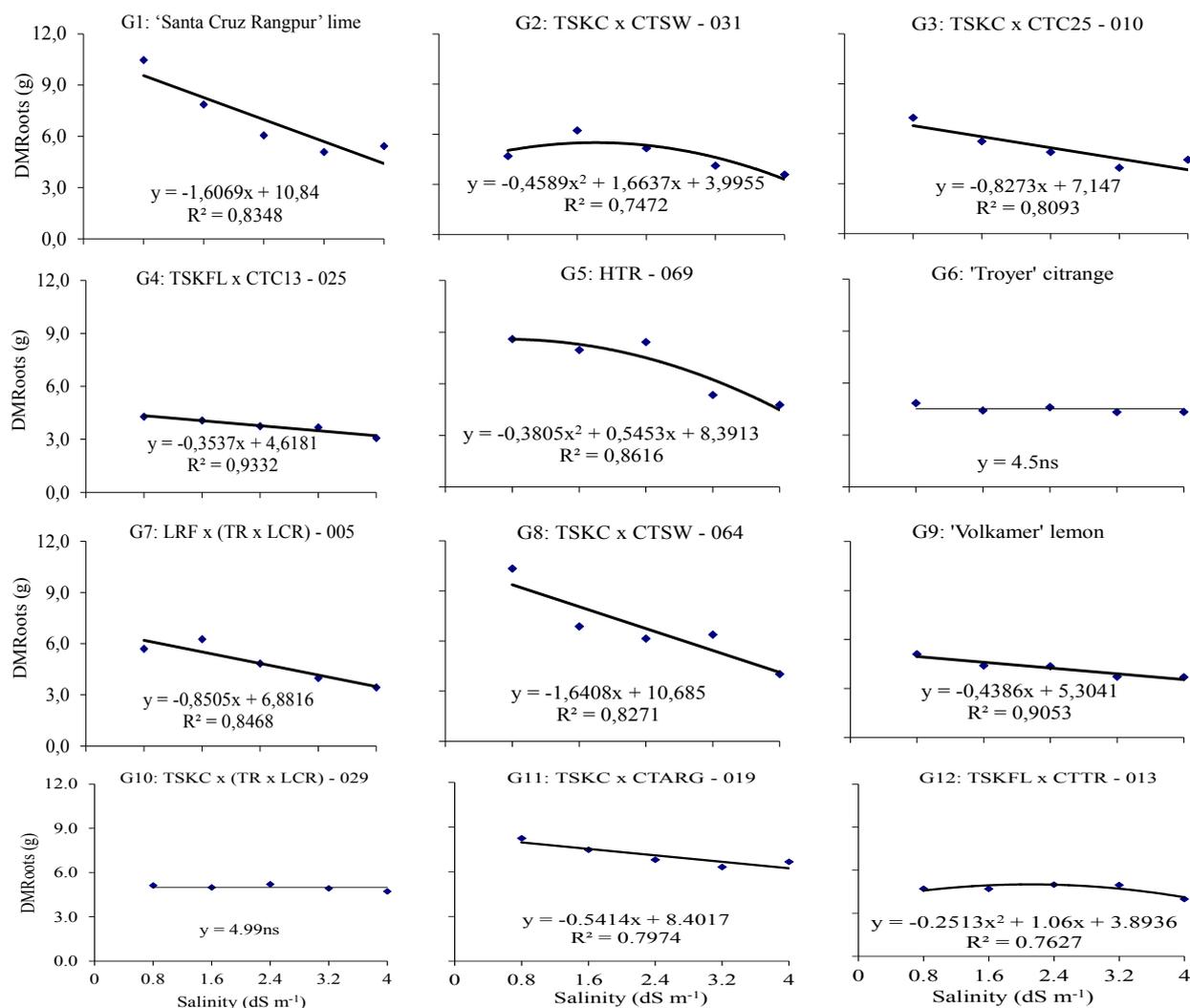


‘Santa Cruz Rangpur’ lime (*Citrus limonia* Osbeck); TSKC: common ‘Sunki’ mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; CTSW: ‘Swingle’ citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.]; TSKFL: ‘Sunki of Florida’ mandarin; CTC25: ‘C25’ citrange [*C. xsinensis* (L.) Osbeck x *P. trifoliata*]; CTC13: ‘C13’ citrange; HTR - 069: trifoliolate hybrid of ‘Pera’ sweet orange (*C. xsinensis* L.) with ‘Yuma’ citrange; CTTR: ‘Troyer’ citrange; LRF: ‘Florida Rough’ lemon (*C. jambhiri* Lush.); TR: *P. trifoliata*; LCR: ‘Rangpur’ lime; LVK: ‘Volkamer’ lemon (*C. volkameriana* V. Ten. & Pasq.); CTARG: ‘Argentina’ citrange.

**Figure 2.** Dry matter of roots (DMRoots) at 330 days after sowing (DAS) of 12 citrus rootstocks grafted with ‘Tahiti’ acid lime [*Citrus latifolia* (Yu. Tanaka) Tanaka] according to the irrigation water salinity (dS m<sup>-1</sup>).

Regarding the dry matter of roots in the combinations between the ‘Star Ruby’ grapefruit and the different rootstocks (Figure 3), the sensitivity to salinity was lower among the combinations because a greater number of genotypes showed a relative reduction of less than 10% as the salinity of the water increased.

Thus, the rootstocks TSKC x CTSW - 031, TSKFL x CTTR - 013, ‘Volkamer’ lemon, TSKC x CTARG – 019, and LRF x (LCR x TR) - 005 were considered less sensitive to salinity, as they showed reductions of 1.0, 9.5, 8.3, 6.4, and 6.7%, respectively, with per unit increase in water salinity. On the other hand, the rootstocks TSKC x (LCR x TR) – 029, TSKFL x CTC13 – 005, and ‘Troyer’ citrange, which were considered more sensitive when grafted with ‘Tahiti’ acid lime, were less sensitive when grafted with ‘Star Ruby’, and decreases of 0, 7.6, and 0% were observed, respectively.



'Santa Cruz Rangpur' lime (*Citrus limonia* Osbeck); TSKC: common 'Sunki' mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; CTSW: 'Swingle' citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.]; TSKFL: 'Sunki of Florida' mandarin; CTC25: 'C25' citrange [*C. xsinensis* (L.) Osbeck x *P. trifoliata*]; CTC13: 'C13' citrange; HTR - 069: trifoliate hybrid of 'Pera' sweet orange (*C. xsinensis* L.) with 'Yuma' citrange; CTTR: 'Troyer' citrange, LRF: 'Florida Rough' lemon (*C. jambhiri* Lush.); TR: *P. trifoliata*; LCR: 'Rangpur' lime; LVK: 'Volkamer' lemon (*C. volkameriana* V. Ten. & Pasq.); CTARG: 'Argentina' citrange.

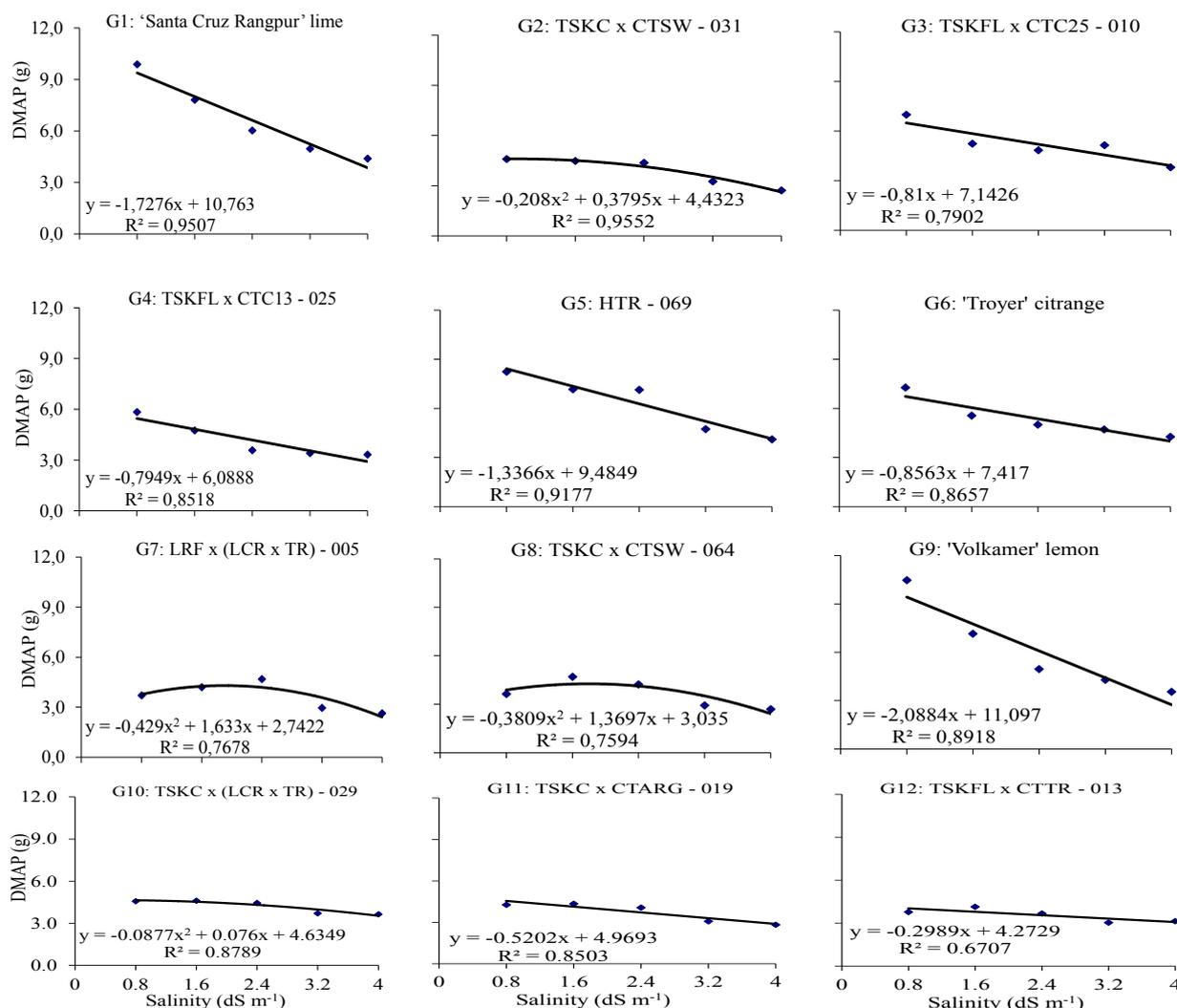
**Figure 3.** Dry matter of roots (DMRRoots) at 330 days after sowing (DAS) of 12 citrus rootstocks grafted with 'Star Ruby' grapefruit (*Citrus paradisi* Macfad.) according to the irrigation water salinity (dS m<sup>-1</sup>).

TSKFL x CTC25 - 010 and TSKC x CTSW - 064 were notable among the rootstocks that were most sensitive to salinity when grafted with 'Star Ruby' because they showed reductions of 11.6 and 15.3% in root dry matter per unit increase in the salinity of the irrigation water, respectively. These genotypes were also sensitive when grafted with 'Tahiti' acid lime.

In addition, the rootstock 'Santa Cruz Rangpur' lime displayed a reduction of 14.8% in DMRRoots per unit increase in EC<sub>w</sub>. HTR - 069 showed mean reduction of 14.5% per unit increase in water salinity compared to an EC<sub>w</sub> of 0.71 dS m<sup>-1</sup>, the level at which the highest formation of root dry matter was observed in this genotype grafted with 'Star Ruby' (Figure 3). Furthermore, these rootstocks were less sensitive when grafted with 'Tahiti' acid lime, indicating the importance of the scion-rootstock combination.

The effect of water salinity on scion dry matter formation was more obvious (Figures 4 and 5), regardless of the scion variety used. In the 'Tahiti' acid lime graft, lower sensitivity was observed when the rootstocks were TSKFL x CTTR - 013 and TSKC x (LCR x TR) - 029, with reductions of 6.99 and 6.72%, respectively, per unit increase in EC<sub>w</sub> (Figure 4).

Among the combinations considered sensitive according to DMRRoots, the TSKC x CTSW - 064 displayed a reduction in DMAP of 19.65% when grafted with 'Tahiti' acid lime, which allowed the classification of this rootstock as one of the most sensitive varieties.



'Santa Cruz Rangpur' lime (*Citrus limonia* Osbeck); TSKC: common 'Sunki' mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; CTSW: 'Swingle' citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.]; TSKFL: 'Sunki of Florida' mandarin; CTC25: 'C25' citrange [*C. xsinensis* (L.) Osbeck x *P. trifoliata*]; CTC13: 'C13' citrange; HTR - 069: trifoliolate hybrid of 'Pera' sweet orange (*C. xsinensis* L.) with 'Yuma' citrange; CTTR: 'Troyer' citrange, LRF: 'Florida Rough' lemon (*C. jambhiri* Lush.); TR: *P. trifoliata*; LCR: 'Rangpur' lime; LVK: 'Volkamer' lemon (*C. volkameriana* V. Ten. & Pasq.); CTARG: 'Argentina' citrange.

**Figure 4.** Dry matter of the aerial parts of the scion-rootstock combinations (DMAP) at 330 days after sowing (DAS) of 12 citrus rootstocks grafted with 'Tahiti' acid lime [*Citrus latifolia* (Yu. Tanaka) Tanaka] according to the irrigation water salinity (dS m<sup>-1</sup>).

Based on the effect of salinity on the scion-rootstock combinations with the 'Star Ruby' grapefruit scion, the rootstocks TSKFL x CTTR - 013 and TSKC x (LCR x TR) - 029 displayed intermediate reductions of 12.45 and 11.94%, respectively, with each unit increase in EC<sub>w</sub> (dS m<sup>-1</sup>) (Figure 5). For this scion, the least sensitive rootstocks were TSKC x CTARG - 019 and TSKC x CTSW - 031, which exhibited relative reductions of 9.42 and 7.59%, respectively, per unit increase in EC<sub>w</sub>.

The scion-rootstock dry matter (Figures 4 and 5) of the 'Volkamer' lemon (LVK) and 'Santa Cruz Rangpur' lime (LCRSTC), regardless of the grafted scion, were among the rootstocks that were most sensitive to salinity, with per unit reductions of 18.82 and 16.05%, respectively, when grafted with 'Tahiti' lime and 19.69 and 19.10%, respectively, when grafted with 'Star Ruby'.

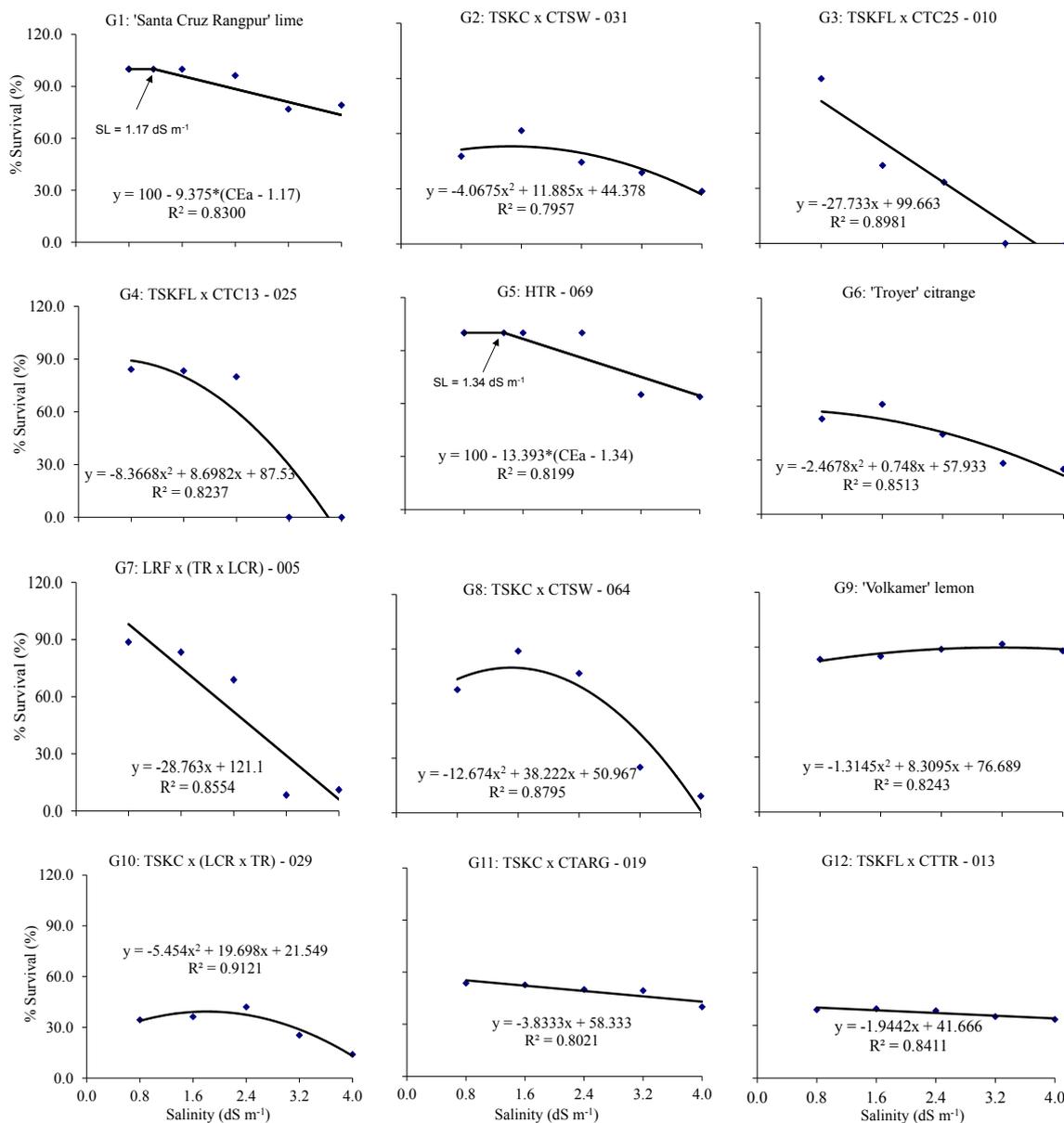
The other combinations showed reductions in DMT<sub>Total</sub> ranging from 11 to 15% per unit increase in water salinity (Figures 4 and 5). Therefore, rootstocks such as HTR - 069 and 'Troyer' citrange were noted for their intermediate behavior in root dry matter formation.

To summarize the results, the Hierarchical Cluster Diagram was obtained using the Euclidean distance method based on data for relative yield in dry matter of the plants to summarize the results and compare mean data obtained from the highest water salinity level (4.0 dS m<sup>-1</sup>) with the lowest level (0.8 dS m<sup>-1</sup>). Figure 6A shows the diagram for the rootstocks grafted with 'Tahiti' acid lime, distinguishing four similar groups. The first was formed by the rootstocks TSKFL x CTTR - 013 and TSKC x CTSW - 031, which were described as less sensitive to salinity.



Four groups were also observed in the Hierarchical Cluster Diagram analyzing the combinations with the ‘Star Ruby’ grapefruit (Figure 6B), but a reduced number of similar genotypes was clustered in the group with the lowest susceptibility to salinity, namely, the rootstocks TSKC x CTSW - 031, TSKFL x CTTR - 013, TSKC x CTARG - 019, ‘Troyer’ citrange, and TSKC x (LCR x TR) - 029. ‘Volkamer’ lemon and ‘Santa Cruz Rangpur’ lime were also similar when grafted with ‘Star Ruby’ and were highlighted as moderately susceptible to salinity. The third group of rootstocks, TSKFL x CTC25 - 010, HTR - 069, LRF x (LCR x TR) - 005, and TSKFL x CTC13 - 005, showed moderate sensitivity to salinity. The rootstock TSKC x CTSW - 064 was not similar to any other group in the cluster and was the material with highest sensitivity to salinity when grafted with ‘Star Ruby’.

In addition to sensitivity to salinity, one must consider resistance, which was measured by calculating the survival index (%Survival) of the plants (Figure 7).



‘Santa Cruz Rangpur’ lime (*Citrus limonia* Osbeck); TSKC: common ‘Sunki’ mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; CTSW: ‘Swingle’ citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.]; TSKFL: ‘Sunki of Florida’ mandarin; CTC25: ‘C25’ citrange [*C. xsinensis* (L.) Osbeck x *P. trifoliata*]; CTC13: ‘C13’ citrange; HTR - 069: trifoliolate hybrid of ‘Pera’ sweet orange (*C. xsinensis* L.) with ‘Yuma’ citrange; CTTR: ‘Troyer’ citrange; LRF: ‘Florida Rough’ lemon (*C. jambhiri* Lush.); TR: *P. trifoliata*; LCR: ‘Rangpur’ lime; LVK: ‘Volkamer’ lemon (*C. volkameriana* V. Ten. & Pasq.); CTARG: ‘Argentina’ citrange.

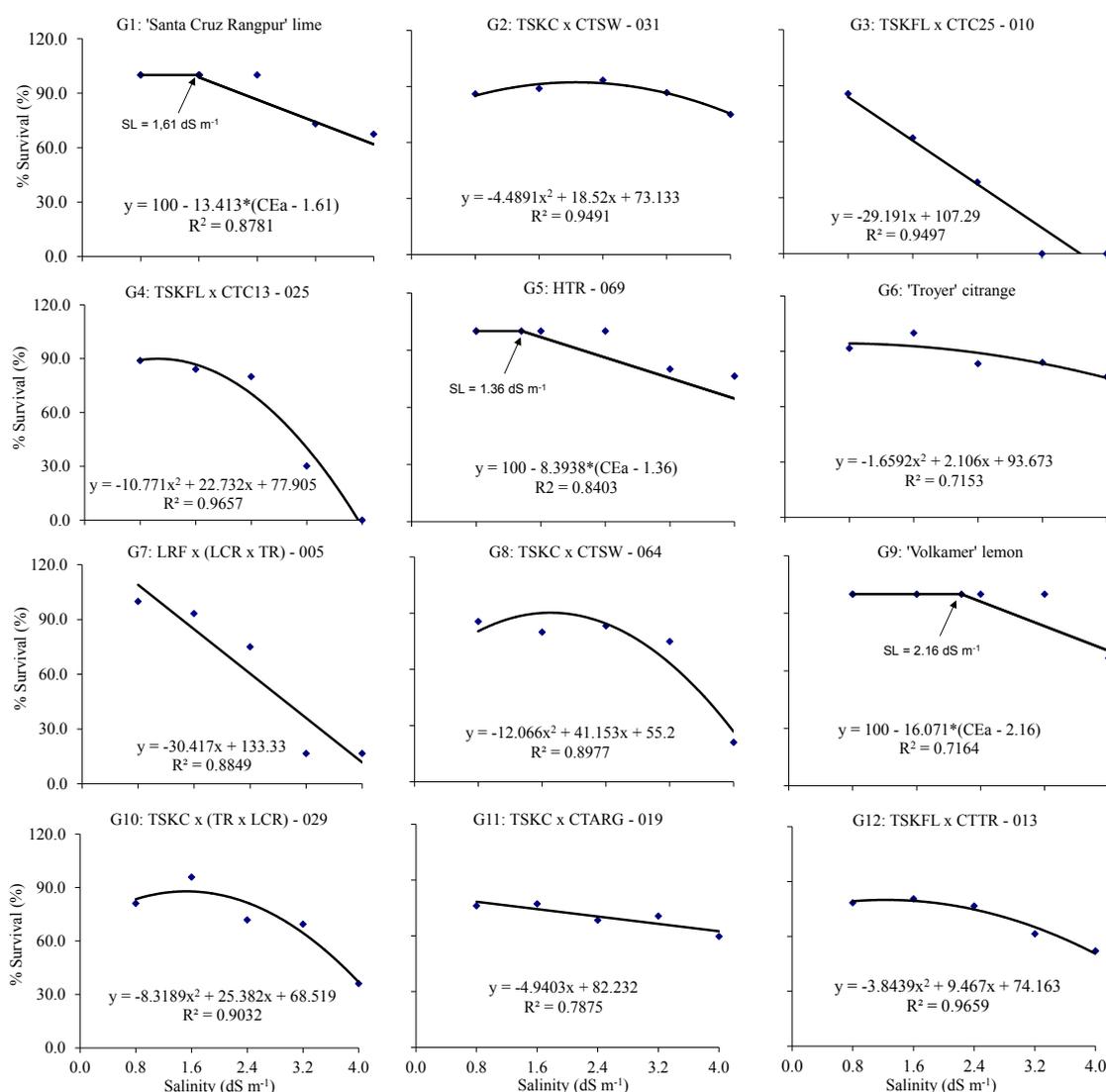
**Figure 7.** Percentage of survival (% Survival) at 330 days after sowing (DAS) of 12 citrus rootstocks grafted with ‘Tahiti’ acid lime [*Citrus latifolia* (Yu. Tanaka) Tanaka] according to the irrigation water salinity (dS m<sup>-1</sup>).

Thus, when the rootstocks were grafted with ‘Tahiti’ acid lime (Figure 7), higher percentages of survival were observed in the genotypes TSKFL x CTTR - 013, TSKC x CTARG - 019, ‘Santa Cruz Rangpur’ lime, HTR

- 069, and 'Volkamer' lemon, and the salinity stress caused reductions of 4.67, 6.57, 5.74, 13.10, and 1.20%, respectively, in the survival of the plants per unit increase in water salinity. Thus, the percentages of survival were 33.9, 43.0, 75.9, 59.0, and 88.9%, respectively, when plants were irrigated using water with a salinity of 4.0 dS m<sup>-1</sup>.

In the other combinations with 'Tahiti' acid lime, irrigation with water of 4.0 dS m<sup>-1</sup> salinity resulted in the death of all plants combined with the rootstocks TSKFL x CTC25 - 010 and TSKFL x CTC13 - 005. In the rootstocks TSKC x CTSW - 031, 'Troyer' citrange, and TSKC x (LCR x TR) - 029, the survival percentage was less than 30% (Figure 7), suggesting that the use of these genotypes is not viable under these saline conditions.

Regarding the survival of plants grafted with 'Star Ruby', a higher number of rootstocks maintained a survival greater than 50%, despite the irrigation with water of 4.0 dS m<sup>-1</sup> salinity, namely, HTR - 069, 'Volkamer' lemon, 'Santa Cruz Rangpur' lime, TSKC x CTARG - 019, 'Troyer' citrange, TSKFL x CTTR - 013, and TSKC x CTSW - 031, which showed survival percentages of 73.96, 70.47, 64.26, 62.47, 75.55, 50.52, and 75.38%, respectively (Figure 8).



'Santa Cruz Rangpur' lime (*Citrus limonia* Osbeck); TSKC: common 'Sunki' mandarin [*C. sunki* (Hayata) hort. ex Tanaka]; CTSW: 'Swingle' citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.]; TSKFL: 'Sunki of Florida' mandarin; CTC25: 'C25' citrange [*C. xsinensis* (L.) Osbeck x *P. trifoliata*]; CTC13: 'C13' citrange; HTR - 069: trifoliolate hybrid of 'Pera' sweet orange (*C. xsinensis* L.) with 'Yuma' citrange; CTTR: 'Troyer' citrange, LRF: 'Florida Rough' lemon (*C. jambhiri* Lush.); TR: *P. trifoliata*; LCR: 'Rangpur' lime; LVK: 'Volkamer' lemon (*C. volkameriana* V. Ten. & Pasq.); CTARG: 'Argentina' citrange.

**Figure 8.** Percentage of survival (% Survival) at 330 days after sowing (DAS) of 12 citrus rootstocks grafted with 'Star Ruby' grapefruit (*Citrus paradisi* Macfad.) according to the irrigation water salinity (dS m<sup>-1</sup>).

The scion-rootstock combinations with the 'Star Ruby' variety that did not survive under an ECw of 4.0 dS m<sup>-1</sup> included the rootstocks TSKFL x CTC25 - 010 and TSKFL x CTC13 - 005, denoting the low tolerance of these materials. In addition, the genotype TSKC x CTSW - 064 was the most sensitive to salinity since its survival percentage was only 26%.

## Discussion

The effects of the interactions observed in Table 2 confirm that salinity alters the growth of the citrus scion-rootstock combinations in different ways. In this case, two scions, 'Tahiti' acid lime and 'Star Ruby' grapefruit, were grafted onto 12 rootstocks and produced ten hybrids whose parents are related to 'Rangpur' lime, 'Sunki' mandarin, 'Volkamer' lemon, 'Rough' lemon, 'Star Ruby' grapefruit, and *P. trifoliata*, which generated genetic variability and thus different responses to salinity. Similarly, Simpson et al. (2014) observed different responses of combinations between 'Valencia' sweet orange grafted onto three rootstocks, bitter orange (*C. aurantium* L.), and two citranges, C22 and C146.

In these cases, the physiological response of citrus plants to salinity differs because some genotypes, particularly rootstocks, have a selective absorption capacity that limits growth, due to the lower cell turgor pressure. On the other hand, an effect of dilution might occur, in which plants are able to compartmentalize toxic ions, such as chloride and sodium, in leaves. These leaves will be lost through senescence, causing a dilution effect (Simpson et al., 2014).

Since the responses of the plants to salinity differed, a PCA was performed to identify the main components responsible for distinguishing the scion-rootstock combinations in an optimized manner. The main components were DMRoots and DMAP. The PCA has been used in studies to separate genotypes subjected to stress conditions and is sufficient for the analysis reported in the present study (Witzel, Wiedner, Surabhi, Börner, & Mock, 2009; Wei et al., 2013; Kohan et al., 2016).

The dry matter of the aerial parts of the scion-rootstock combination (DMAP) is the most representative variable because it is the sum of the dry matter of roots (DMRoots), rootstock stem (DMRS), the stem on the grafting line (DMGraft), scion stem and branches (DMScion), and scion leaves (DMLeaves). In addition, in citrus, the growth of the aerial parts of the scion-rootstock combination is more pronounced than the growth of the rootstock root; therefore, the effect of salinity will be more easily observed in the DMAP. On the other hand, although plants were cultivated in recipients that limited the growth of roots, their dry matter was the second component that allowed greater distinction of the combinations, suggesting that the mechanisms of tolerance to salinity may differ between different rootstocks. Thus, some plants may limit root growth to decrease the absorption of salts or prioritize the growth of their aerial parts to guarantee an increase in the number and size of vacuoles and dilute the salts (Simpson et al., 2014; Syvertsen & Garcia-Sanchez, 2014).

According to the dry matter of the roots (DMRoots) and the dry matter of the aerial parts of the scion-rootstock combination (DMAP), salinity exerted different effects on the scion-rootstock combinations studied, with the rootstocks grafted with 'Tahiti' acid lime displaying a higher sensitivity. These results may be interesting for citrus breeding programs, which can use the 'Tahiti' acid lime as a special scion to identify the tolerance of rootstocks in production systems in field. In this case, rootstocks with good tolerance to salinity when grafted with 'Tahiti' acid lime might exhibit efficient production in combination with other scion varieties since the 'Tahiti' variety is very sensitive to salinity.

The least sensitive rootstocks to salinity in both scions, based on the dry matter of root, included TSKC x CTSW - 031 and TSKFL x CTTR - 013, as well as TSKC x CTARG - 019, which showed the highest mean values for DMRoots and a low DMAP. The restriction of the dry matter formation of the aerial parts of the scion-rootstock combinations using these rootstocks may be attributed to the higher efficiency in the absorption of nutrients to meet the requirements for the growth of the scions.

These rootstocks share the 'Sunki' mandarin as the female parent (common 'Sunki' mandarin - TSKC and 'Florida Sunki' mandarin - TSKFL) and hybrids of *Poncirus* as male parents: 'Swingle' citrumelo (CTSW), 'Troyer' (CTTR), and 'Argentina' (CTARG) citranges. The literature has reported a higher tolerance of citranges and 'Sunki' mandarin rootstocks to salinity than, for example, bitter orange, which has been reported to display a reduced capacity to transport sodium and chloride ions to the scions (Maas, 1993; Hussain, Luro, Costantino, Ollitrault, & Morillon, 2012; Wei et al., 2013).

The most sensitive genotypes, regardless of the grafted scion, were TSKFL x CTC25 - 010 and TSKC x CTSW - 064, which also originated from 'Sunki' mandarin and 'Swingle' citrumelo. However, genetic variability may exist in the cross since many species exhibit a low degree of genetic diversification due to the accumulation of somatic mutations (Hussain et al., 2015). Nevertheless, these genotypes may have acquired the potential to respond to other types of biotic and abiotic stresses.

Although the studied materials displayed different levels of sensitivity to salinity, all rootstocks from the crosses between 'Sunki' mandarin and 'Swingle' citrumelo showed better behavior than 'Santa Cruz Rangpur' lime and 'Volkamer' lemon, according to the Hierarchical Cluster Dendrogram obtained using the Euclidean distance method. This diagram displays the potential of these rootstocks, particularly TSKC x CTSW - 031 and TSKFL x CTTR - 013, but without disregarding genotypes such as TSKC x CTARG - 019, 'Troyer' citrange and TSKC x (LCR x TR) - 029, HTR - 069, and LRF x (LCR x TR) - 005, which must be studied in other stages and in combinations with other scion varieties.

In addition to tolerance, which is considered an adaptation of the plant to stress, another important aspect is the resistance of the material to salinity. Resistance depends on the survival capacity, which is potentially modulated by the concentration or duration of the stress. In the present study, plants were subjected to salt stress from 60 to 330 DAS, which caused the death of many plants, particularly after grafting. All plants of the genotypes TSKFL x CTC25 - 010 and TSKFL x CTC13 - 005 died after irrigated with water of 4.0 dS m<sup>-1</sup> salinity.

The highest plant (scion-rootstock combinations) survival percentages were observed in 'Volkamer' lemon, 'Santa Cruz Rangpur' lime and in the trifoliolate hybrid HTR - 069 for both scions. In the combination with the most tolerant scion, 'Star Ruby', the rootstocks TSKC x CTSW - 031, TSKFL x CTTR - 013, and TSKC x CTARG - 019 were tolerant to irrigation with saline water; notably, the survival percentage was maintained.

Importantly, the low survival percentage observed in the rootstocks TSKFL x CTTR - 013 and TSKC x CTARG - 019 grafted with 'Tahiti' acid lime at the end of the experiment was due to the limited number of plants with successful grafting, which depended on sap flow and turgor. In this case, although these materials display a potential tolerance to salinity, an important strategy would be to refrain from using water with a higher electrical conductivity in the period before grafting and during graft establishment, which will improve the establishment of the plants. This strategy aims to establish a period of adaptation since the effect of salinity on citrus depends on multiple stresses (Syvertsen & Garcia-Sanchez, 2014).

Figure 9 shows the effect of salinity on some combinations. Scions grafted on 'Santa Cruz Rangpur' lime (Figure 9A) and HTR - 069 (Figure 9B), both under an EC<sub>w</sub> of 0.8 and 4.0 dS m<sup>-1</sup>, showed reductions in growth with the increase in EC<sub>w</sub>, but survived the stress. However, for CTC13 - 025, which was under an EC<sub>w</sub> of 0.8 and 4.0 dS m<sup>-1</sup>, plants did not withstand irrigation with water of 4.0 dS m<sup>-1</sup> (Figure 9C).



**Figure 9.** Comparison between different scion-rootstock combinations under S1 (EC<sub>w</sub>=0.8 dS m<sup>-1</sup>) and S5 (EC<sub>w</sub>=4.0 dS m<sup>-1</sup>) at 330 days after sowing: (A) 'Santa Cruz Rangpur' lime (*Citrus limonia* Osbeck) grafted with 'Tahiti' acid lime [*C. latifolia* (Yu Tanaka) Tanaka], (B) the trifoliolate hybrid HTR - 069 grafted with 'Star Ruby' grapefruit (*C. paradisi* Macfad.) and (C) TSKFL ['Sunki of Florida' mandarin [*C. sunki* (Hayata) hort. ex Tanaka]] x CTC13 [C13 citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.]] - 025 grafted with 'Star Ruby' grapefruit. HTR - 069 is a hybrid of 'Pera' sweet orange *C. sinensis* with 'Yuma' citrange.

## Conclusion

Salinity exerts different effects on dry matter formation, depending on the citrus scion-rootstock combination.

The dry matter of the aerial parts of a citrus scion-rootstock combination is the most representative variable reflecting the effect of salinity.

The rootstocks TSKFL x CTTR - 013, TSKC x CTARG - 019, TSKC x CTSW - 031, 'Volkamer' lemon, HTR - 069, and 'Santa Cruz Rangpur' lime are less susceptible to salinity when grafted in combination with 'Star Ruby' grapefruit.

The rootstocks TSKFL x CTTR - 013 and TSKC x CTARG - 019 are the least sensitive to salinity when grafted with 'Tahiti' acid lime.

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