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PROPAGATION OF YELLOW PASSION FRUIT SEEDLINGS BY CUTTING, GRAFTING AND SEEDS UNDER SALT STRESS

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Abstract
Salinity is one of the most limiting abiotic stresses in the global agricultural sector. The objective of this study was to evaluate the effect of irrigation water salinity on the initial development of *P. edulis* Sims. seedlings propagated by seeds, grafting and cuttings. Treatments were distributed in a completely randomized design, in a 5 x 3 factorial arrangement, corresponding to 5 levels of irrigation water electrical conductivity (0.3 - control, 1.8, 3.3, 4.8, and 6.3 dS m^{-1}) and 3 propagation methods (seeds, cuttings and grafting), with four replicates and one plant per plot. Fifty days after the imposition of treatments with saline water, plant height, number of leaves, stem diameter, total chlorophyll, stomatal conductance, relative leaf water content, total water consumption, dry matter of root, shoot and total, root length and root volume were evaluated. The increase in water salinity affected with greater intensity the growth and development variables (height, total dry matter and root volume of the plant) and the physiological characteristics (stomatal conductance) of the species *Passiflora edulis*, regardless of the method of propagation. Seedlings propagated by grafting showed better development compared to the other propagation methods (seeds and cuttings). The interaction between the propagation methods and water salinity affected seedlings propagated by seeds and cuttings with greater intensity.

Keywords: *Passilora edulis* sims. Salinity. Vegetative propagation.

1. Introduction

Yellow passion fruit (*Passiflora edulis* Sims) is a tropical fruit crop with significant production in Brazil, equal to 602,651 thousand tons in 2018 (IBGE, 2018). This crop can be propagated sexually by seeds (non-grafted) and asexually by cuttings and grafting. Vegetative propagation is mainly performed by the cutting method, which is more feasible to establish clonal plantations, because it makes possible to multiply selected genotypes in short time and at low cost, especially considering that this crop's lifetime has been reduced in the last years due to biotic and abiotic problems (Souza and Gentil, 2013; Morgado et al. 2015).

Nonetheless, the Brazilian Northeast region is responsible for the highest passion fruit production in the country *(*Cavichioli et al. 2020)*,* where its management depends on irrigation, frequently performed using low-quality water (high contents of salts), which may alter physiology and compromise plant growth and development, consequently resulting in low-quality production and yield (Cavalcante et al. 2011; Freire et al. 2014).

Salt stress is the most limiting factor for crop growth, development and yield in arid and semi-arid regions (Gadelha, 2020). The use of high-salinity water has been studied as one alternative for the agricultural production in general, especially in regions where water sources with high concentrations of salts particularly that of sodium, commonly occur (Jiang et al. 2012). However, inadequate management and low-quality water, associated with high crop evapotranspiration, contribute to increase soil salinity, directly affecting yield (Lima et al. 2014). Although several studies have contributed with important information related to the negative effects of salinity on seedlings and adult plants of yellow passion fruit, such as reduction in biometric parameters, physiological and anatomical changes (Freire et al. 2010; Freire et al. 2014; Oliveira et al. 2015; Bezerra et al. 2016; Moura et al. 2016; Moura et al. 2017; Nascimento et al. 2017, Moura et al. 2019, Andrade et al. 2019, Lima et al. 2020a, Lima et al., 2020b), most of these studies have evaluated only morphological and physiological responses of seedlings propagated by seeds (non-grafted) and irrigated using waters of different salinity levels. Therefore, further research is needed to understand the behavior of passion fruit plants asexually propagated (grafting and cutting) subjected to salinity and the alterations caused by this abiotic stress particularly in the initial stage of seedling formation.

Thus, this study aimed to evaluate the effect of irrigation water salinity on the initial development of *Passiflora. edulis* Sims. seedlings propagated by cuttings, grafting and seeds.

2. Material and Methods

The experiment was carried out in a greenhouse with mean temperature adjusted to 28±2 °C and relative humidity of 60±5%, at Embrapa Cassava & Fruits, located in the municipality of Cruz das Almas-BA, Brazil (12°48’19” S; 39°06’23” W; 225 m), from April to June 2016. The climate, according to the classification of Thornthwaite (1984), is C1dA’a’, dry and sub-humid (Embrapa 2016), with annual means of rainfall, temperature and relative humidity of 1,224 mm, 24.5 °C and 80%, respectively.

The experimental design was completely randomized, in 5 x 3 factorial scheme, corresponding to 5 levels of irrigation water salinity (ECw): 0.3 - control; 1.8; 3.3; 4.8; and 6.3 dS m^{-1}, and 3 propagation methods (seeds, grafting and cuttings), with four replicates. These levels of water salinity were based on the study of Moura et al. (2016) on the tolerance of passion fruit seedlings to water salinity considering *Passiflora edulis* as tolerant crop up to irrigation water salinity of 2.14 dS m^{-1} compared to control treatment irrigated with water of 0.3 dS m^{-1}. Waters of different salinity levels were prepared by dissolving NaCl in public-supply water (ECw=0.3 dS m^{-1}), and the quantity was calculated using the following equation:

$$\text{mg L}^{-1} = 640 \text{ x } (\text{Desired EC} - \text{Current EC})$$

The substrate (mixture of soil and decomposed bovine manure, at 10:1 proportion, on mass basis) was placed in containers made with of 2-dm^3 PET bottles. The bottom part of bottle was removed, and it was placed upside down on a metal frame, with the hole facing the ground and working as a drain. The containers were filled up to 3 cm with gravel and with 2 kg of substrate above it. The soil used was collected in the 0-100 cm depth, and is classified as YELLOW LATOSOL with clay loam texture (Embrapa 2006). The substrate showed following chemical attributes: pH = 7.0; P = 139.00 mg dm^{-3}; exchangeable K^+, Ca^{2+}, Mg^{2+} and Na^+ = 3.08, 1.65, 0.87 and 0.87 $cmol_c$ dm^{-3} respectively, and organic matter = 7.00 g kg^{-1}, and the analysis were performed according to methodologies recommended by Embrapa (1997). The species *Passiflora edulis* Sims. (‘BRS Gigante Amarelo’) was used in the three propagation methods. Initially, cuttings were collected from adult plants of *P. edulis* Sims. (15-cm long, with two apical buds) and placed to root on polyethylene trays containing the commercial substrate Vivato®, remaining for 65 days until transplantation.

The parent plants that gave rise to the cuttings of *Passiflora edulis* were grown in a protected environment and in a pot (20 L) containing a substrate containing soil + bovine manure in the proportion of 3:1 (v/v). For seedlings propagated by grafting and seeds (non-grafted), *Passiflora edulis* Sims. plants were sown twice; the first one to produce the rootstocks and, 30 days after, the second one to produce seedlings to be used as scion for grafted seedlings, and non-grafted seedlings. Grafting was performed using the hypocotyledonary top cleft method. Twenty days after grafting, seedlings with similar height and vigor from the three propagation methods were selected for transplanting to the PET bottle containers. From sowing to up to 10 days after transplanting (DAT), seedlings were irrigated with public-supply water. After this period, waters with the saline concentrations corresponding to the respective treatments were used in the irrigations. Irrigations were carried out on alternate days and the volume of water applied was calculated using the following equation:

$$V = \frac{(VA - VD)}{0.9}$$

where V is the volume of water to be applied in irrigation; VA is the water volume applied in the previous irrigation; and VD is the water volume drained in the previous irrigation. The value "0.9" corresponds to the factor that fixes the leaching fraction at 10%, to avoid excessive accumulation of salts in the substrate. Drainage was monitored in each irrigation, by attaching a collector to the bottom of each container. Total water consumption (TWC) during the experiment was estimated by subtracting volume of water drained from the total volume of water applied.

At 50 days after the beginning of irrigations with water of different salinities, passion fruit seedlings were evaluated for growth and development based on plant height (PH), measured as the distance from the base to the apical meristem; number of leaves (NL); stem diameter (SD), measured 3 cm above the grafting point, and at 10 cm from the soil for seedlings grown from cuttings and seeds, using a digital caliper; total chlorophyll (TC), measured with a digital chlorophyll meter; stomatal conductance (gs), determined using a digital leaf porometer (SC-1 Decagon Devices); dry matter of root (RDM), shoot (SDM) and total (TDM), were determined after drying the harvested material in forced-air oven at temperature of 65 ºC; RDM/SDM ratio and root length (RL) were estimated and root volume (RV) was determined by water displacement in a graduated cylinder.

The obtained data were subjected to analysis of variance by F test, comparing the means by Tukey test ($p \leq 0.05$). For the factor irrigation water salinity, whenever the effect was found significant, polynomial regression analysis was performed using the "Agricolae" package implemented in the R program.

3. Results

Irrigations with different water salinity levels influenced plant height ($p \leq 0.01$), stomatal conductance ($p \leq 0.05$) and total water consumption ($p \leq 0.01$) (Table 1). There was significant effect ($p \leq 0.01$) of propagation methods on the variables plant height, stem diameter, total chlorophyll, total water consumption and stomatal conductance ($p \leq 0.05$). There was interaction between salinity levels and propagation methods only for number of leaves ($p \leq 0.01$) and total water consumption ($p \leq 0.01$).

Table 1. Summary of the analysis of variance for plant height (PH), number of leaves (NL), stem diameter (SD), stomatal conductance (g*s*), total chlorophyll (TC) and total water consumption (TWC) of seedlings of *Passiflora edulis* Sims. propagated by seeds, cuttings and grafting, subjected to different water salinity levels, at 50 days after transplanting (DAT).

	Mean squares					
SV	PH	NL	SD	g*s*	TC	TWC
Salinity (S)	3562.07^{**}	12.27^{ns}	0.76^{ns}	383.31^{*}	84.28^{ns}	1.61^{**}
Propagation (P)	32965.06^{**}	2.95^{ns}	14.87^{**}	408.30^{*}	414.3^{**}	7.08^{**}
Interaction S x P	1580.04^{ns}	21.84^{**}	0.16^{ns}	116.75^{ns}	89.77^{ns}	0.12^{**}
CV (%)	14.9	8.93	2.41	18.46	11.61	7.75
Propagation	cm		mm	mmol m^2 s^{-1}	µmol g^{-1}	L $planta^{-1}$
Cutting	76.20^{b}	12.60^{a}	3.82^{c}	33.40^{a}	22.17^{b}	2.32^{b}
Seed	76.73^{b}	13.33^{a}	4.94^{b}	23.36^{b}	25.59^{b}	2.05^{c}
Grafting	157.66^{a}	13.40^{a}	5.80^{a}	25.91^{ab}	32.49^{a}	3.19^{a}

^{ns,} * and ** = not significant, significant at 0.05 and 0.01 probability level, respectively.

For the propagation methods, the height of plants produced by grafting (157,66 cm) was approximately two times that of plants propagated by the other methods (cutting 76,20 cm and seed 76.73cm) (Table 1). Irrigation water salinity negatively affected passion fruit leaf production with higher intensity in plants propagated by seeds (reduction of 2.3% per unit increase in water salinity) and did not influence plants propagated by grafting and cuttings (Table 1 and Figure 1B).

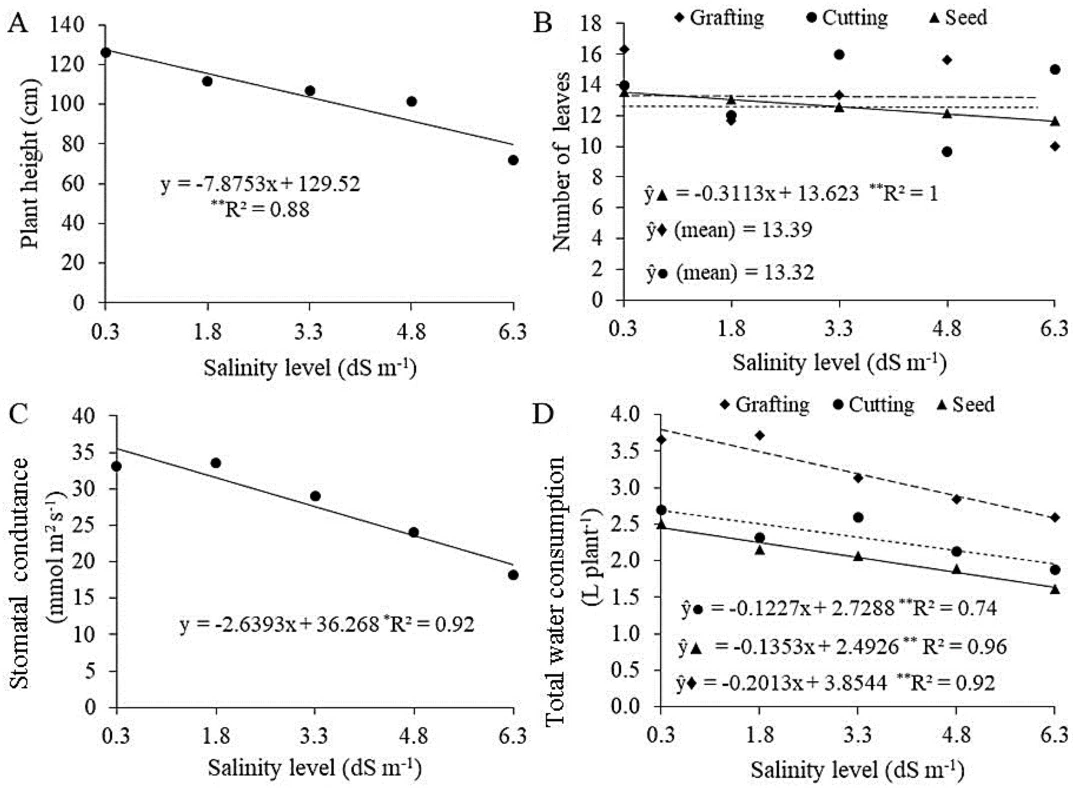

Figure 1. Influence of water salinity on biometric and physiologic characteristics of yellow passion fruit: A - plant height, C - stomatal conductance and B - interaction between water salinity and propagation method on number of leaves, and on D - total water consumption.

Passiflora. edulis height decreased by 6.08% per unit increase in irrigation water electrical conductivity (ECw) (Figure 1 A). Water salinity also caused 7.27% reduction in stomatal conductance (gs) with per unit increase in ECw (Figure 1C) corresponding to a 44.64% reduction at the level of 6.3 dS m^{-1}, compared to ECw=0.3 dS m^{-1}. There were linear reductions of 4.49, 5.42 and 5.22% in total water consumption (TWC) per unit increase in ECw, for the propagation methods grafting, seeds, and cuttings, respectively (Figure 1D).

The analysis of variance presented in Table 2 revealed that waters of different salinity levels influenced root dry matter and root volume ($p \le 0.05$) and shoot dry matter and total dry matter ($p \le 0.01$). Regarding the propagation methods, all variables evaluated showed significant response ($p \le 0.01$) except root volume, which was significant at $p \le 0.05$ by the F test. On the other hand, the interaction between salinity levels and propagation methods had significant effect ($p \le 0.05$) only on dry matter of root, shoot and RDM/SDM ratio.

The salinity affected the root dry matter with a negative linear effect (Figure 2A) on seedlings propagated by cuttings and seeds, with drastic reductions of 10.33 and 8.09% per unit increase in irrigation water salinity, respectively. However, there was no influence on plants propagated by grafting, and mean root dry matter was equal to 4.93 g, regardless of the ECw used in irrigation. Only seedlings propagated by cuttings and seeds were affected by salinity in shoot dry matter (Figure 2B), which decreased by 5.29 and 5.85% per unit increase in ECw, respectively.

For the propagation by grafting, water salinity had no effect on shoot dry matter, which showed mean value of 14.53 g (Figure 2B). Likewise, total dry matter decreased with the increment in the electrical conductivity of water (Figure 2C), on average by 5.90% per unit increase in ECw. RDM/SDM ratio in seedlings propagated by grafting and cuttings was not affected ($p \ge 0.05$) by irrigation water salinity, indicating that both plant parts were similarly influenced at different levels of water salinity (Figure 2D). Root volume (RV) linearly decreased on average by 7.12% per unit increase in ECw (Figure 2E) and propagation by grafting promoted higher volume compared to other methods due to difference in the age of plants used as rootstock.

Table 2. Summary of the analysis of variance for dry matter of root (RDM), shoot (SDM), and total (TDM), root dry matter/shoot dry matter ratio (RDM/SDM), root length (RL), and root volume (RV) of *Passiflora edulis* Sims. seedlings propagated by seeds, cuttings, and grafting, subjected to different salinity levels, at 50 days after transplanting (DAT).

	Mean squares					
SV	RDM	SDM	TDM	RDM/SDM	RL	RV
Salinity (S)	7.37*	26.26**	50.35**	0.03^{ns}	40.42^{ns}	207.63*
Propagation (P)	40.14**	275.18**	497.44**	0.18**	352.62*	786.02*
Interaction S x P	5.01*	10.33*	8.76^{ns}	0.05*	115.20^{ns}	79.55^{ns}
CV	13.8	9.82	9.03	4.98	12.01	17.10
Propagation (mean)	g		g	g	cm	cm^3
Cutting	1.75^{b}	8.31^{b}	10.06^{b}	0.21^{b}	31.26^{b}	14.66^{b}
Seed	2.68^{b}	6.32^{c}	9.00^{b}	0.42^{a}	39.00^{ab}	20.06^{b}
Grafting	4.93^{a}	14.53^{a}	19.46^{a}	0.36^{a}	40.20^{a}	29.00^{a}

^{ns}, * and ** = not significant, significant at 0.05 and 0.01 probability level, respectively.

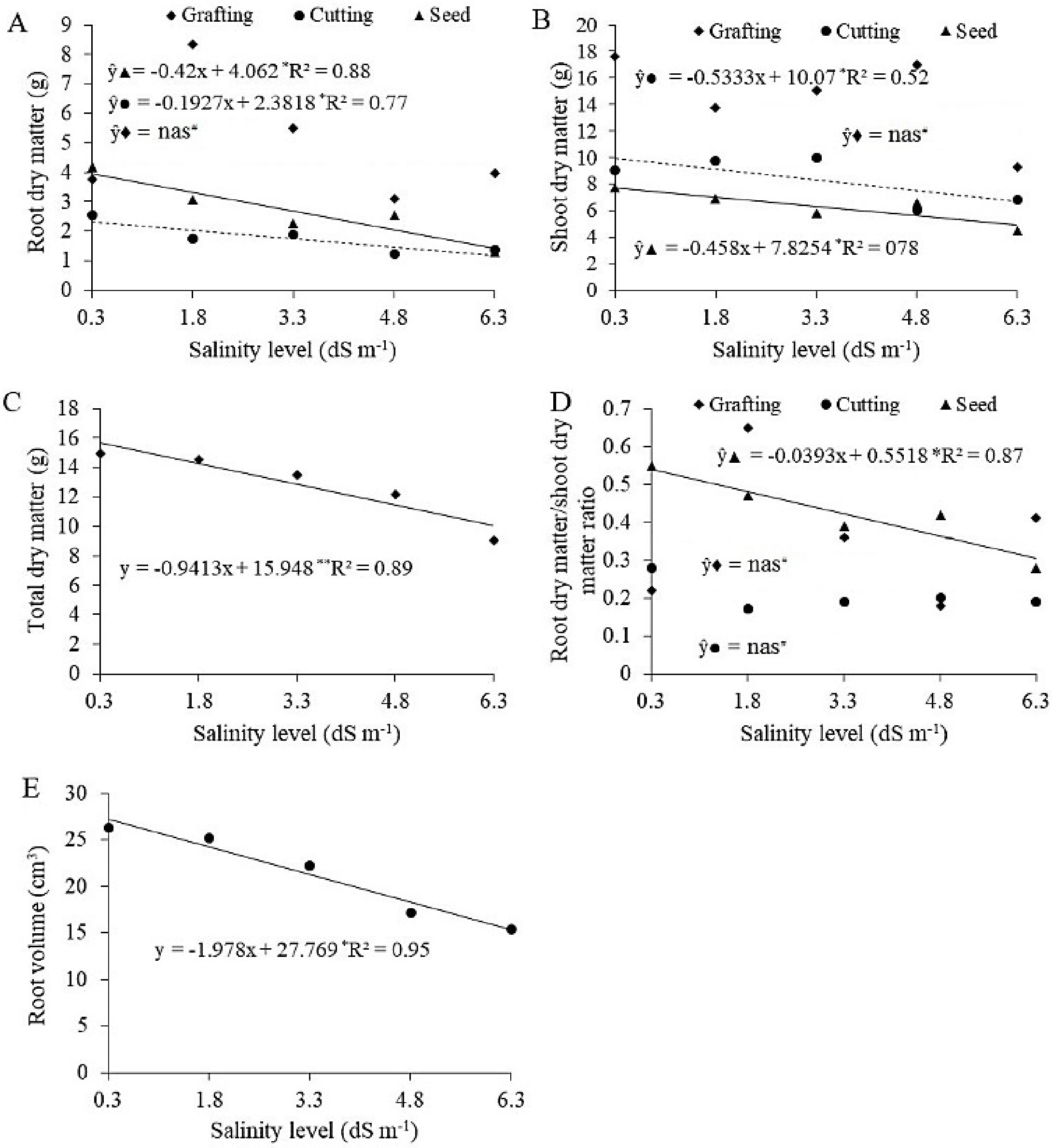

Figure 2. Influence of water salinity on biometric characteristics of yellow passion fruit: A - root dry matter; B - shoot dry matter; C - total dry matter; D - root dry matter/shoot dry matter ratio and E - root volume. nas - no satisfactory adjustment.

In comparison to seedlings propagated by cuttings and seeds, grafting propagation led to seedlings with 93.43 and 116.22% higher TDM, respectively (Table 2). Such superiority was also observed in plant height and stem diameter (Table 1), and it may have occurred due to the difference of age of the seedlings between the propagation methods.

4. Discussion

The increase in water salinity affected negatively the height of plants of *Passiflora edulis*. Similar results have been reported by Moura et al. (2016, 2017, 2019) in *Passiflora* edulis and Lima et al. (2020a) in BRS Rubi do Cerrado, who observed that yellow passion fruit growth was affected by the increase in the levels of irrigation water salinity. Reduction in crop yield caused by salt stress basically occurs in two ways: salinity may cause water deficit because of reduced soil water potential or due to absorption of toxic ions, such as Na^+ (Patade et al. 2011; James et al. 2012).

The results found differ from those of Morgado et al. (2015), who demonstrated that the *Passiflora edulis*/*Passiflora edulis* combination led to lower height in comparison to plants grown from seeds (non-grafted) in the second evaluation (45 days after grafting). Lima et al. (2017) compared grafted seedlings of *Passiflora edulis*/*Passiflora edulis* with seedlings grown from seeds and observed greater height in plants propagated by seeds. This indicates that the grafting practice alone does not significantly influence plant growth and, consequently, plant height. However, this difference between propagation methods in the present study was expected, due to the difference of age (30 days) of the rootstock of seedlings propagated by grafting and seeds.

Salt stress causes overall reduction in the growth of cultivated plants due to the toxic effects of sodium, leading to serious damages to their development (Cavalcante et al. 2010), as evidenced by the number of leaves in the present study. Similar result was obtained by Nascimento et al. (2017), who also found significant influence of water salinity (0.43 and 4.50 dS m^{-1}) on the number of leaves in *Passiflora edulis* (grown from seeds), corroborating the present study. For stem diameter, grafted seedlings were superior to those propagated by the other two methods. Lima et al. (2017) also found greater stem diameter in the self-grafting of *Passiflora edulis*/*Passiflora edulis* in comparison to seedlings grown from seeds.

Stomatal conductance was affected by the increase in water salinity, Freire et al. (2014) found 50% reduction in the stomatal conductance of *Passiflora edulis*, between the highest salinity level (4.5 dS m^{-1}) and the control treatment (0.5 dS m^{-1}). Andrade et al. (2019) observed 15.37% reduction in stomatal conductance (gs) per unit increase in ECw. Water salinity affects stomatal opening in response to the reduction in soil osmotic potential. It is known that CO_2 inflow occurs through the stomata in the photosynthesis, whereas water outflow occurs through transpiration, and stomatal movement is the main mechanism of control of gas exchanges in higher plants (Silva et al. 2015). Thus, low water availability in the soil, due to the osmotic effect, can cause stomatal closure, limiting stomatal conductance and transpiration, which consequently reduces the photosynthetic rate (Silva et al. 2010), contributing to the reductions in plant growth caused by saline stress.

Regarding passion fruit propagation methods, total chlorophyll (TC) content was on average 26.5% higher ($p \leq 0.01$) in plants propagated by grafting, compared with the other two methods (Table 1). Thus, it can be inferred that the variations found between the propagation methods for TC are due to the difference in absorption of nutrients promoted by the root system of plants in each propagation method, as shown in Table 2, in which grafting led to greater root length (RL) and root volume (RV). Moura et al. (2017) observed that the water consumption of passion fruit seedlings (grown from seeds) was negatively affected by the increment in the levels of irrigation water salinity. High concentrations of salts in the substrate reduce its osmotic potential, which is responsible for water direction, thus limiting water absorption by the crops (Dias et al. 2016). Such reduction in TWC results from the multiple response of plants to salt stress, with effects on plant height, number of leaves, and stomatal conductance (Figure 1A, 1B and 1C).

Referring to shoot dry matter, only seedlings propagated by cuttings and seeds were affected by water salinity. Oliveira et al. (2015), Moura et al. (2017), and Lima et al. (2020b) also found reduction in root dry matter and shoot dry matter in passion fruit (grown from seeds) as a function of the increase in NaCl level in the irrigation water, under greenhouse conditions. Such reduction in the total dry matter of plant occurred because NaCl negatively affects the synthesis and translocation of nutrients and hormones from roots to shoots, which results in loss of dry matter (Moura et al., 2020). Similar results have been reported in other species such as *Passiflora tripartita* var. Mollissima (Casierra-Posada et al. 2013) and *Talisia esculenta* (Melo Filho et al. 2017), which showed drastic reductions in total dry matter accumulation as salinity increased (5 dS m^{-1}).

The interaction between water salinity and propagation method revealed that the RDM/SDM ratio in seedlings propagated by seeds decreased linearly by 7.12% per unit increase in ECw. Such behavior may be due to the high Na^+ concentrations in the water, negatively interacting with plant physiology, leading to ionic, osmotic and nutritional reactions that are harmful to plants, compromising root growth and biomass accumulation more than the shoots (SÁ et al., 2013). The RDM/SDM ratio in seedlings propagated by grafting and cuttings was not significantly affected by the salinity of the irrigation water, indicating that both parts of the plant were similarly influenced at different levels of water salinity. All propagation methods showed RDM/SDM ratio less than 1.0, indicating that SDM was superior to RDM, regardless of the salinity level.

Shorter root length was observed in seedlings propogated by cuttings being significantly inferior (22.2%) compared to grafted seedlings while the propogated by seeds did not differ significantly from other two methods of propogation (Table 2). Santos et al. (2017) observed in citrus ('Santa Cruz' Rangpur lime) that self-grafted plants obtained greater root length in comparison to plants grown from seeds. Such lower development of seedlings grown from cuttings may be related to the fact that they exhibit features of adult plants with lower vigor, compared to seedlings grown from seeds and grafted, which show juvenile characteristics, besides having different physiological ages. Root volume (RV) linearly decreased, on average by 7.12% per unit increase in ECw (Figure 2E) and propagation by grafting promoted higher volume compared to other methods due to difference in the age of plants used as rootstock.

In general, the osmotic and toxic effects caused by high concentrations of salts affected physiological processes in *Passiflora edulis* and, consequently, limited seedling production by inhibiting plant height, stomatal conductance, total water consumption, dry matter of root, shoot, and total and root volume (Figures 1 and 2). Among the propagation methods, grafting for the reasons explained earlier, led to the best results in seedling production, and propagation by seeds proved to be more sensitive to the effects of salinity.

5. Conclusions

The increase in water salinity affected with greater intensity the growth and development variables (height, total dry matter, and root volume of the plant) and the physiological characteristics (stomatal conductance) of the species *Passiflora edulis*, regardless of the method of propagation.

Seedlings propagated by grafting showed better development compared to the other propagation methods (seeds and cuttings).

The interaction between the propagation methods and water salinity affected seedlings propagated by seeds and cuttings with greater intensity.

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