

Tolerance of Varieties and Hybrid of Pumpkin and Squash to Salt Stress

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Abstract

Irrigated agriculture in arid and semi-arid regions is a practice encouraged to maximize food production, however these regions hesitate a qualitative shortage of water resources, often being necessary the use of saline water, affecting soil and sensitive plants to saline stress. So this study aimed to evaluate emergence, growth, phytomass accumulation and tolerance of the Tetsukabuto hybrid and varieties of pumpkin and squash under salt stress. The experiment was carried out in protected environment (greenhouse), located in the municipality of Pombal, Paraíba, Brazil. Treatments were arranged in completely randomized experimental design with four replicates, in a 5×3 factorial scheme, relative to five levels of irrigation water salinity and three genotypes of pumpkin and squash. Pumpkin and squash seedlings were grown on trays of 30 cells with capacity for 0.1 dm^3 of substrate, during the first 20 days after sowing, and were monitored for emergence, initial growth, dry matter accumulation and tolerance to salinity. Increment in irrigation water salinity reduces emergence and initial growth of the pumpkin and squash genotypes. The genotypes G_2 - 'Squash cv. Coroa' and G_3 - Tetsukabuto hybrid are more tolerant to salinity than G_1 - 'Pumpkin cv. Jacarezinho' in the initial growth stage.

Keywords: *Cucurbita* sp., irrigation, saline water

1. Introduction

The botanical family *Cucurbitaceae* has various representatives, including pumpkins (*Cucurbita moschata* and *Cucurbita pepo*) and squash (*Cucurbita maxima*), species of great economic and food value, since the *Cucurbitaceae* stands out as the second family with highest economic expression in the world (Resende et al., 2013; FAO, 2014). In Brazil, these crops are widely spread, especially in the Northeastern region of the country, where they are in full expansion, playing a substantial role as subsistence crop (Carmo et al., 2011).

Due to the low rainfall in this region, which leads to scarcity of water resources, irrigation is considered as the technique that most contributes to the increase of yield. However, due to such scarcity, lower-quality waters have been used and they can salinize the soil and reduce the yield of crops (Oliveira et al., 2014). Salinity affects plants in three different ways: first, reducing the osmotic potential of the environment, which reduces water availability in the soil; second, causing toxicity through the accumulation of specific ions; and third, it may cause an indirect effect of nutritional order, including the one due to soil destructuring (Martins et al., 2013; Sá et al., 2017).

For successful use of water with high saline levels, it is necessary to know the effects of salinity on the development of the species to be used and its tolerance level to such adverse condition (Freire et al., 2010). The ability of a plant to withstand certain levels of salts, which varies according to species, growth stage, irrigation and crop managements and stress nature and intensity, defines the meaning of crop tolerance to salinity (Sá et al., 2013; Brito et al., 2014; Oliveira et al., 2015; Albuquerque et al., 2016).

In the search for strategies of coexistence with the semi-arid region, some authors have sought plants that best adapt to saline environments, especially vegetables such as: watermelon (Martins et al., 2013), cucumber (Albuquerque et al., 2016), squashes (Oliveira et al., 2014), beet (Oliveira et al., 2015) and lettuce (Oliveira et al.,

2011). However, for the squash crop, there are only a few studies on the tolerance of cultivars to salinity, especially regarding the Tetsukabuto hybrid, thus requiring further research on this crop. Hence, the objective was to evaluate emergence, growth, phytomass accumulation and tolerance of this hybrid and varieties of pumpkin and squash under salt stress.

2. Material and Methods

The experiment was carried out in protected environment (greenhouse) at the Center of Sciences and Agri-food Technology - CCTA of the Federal University of Campina Grande - UFCG, located in the municipality of Pombal, Paraíba, Brazil (6°47'20" S; 37°48'01" W; 194 m) from August to September 2014.

The pumpkin and squash genotypes with greatest commercial importance in the Northeastern semi-arid region were evaluated, namely: (G1 - 'Pumpkin cv. Jacarezinho' - *Cucurbita moschata*; G2 - 'Squash cv. Coroa' - *Cucurbita maxima*; and G3 - Tetsukabuto hybrid - *Cucurbita moschata* × *Cucurbita maxima*) and levels of irrigation water salinity (0.6 (control), 1.2, 1.8, 2.4 and 3.0 dS m⁻¹), which are common in waters used for irrigation in Northeast Brazil (Medeiros et al., 2003). The treatments were arranged in 5 × 3 factorial scheme, in a randomized block design, with four replicates and 10 plants per plot, totaling 600 experimental plants.

Pumpkin and squash plants were grown on trays of 30 cells with capacity for 0.1 dm³ of substrate, until 20 days after sowing (DAS). The substrate for seedling production was composed of soil (Fluvisol Neosol) (Santos et al., 2013) and commercial substrate at proportion of 1:1, respectively (Table 1). Ten tray cells were allocated for each treatment, so that each cell received three seeds, totaling 30 seeds per treatment. After total emergence, thinning was performed to leave only the most vigorous plant per cell. The seeds of both species were purchased in a commercial establishment, with 99% purity and 95% germination.

Table 1. Chemical characteristics of the substrate components used in the cultivation of pumpkins and squashes

	EC	pH	P	K ⁺	Ca ⁺²	Mg ⁺²	Na ⁺	Al ³⁺	H ⁺ +Al ³⁺	SB	T	OM
	dS m ⁻¹	H ₂ O	mg dm ⁻³	----- cmol _c dm ⁻³ -----							g kg ⁻³	
A	0.09	8.07	3.00	0.32	6.40	3.20	0.18	0.00	0.00	10.49	10.49	16.0
B	1.65	5.75	86.00	1.67	11.60	28.50	17.84	0.00	11.88	59.61	71.49	570.0

Note. SB = sum of bases; EC = electrical conductivity; T = total cation exchange capacity; OM = organic matter; A = Soil; B = commercial substrate.

Irrigation solutions with various salinity levels were prepared considering the equivalent relationship between EC_w and concentration of salts (10*mEq L⁻¹ = 1 dS m⁻¹ of EC_w) according to Rhoades et al. (1992), valid for EC_w range from 0.1 to 5.0 dS m⁻¹, which comprehends the tested levels. Public-supply water available in the area (EC_w = 0.3 dS m⁻¹) was used, mixed with NaCl salts as necessary (Table 2).

Table 2. Chemical analysis of the public-supply water used to prepare the solutions

Water	EC _a	pH	K	Ca	Mg	Na	SO ₄ ⁻²	CO ₃ ⁻²	HCO ₃ ⁻	Cl ⁻	SAR ¹
	dSm ⁻¹		----- mmol _c L ⁻¹ -----							(mmol _c L ⁻¹) ^{0.5}	
	0.3	7.0	0.3	0.2	0.6	1.4	0.2	0.0	0.8	1.3	2.21

Note. EC = electrical conductivity; SAR = Sodium adsorption ratio.

To prepare the solutions with the respective values of electrical conductivity (EC), the salts were weighed according to the treatment and water was added until reaching the desired EC level, which was verified using a portable conductivity meter adjusted to temperature of 25 °C. After preparation, the saline solutions were stored in 30-L plastic containers, one for each EC_w level, properly protected to avoid evaporation, entry of rainwater and contamination by material that could compromise their quality.

Irrigations were daily performed to keep soil moisture close to the maximum retention capacity, based on drainage lysimetry, and the applied water depth was added of a leaching fraction (LF) of 20%. The volume applied per container (V_a) was obtained by difference between the previous volume applied (V_{prev}) minus the average drainage (d), divided by the number of containers (n), as shown in Equation 1:

$$V_a = \frac{V_{prev} - (D/n)}{(1-LF)} \quad (1)$$

During the experiment, pumpkin and squash emergence was monitored by counting the number of emerged seedlings, *i.e.*, with cotyledons above the soil level. Counts were daily made, without discarding the seedlings, thus obtaining a cumulative value. Hence, the number of emerged seedlings relative to each count was obtained by subtracting the previous reading from the current one. Thus, the number of emerged seedlings referring to each reading was used to calculate the emergence speed index (ESI) using Equation 2, described by Schuab et al. (2006).

$$ESI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \dots + \frac{G_n}{N_n} \quad (2)$$

Where, ESI = emergence speed index; G = number of emerged seedlings observed in each count; N = number of days from sowing to each count.

After stabilization of emergence, emergence percentage (EP) (%) was determined based on the ratio between the number of emerged plants and number of seeds sown.

The morphological aspects of the crop were monitored by growth analysis at 20 DAS: plant height (PH) (cm), measured using a ruler as the distance from soil to plant apex; stem diameter (SD), measured with a digital caliper, at height of 1 cm from soil surface; and number of leaves (NL), through the count of mature leaves.

After growth analysis, plants were collected, separated into shoots and roots, and dried in a forced-air oven at 65 °C until constant weight. Subsequently, the material was weighed on analytical scale to determine total dry matter (TDM). TDM production data were used to calculate the percentages partitioned between vegetative organs and the salinity tolerance index (STI), comparing the data of saline treatments with those of the control ($EC_w = 0.6 \text{ dS.m}^{-1}$), according to the methodology of Fageria et al. (2010), based on four levels of classification: T (tolerant; 0-20%), MT (moderately tolerant; 21-40%), MS (moderately sensitive; 41-60%) and S (Sensitive; > 60%), as demonstrated in Equation 3:

$$STI(\%) = \frac{\text{TDM production in the saline treatment}}{\text{TDM production in the control treatment}} \times 100 \quad (3)$$

The total dry matter production data of the genotypes were used as the main parameters to determine their tolerance to salt stress.

The obtained data were subjected to analysis of variance by F test and, in cases of significance, regression analyses were applied to the factor irrigation water salinity levels and Tukey test was applied to the factor species, both at 0.05 probability level, using the statistical software SISVAR® (Ferreira, 2011). The data were standardized to show mean equal to zero ($\bar{X} = 0$) and variance equal to one ($\sigma = 1$). Subsequently, cluster analysis was performed through the hierarchical method, Ward's minimum variance, using the Euclidean Distance as the measure of dissimilarity (Hair et al., 2009).

3. Results and Discussion

The interaction between pumpkin and squash genotypes and irrigation water salinity levels had significant effect ($p < 0.05$) on all studied variables, except the number of leaves, which was not significantly affected by the studied sources of variation (Figures 1A-1F). Significant effects on *Cucurbitaceae* species have also been observed by Albuquerque et al. (2016), studying the tolerance of cucumber cultivars to salt stress; Araújo et al. (2016), in melon cultivars; and Oliveira et al. (2014), in pumpkin and squash cultivars. According to these authors, the increase in irrigation water salinity reduces the initial development of these crops, varying according the studied cultivar.

Linear reduction was observed in the variables EP and ESI of the pumpkin genotypes as a function of the increase in irrigation water salinity. Emergence percentage reduced by approximately 20.27, 16.66 and 15.27% for every unit increase in irrigation water salinity for the genotypes G₁ ('Pumpkin cv. Jacarezinho'), G₂ ('Squash cv. Coroa') and G₃ (Tetsukabuto hybrid) (Figure 1A). In addition, the genotypes 'Squash cv. Coroa' and Tetsukabuto hybrid showed the highest emergence percentage and emergence speed index, thus proving to be more tolerant in saline environments, especially the Tetsukabuto hybrid, which stood out from the others, obtaining emergence percentage of 65.00% when irrigated with water of highest salinity level (3.0 dS m^{-1}).

Regarding emergence speed index (ESI), there were reductions from 0.43 to 0.27, 0.57 to 0.32 and 0.57 to 0.39 days for the genotypes G₁ ('Pumpkin cv. Jacarezinho'), G₂ ('Squash cv. Coroa') and G₃ (Tetsukabuto hybrid), respectively, between the highest (3.0 dS m^{-1}) and lowest salinity level (0.6 dS m^{-1}) (Figure 1B). According to

Albuquerque et al. (2016), this behavior is possibly related to the reduction in seed imbibition capacity as water salinity increases, because the osmotic potential of the substrate also increases, retarding or reducing germination and, consequently, it eventually affects seedling emergence.

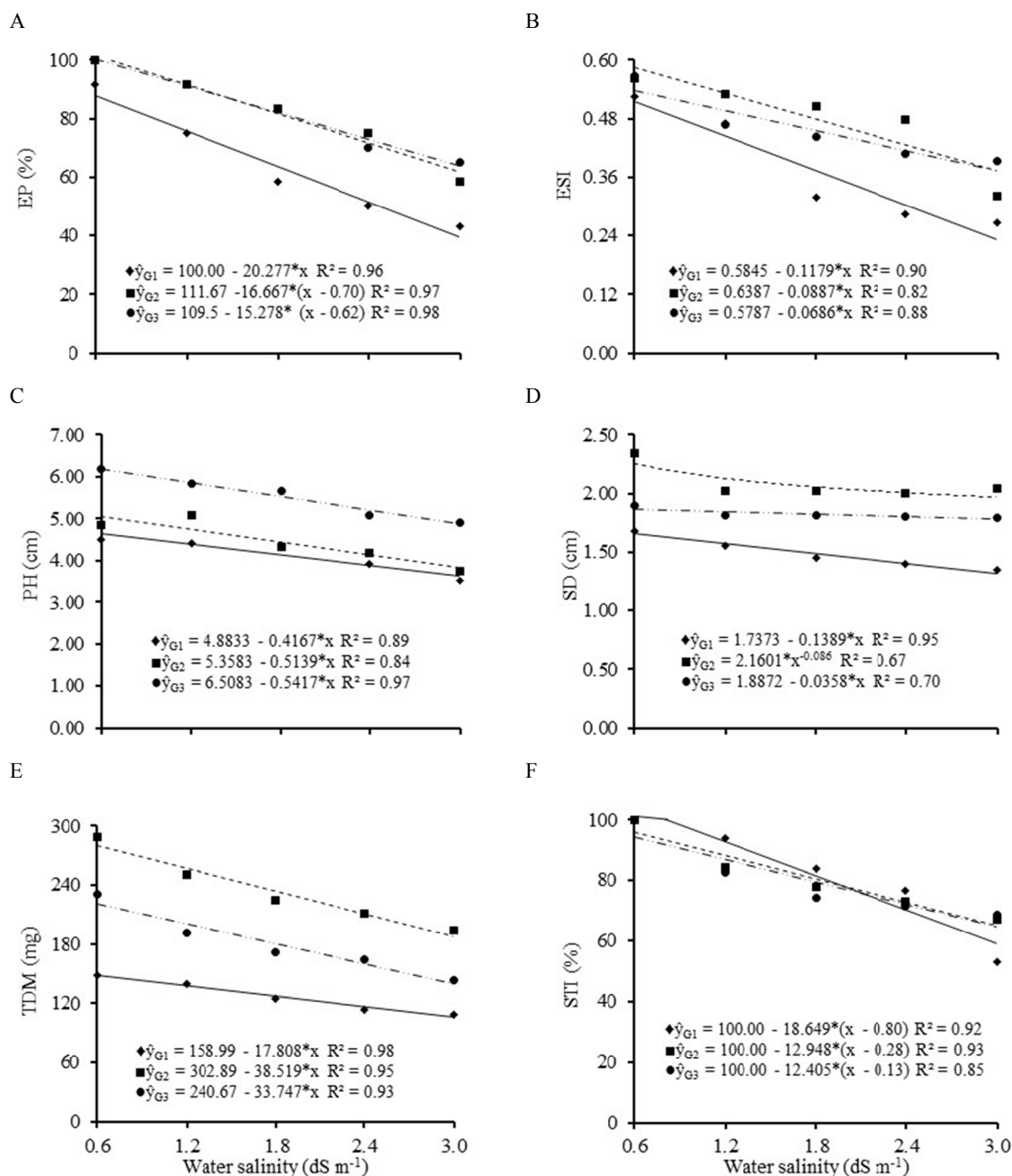


Figure 1. Emergence percentage, EP (A), emergence speed index, ESI (B), Plant height, PH (C), stem diameter, SD (D), total dry matter, TDM (E) and salinity tolerance index, STI (F) of pumpkin and squash genotypes under different levels of irrigation water salinity

Note. * = significant at 5% ($p < 0.05$) of probability. G₁ - ‘Pumpkin cv. Jacarezinho’; G₂ - ‘Squash cv. Coroa’ and G₃ - Tetsukabuto hybrid.

These results are consistent with those of Araújo et al. (2016), who studied the tolerance of melon cultivars to irrigation water salinity and observed that the increment in the saline level compromised seedling emergence, regardless of the studied genotype. However, there was an increment in the emergence speed index, differing

from the results obtained in the present study. These same authors did not find effect of the interaction Salinity x Genotype on these variables, which is different from the present study.

The increase in irrigation water salinity reduced the growth of the pumpkin genotypes. Plant height decreased by 0.41, 0.51 and 0.54 cm in the genotypes G₁, G₂ and G₃ for every unit increase in irrigation water salinity (Figure 1C). Stem diameter also showed reductions due to the increment in irrigation water salinity, but they were lower than those observed on the growth in height. The reductions were equal to 8.0%, 8.4% and 1.9% in the genotypes G₁, G₂ and G₃ respectively, as a function of the increase in irrigation water salinity (Figure 1D).

Albuquerque et al. (2016) report that irrigation water salinity affects emergence, growth and dry matter accumulation of cucumber plants, as well as Oliveira et al. (2014) working with melon, pumpkin and squash plants subjected to salt stress and Oliveira et al. (2012), working with the gherkin crop. Vegetables are considered as the most sensitive plants to salinity; therefore, any increment in salinity levels can reduce their production, in both quantity and quality. However, these reductions occur according to the cultivar used, because these studies demonstrate that some of the evaluated cultivars are more tolerant to salinity than others.

Again, the Tetsukabuto hybrid stood out from the other genotypes, showing lower growth reductions, which indicates higher tolerance. These results are a sign of occurrence of genetic dissimilarity, so that genotypes that are superior and genetically dissimilar, when used in a cross, have great probability to originate populations with wide genetic variability and with higher possibility of selection of transgressive segregants for the character of interest (Carvalho et al., 2001). In this case, although the focus of origin of the Tetsukabuto hybrid is not related to salinization problems and the progenies are not exposed to any type of pressure of selection, it was possible to obtain a hybrid with high potential of tolerance to salinity. This fact will be confirmed hereinafter with the study on the salinity tolerance index.

For every unit increase in irrigation water salinity, there were reductions of 17.8, 38.5 and 33.7 mg of dry matter per plant for the genotypes G₁ ('Pumpkin cv. Jacarezinho'), G₂ ('Squash cv. Coroa') and G₃ (Tetsukabuto hybrid), respectively (Figure 1E), with values ranging from 148.62 to 108.18, 289.03 to 193.22 and 230.72 to 142.58 mg, between the highest (3.0 dS m⁻¹) and lowest (0.6 dS m⁻¹) salinity levels. The genotype 'Squash cv. Coroa' exhibited the highest dry matter accumulation, compared with the others, regardless of the saline levels.

Linear reductions of growth and biomass accumulation in response to the increase in irrigation water salinity have been observed in other cucurbits, such as watermelon (Martins et al., 2013), melon (Araújo et al., 2016) and cucumber (Albuquerque et al., 2016). These responses can be related to the reduction in the photosynthetic capacity of the plants, through ionic interactions promoted by the excess of sodium salts, which eventually reduces the accumulation of photoassimilates due to their lower production, also associated with the reduction in the osmotic potential, which causes decrease in the availability of water for plant growth (Taiz & Zeiger, 2013).

Regarding the salinity tolerance index, despite the losses with the increment in irrigation water salinity, the genotypes G₁ ('Pumpkin cv. Jacarezinho'), G₂ ('Squash cv. Coroa') and G₃ (Tetsukabuto hybrid) reduced their tolerance, as salinity increased, from 100.0 to 58.9%, from 100.0 to 64.8% and from 100.0 to 64.4%, respectively, between the lowest (0.6 dS m⁻¹) and highest (3.0 dS m⁻¹) salinity levels, evidencing a moderate tolerance for the genotypes G₂ and G₃, and moderate sensitivity to salinity for G₁, according to the classification of Fageira et al. (2010) (Figure 1F). It is possible to infer that the allele(s) that encode the dominant character for tolerance to salinity in the Tetsukabuto hybrid may have come from the squash, given its higher tolerance to salinity compared with pumpkin.

The study on the clusters, based on the Euclidean Distance as the measure of dissimilarity, evidenced the formation of four different groups between the combinations of salinity levels (S) and pumpkin and squash genotypes (G) (Figure 2). The first (I) and second (II) clusters can be characterized by low salinity and high performance for germination and allocation of shoot and root phytomass. In both groups, the genotypes G₂ and G₃ stood out, showing high performance even at high salinity level (2.4 dS m⁻¹), which indicates their tolerance to salinity. The third (III) and fourth (IV) clusters are characterized by high salinity and low performance for germination and accumulation of shoot and root dry matter. The genotype G₃ exhibits low performance already at the S3 salinity level (1.8 dS m⁻¹), standing out as the most sensitive, confirming the tolerance hierarchy observed in the STI, with expressive reduction of performance in the following order: G₃ > G₂ > G₁. These results reinforce that the higher tolerance shown by the Tetsukabuto hybrid comes from the squash, which has higher degree of tolerance compared with pumpkins.

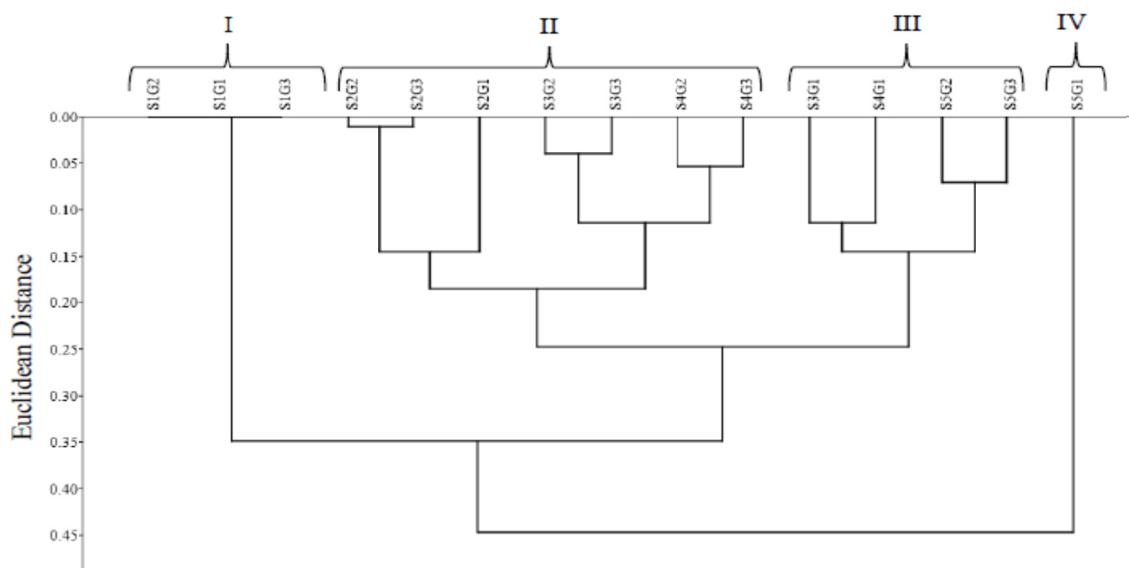


Figure 2. Dendrogram of dissimilarity of the groups formed by the combination of salinity levels (S1 - 0.6; S2 - 1.2; S3 - 1.8; S4 - 2.4 and S5 - 3.0 dS m^{-1}) and genotypes of pumpkins and squashes (G1 - 'Pumpkin cv. Jacarezinho', G2 - 'Squash cv. Coroa' and G3- Tetsukabuto hybrid)

4. Conclusions

The increase in irrigation water salinity reduces emergence and initial growth of the pumpkin and squash genotypes.

The genotypes G₂ - 'Squash cv. Coroa' and G₃ - Tetsukabuto hybrid are more tolerant to salinity compared with G₁ - 'Pumpkin cv. Jacarezinho', in the initial growth stage.

Waters with salinity of up to 3.0 dS m^{-1} can be used to produce seedlings of 'Squash cv. Coroa' and Tetsukabuto hybrid, but with small losses in growth.

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