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INITIAL DEVELOPMENT AND TOLERANCE OF PEPPER SPECIES TO SALINITY STRESS[1]

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ABSTRACT - Salinity is one of the main causes of crop yield decline in arid and semi-arid regions, requiring the use of tolerant species that allow cultivation in these areas. The objective of this study was to determine the emergence, initial growth and tolerance of pepper species irrigated with saline waters. The experiment was conducted in a protected environment (greenhouse), with a 5 x 3 factorial scheme, using four replicates of 30 seeds each. The experimental design was a completely randomized design, involving five irrigation water salinity levels (0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}) and three species of pepper [E1 – *Capsicum annuum* ("Doce Comprida"); E2 – *Capsicum frutescens* ("Malagueta"); E3 – *Capsicum chinense* ("De Bico")]. Pepper seedlings were grown in trays of 30 cells with a capacity of 0.1 dm^3 of substrate. During the first 30 days after sowing, seedlings were monitored for emergence, initial growth and dry matter accumulation. As the salinity of the irrigation water increased, there was a reduction in the emergence, growth and phytomass accumulation of *C. annuum, C. frutescens* and *C. chinense* peppers. These species tolerate saline waters of up to 1.78, 2.71 and 1.55 dS m^{-1} respectively, in the initial development phase, with *C. frutescens* being the most tolerant to saline stress, and *C. chinense* the most sensitive.

Keywords: Solanaceae. *Capsicum sp.* Abiotic stress. Irrigation.

DESENVOLVIMENTO INICIAL E TOLERÂNCIA DE ESPÉCIES DE PIMENTA AO ESTRESSE SALINO

RESUMO - A salinidade é uma das principais causas da queda de rendimento das culturas, em regiões áridas e semiáridas, sendo necessária a utilização de espécies tolerantes que viabilizem o cultivo nestas áreas. Nesse sentido, objetivou-se verificar a emergência, o crescimento inicial e a tolerância de espécies de pimenta irrigadas com águas salinas. O experimento foi conduzido em ambiente protegido (casa de vegetação), sendo os tratamentos formados a partir do esquema fatorial 5 x 3, usando-se quatro repetições de trinta sementes. O delineamento experimental foi o inteiramente casualizado, relativos a cinco níveis de salinidade da água de irrigação (0,6; 1,2; 1,8; 2,4 e 3,0 dS m^{-1}) e três espécies de pimenta [E1 – *Capsicum annuum* ("Doce Comprida"); E2 – *Capsicum frutescens* ("Malagueta"); E3 – *Capsicum chinense* ("De Bico")]. As plântulas de pimenta foram cultivadas em bandejas de 30 células com capacidade de 0,1 dm^3 de substrato, durante 30 dias após a semeadura. Nesse período, as plântulas foram monitoradas quanto à emergência, o crescimento inicial e o acúmulo de matéria seca. O aumento da salinidade da água de irrigação reduz a emergência, o crescimento e o acúmulo de fitomassa das pimentas *C. annuum*, *C. frutescens* e *C. chinense*. As espécies *C. annuum*, *C. frutescens* e *C. chinense* toleram CE_a de até 1,78, 2,71 e 1,55 dS m^{-1} na fase de desenvolvimento inicial, respectivamente, sendo a *C. frutescens* a mais tolerante ao estresse salino, e a *C. chinense*, a mais sensível dentre as espécies verificadas.

Palavras-chave: Solanaceae. *Capsicum sp.* Estresse abiótico. Irrigação.

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INTRODUCTION

Water is a fundamental factor for agricultural production, being crucial for plant germination, development and production (CARVALHO; NAKAGAWA, 2012). However, in regions with low availability of good-quality water, such as semi-arid regions, the development of agricultural crops is normally hampered due to the limitation of this resource for irrigation and the presence of salts, which cause a series of physiological and nutritional disorders (ESTEVES; SUZUKI, 2008; SÁ et al., 2017; TAIZ et al., 2017).

Salinity in either the soil or water is one of the main causes of reductions in crop yield in semi-arid regions, but such reductions depend on several factors such as species, intensity, types of salts, phenological stage, crop management, irrigation and edaphoclimatic conditions (MUNNS; TESTER, 2008). Osmotic effects result from the reduction in the water potential of the growth medium, thus decreasing water availability to the seed and seedling (ESTEVES; SUZUKI, 2008). Ionic effects, in turn, result from the excessive absorption of ions, especially Na^+ and Cl^-, which alter cell homeostasis when they are at high concentrations (MUNNS; TESTER, 2008; SYVERTSEN; GARCIA-SANCHEZ, 2014).

Some crops are sensitive to salinity, even at low levels, while others produce significant yields under high levels of salinity; this is due to the higher capacity for osmotic adaptation found in some plants, which allows them to absorb a sufficient volume of water even in a saline medium (AYERS; WESTCOT, 1985). In general, crops are initially tolerant to salinity at the germination stage, but they become more sensitive throughout their development, especially at emergence and in the initial growth stage (SÁ et al., 2017; SANTOS et al., 2018).

Pepper belongs to the genus *Capsicum*, family Solanaceae, and has economic importance in the Brazilian agribusiness, with 27 species of commercial interest distributed all over the country (FILGUEIRA, 2013). In this context, the species *C. annuum* (“Doce Comprida”), *C. frutescens* (“Malagueta”) and *C. chinense* (“De Bico”) are the most economically important in Brazil (FILGUEIRA, 2013).

The main producers of pepper are the states of Pará, Bahia, Minas Gerais and Goiás; peppers are highly important for the economy and as food in the agricultural scenario of Brazil, generating income through their high levels of production and use of labour (FILGUEIRA, 2013).

Information about pepper cultivation is scarce and there are few studies on its management and varieties, especially related to salinity tolerance. The effects of salinity on plants vary among species and even among individuals of the same species, depending on the phenological stage and on the intensity and duration of salt stress, with the initial growth stage being one of the most sensitive to stress in most agricultural crops (AYERS; WESTCOT, 1985; SÁ et al., 2017). Therefore, the objective of this study was to assess the emergence, initial growth and tolerance of pepper species irrigated with saline water.

MATERIAL AND METHODS

The experiment was carried out in a protected environment (greenhouse) at the Centre of Sciences and Agri-Food Technology – CCTA of the Federal University of Campina Grande – UFCG, Pombal, Paraíba, Brazil (6º47’20’’S, 37º48’01’’W and altitude of 194 m). Three species of pepper (*C. annuum*, *C. frutescens* and *C. chinense*) were subjected to five levels of irrigation water salinity (0.6 (control), 1.2, 1.8, 2.4 and 3.0 dS m^{-1}), distributed in a 5 x 3 factorial arrangement, in a randomized block design, with four replicates. These levels of salinity were chosen because they are common in waters used for irrigation in Northeast Brazil (MEDEIROS et al., 2003).

Pepper seeds were placed to germinate on trays with 30 cells and a capacity for 0.1 dm^3 of substrate for 30 days. The substrate for seedling production was composed of soil (Fluvic Neosol) (SANTOS et al., 2013) and commercial substrate (TOP Plant®) in a 1:1 ratio (Table 1). The experimental unit consisted of 30 cells, each of which contained one seed. The seeds of the three species were purchased from commercial establishments, with 99% purity and 95% germination guaranteed.

Table 1. Chemical characteristics of the substrate components used in the cultivation of pepper species.

	EC	pH	P	K^+	Ca^{+2}	Mg^{+2}	Na^+	Al^{3+}	H^++Al^{3+}	SB	T	OM
	dS m^{-1}	H_20	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

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

Irrigation solutions with the different levels of salinity were prepared by considering the equivalent ratio between EC_w and the concentration of salts (10*meq L^{-1} = 1 dS m^{-1} of EC_w) according to Rhoades, Kandiah and Mashali (1992),which is valid for EC_w from 0.1 to 5.0 and thus encompassed the tested levels. Water from the local supply system (EC_w = 0.3 dS m^{-1}) mixed with NaCl salts as needed was used for all solutions (Table 2). To prepare the solutions with the respective levels of electrical conductivity (EC), the salts were weighed according to the treatment, and water was added until the desired EC level was reached. EC values were checked using a portable conductivity meter adjusted to a temperature of 25 ºC. After preparation, the saline solutions were stored in plastic containers (30 L), and protected from evaporation, entry of rainwater and contamination with materials that could compromise their quality.

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

EC_w dSm^{-1}	pH	K	Ca	Mg	Na	SO_4^{-2}	CO_3^{-2}	HCO_3^-	Cl^-	SAR $(mmol_cL^{-1})^{0,5}$
				....................................($mmol_cL^{-1}$)................................						
0.3	7.0	0.3	0.2	0.6	1.4	0.2	0.0	0.8	1.3	2.21

EC_w = electrical conductivity of water; SAR = Sodium adsorption ratio.

Irrigation was performed daily, from sowing, in order to maintain a soil moisture content close to its maximum retention capacity, based on the drainage lysimetry method, and the irrigation water was applied along with a leaching fraction (LF) of 0.20. The volume applied (V_a) per container was obtained by the difference between the previous volume applied (V_{prev}) minus the average drainage (d), divided by the number of containers (n), as indicated in Equation 1:

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

During the experiment, the emergence of pepper seedlings was monitored by daily counts, and emerged seedlings were considered as those whose cotyledons were above the soil level. The seedlings counted daily were not discarded, so that the cumulative value was obtained. Thus, the number of emerged seedlings in each count was obtained by subtracting the value found on the previous day from the current value. The number of seedlings emerged in each evaluation was then used to calculate the emergence speed index (ESI), using Equation 2 (eq. 2), as proposed by Maguire (1962).

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

where: ESI = emergence speed index; G = number of seedlings emerged 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 seedlings emerged and the number of seeds sown.

For the monitoring of morphological aspects, the following parameters were evaluated at 30 days after sowing (DAS): plant height (PH) (cm), measured using a graduated ruler (mm) as the distance from the collar to the apex of the plant; stem diameter (SD), measured with a digital caliper at 1 cm from the soil surface; and number of leaves (NL), determined by counting.

Plants were then collected, separated into shoots and roots, and dried in a forced air circulation oven at 65 ºC until constant weight. After that, the materials were weighed on an analytical scale (0.0001 g) to determine shoot dry matter (SDM) (g) and root dry matter (RDM) (g). Total dry matter (TDM) was determined by the sum of SDM and RDM.

The total dry matter production data were used to calculate the percentages partitioned among the vegetative organs and the salinity tolerance index. The data from the saline treatments were compared with those of the control (EC_w = 0.6 dS m^{-1}), according to the methodology of Fageria, Soares Filho and Gheyi (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 shown in equation 3.

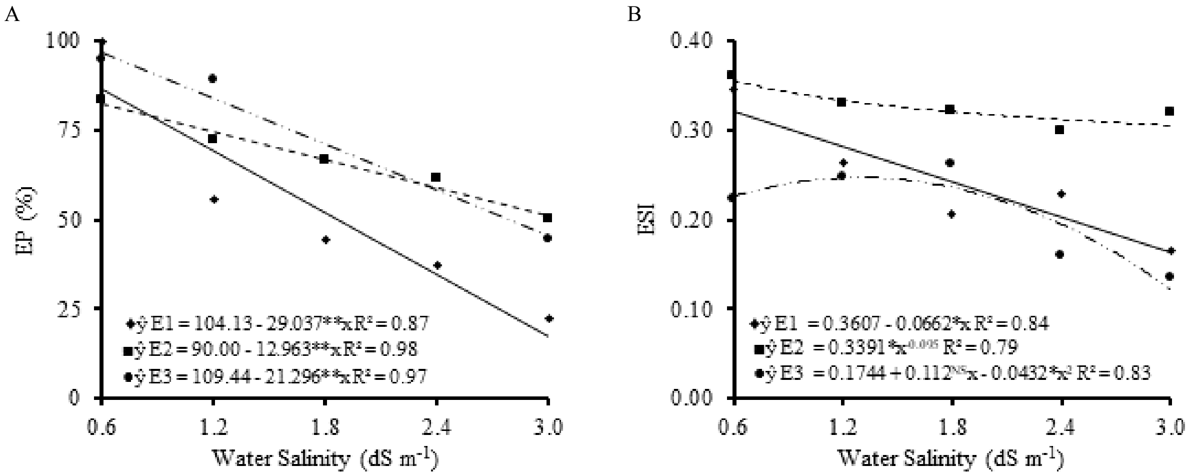
$$STI(\%) = \frac{\text{SDM production in saline treatment}}{\text{SDM production in control treatment}} x100 \quad (3)$$

These indices were calculated using the total dry matter production of each species as the main parameter to determine their tolerance to salt stress.

The obtained data were subjected to analysis of variance (F test) and, in cases of significance, regression analyses were carried out for the factor levels of irrigation water salinity, whereas the Tukey test was applied to the species factor, both at 0.05 probability level, using the statistical program SISVAR® (FERREIRA, 2011).

RESULTS AND DISCUSSION

The emergence of pepper seedlings, regardless of the species studied, decreased linearly as irrigation water salinity increased. The lowest reduction per unit increase in irrigation water salinity was found in *C. frutescens* (12.96%). However, the species *C. annuum* and *C. chinense* showed reductions of 29.03 and 21.29% per unit increase in irrigation water salinity, denoting a higher sensitivity compared to *C. frutescens* at the emergence stage (Figure 1A). As observed for emergence, lower reductions in emergence speed index were observed in *C. frutescens*, compared to the other species (Figure 1B). The emergence speed index of *C. annuum* decreased linearly with the increase in water salinity, showing a reduction of 48.5% when irrigated with the highest level of salinity (3.0 dS m^{-1}) compared to the lowest level studied (0.6 dS m^{-1}), indicating drastic reductions in the vigour of its seeds under salt stress conditions.



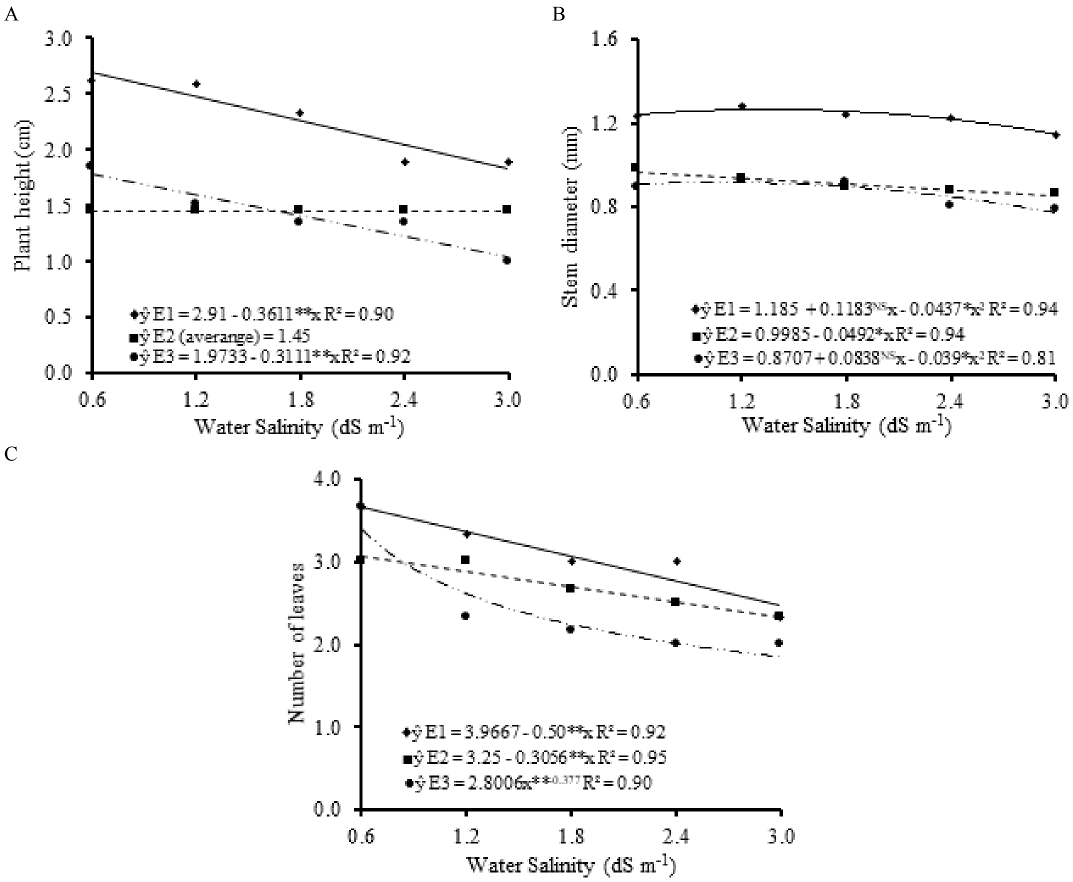
**and* = significant at 1 and 5% (p <0.01 and p <0.05) probability; NS = not significant.

Figure 1. Emergence percentage, EP (A) and emergence speed index, ESI (B) of pepper species (S1 – *C. annuum*; S2 – *C. frutescens*; S3 – *C. chinense*) under different levels of irrigation water salinity.

The emergence speed index of *C. chinense* showed a quadratic response to the increase in irrigation water salinity, and its highest value was obtained under 1.3 dS m^{-1}. These results indicate that *C. chinense* seeds remained vigorous with the initial increase in EC_w. With the osmotic restriction caused by salt stress, these seeds possibly intensified their metabolic activity in order to promote osmotic homeostasis and absorb water satisfactorily, allowing faster emergence and ensuring their survival. However, the increase in salt concentration in irrigation water above 1.3 dS m^{-1} directly affected seed vigour and, under these conditions, the accumulation of salts in the substrate would lead to osmotic restriction that is greater than the capacity for osmotic homeostasis of *C. chinense*.

The emergence results indicate that salt stress affects the physiological potential of the seeds and seedlings that emerge under high salinity, causing a reduction in vigour and possibly death whilst at the germination stage, due to the reduction in osmotic potential caused by the increase in NaCl contents in the substrate, in addition to the toxicity caused by these ions, thus decreasing seed viability and seedling emergence (MUNNS; TESTER, 2008; VOIGT et al., 2009; DANTAS et al., 2011; SYVERTSEN; GARCIA-SANCHEZ, 2014; TAIZ et al., 2017). However, *C. frutescens* was less affected by salt stress in the emergence stage than the other two species (Figures 1A and B).

Linear reductions in height were observed in the species *C. annuum* and *C. chinense* as irrigation water salinity increased, on the order of 0.36 and 0.31 cm for every 1.0 dS m^{-1} increase in EC_w. For *C. frutescens*, no significant fit was observed and the seedlings had an average growth of 1.45 cm at 30 DAS (Figure 2A).



**and* = significant at 1 and 5% ($p < 0.01$ and $p < 0.05$) probability; NS = not significant.

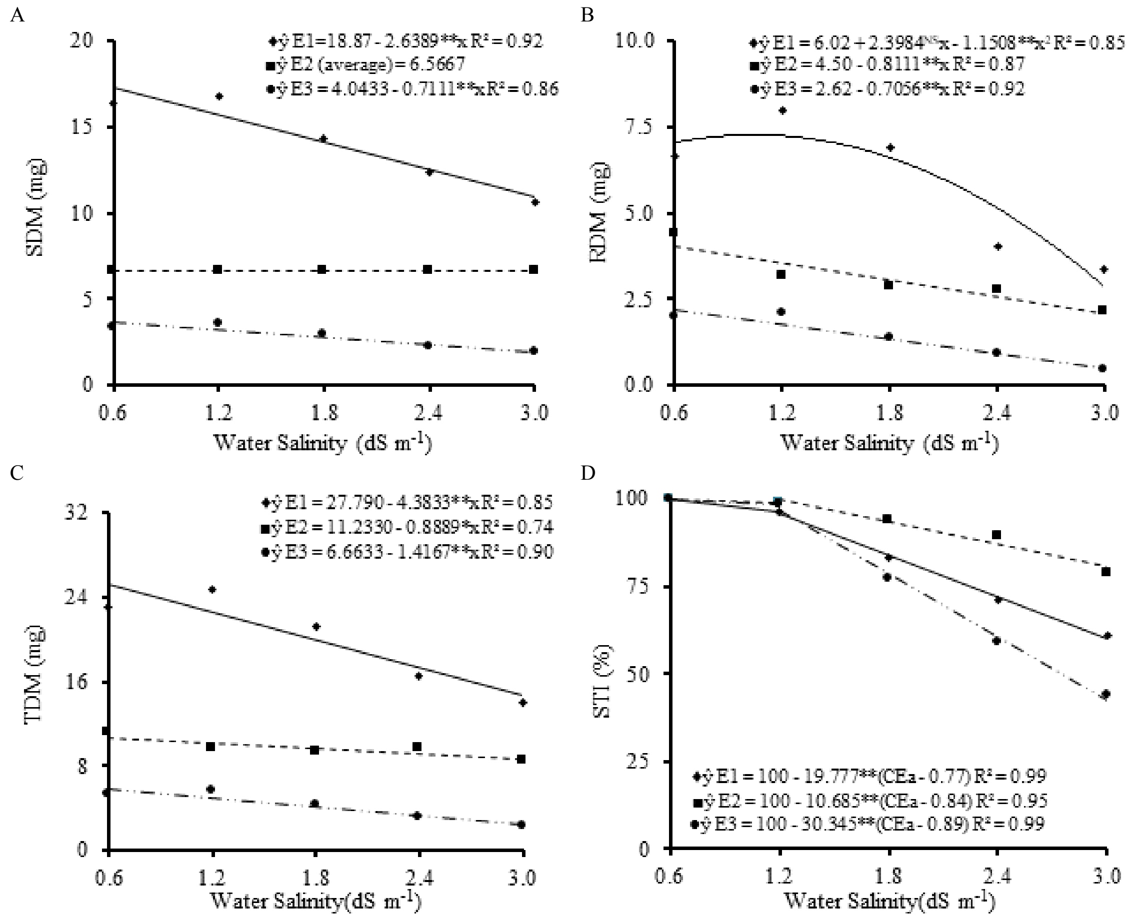
Figure 2. Plant height (A), stem diameter (B) and number of leaves, NL (C) of pepper species (S1 – *C. annuum*; S2 – *C. frutescens*; S3 – *C. chinense*) under different levels of irrigation water salinity.

Stem diameter in the pepper species was affected by the increase in irrigation water salinity; in *C. frutescens*, it decreased linearly by 0.05 mm for every 1.0 dS m^{-1} increase in water salinity (Figure 2B). In the species *C. annuum* and *C. chinense*, stem diameter showed a quadratic response as irrigation water salinity increased, with highest growth in diameter at the levels 1.35 and 1.07 dS m^{-1}, respectively, and a subsequent reduction in growth up to the level of 1.35 dS m^{-1} (Figure 2B).

The increase in irrigation water salinity reduced leaf production in the pepper plants, causing reductions of 32.7, 24.1 and 45.4% between the highest (3.0 dS m^{-1}) and lowest (0.6 dS m^{-1}) levels of salinity, for the species *C. annuum*, *C. frutescens* and *C. chinense*, respectively (Figure 2C).

The results indicate that *C. frutescens* was the least influenced by the deleterious effects of salinity, since it showed lower reductions in growth compared to the other species. The results found for pepper growth corroborate those observed by Sales et al. (2015), Oliveira et al. (2015a) and Albuquerque et al. (2016), who evaluated the emergence and initial growth of coriander, beet and cucumber seedlings, respectively, under different levels of irrigation water salinity. These authors pointed out that an excessive increase in substrate salinity caused by irrigation with saline water directly affects the physiological responses of seeds, as well as leading to hormonal disorders in young plants, thus reducing emergence and initial growth.

The increase in water salinity significantly influenced phytomass accumulation in the shoots, roots and consequently in the entire plant in all three species of pepper (Figures 3A, B and C). *C. annuum* showed the highest values of shoot, root and total phytomass accumulation, but was the most affected by the increase in irrigation water salinity, showing reductions of 36.7, 59.3 and 41.8% in shoot, root and total dry matter respectively between the highest (3.0 dS m^{-1}) and lowest (0.6 dS m^{-1}) levels of irrigation water salinity (Figures 3A, B and C). Phytomass accumulation in *C. chinense* also decreased drastically as irrigation water salinity increased, with reductions of 47.2, 77.3 and 58.5% between the highest (3.0 dS m^{-1}) and lowest (0.6 dS m^{-1}) levels of irrigation water salinity (Figures 3A, B and C). However, in *C. frutescens*, the levels of water salinity had no influence on shoot phytomass accumulation, but there were reductions of 48.4 and 19.9% in root and total dry matter accumulations between the highest (3.0 dS m^{-1}) and lowest (0.6 dS m^{-1}) levels of irrigation water salinity (Figures 3A, B and C).



**and* = significant at 1 and 5% ($p < 0.01$ and $p < 0.05$) probability; NS = not significant.

Figure 3. Shoot dry matter, SDM (A), root dry matter, RDM (B), total dry matter, TDM (C) and salinity tolerance index, STI (D) of pepper species (S1 – *C. annuum*; S2 – *C. frutescens*; S3 – *C. chinense*) under different levels of irrigation water salinity.

The reduction in phytomass accumulation is related to the deleterious effects caused by salt stress, since high concentrations of sodium salts negatively affect physiological aspects of the plant, causing ionic, osmotic, hormonal and nutritional alterations, which can be deleterious to plants, or a cause reduction in growth and consequently of phytomass accumulation (MUNNS; TESTER, 2008; ESTEVES; SUZUKI, 2008; TAIZ et al., 2017). A reduction in phytomass accumulation with increasing irrigation water salinity has also been observed in other vegetable crops such as coriander (REBOUÇAS et al., 2013), lettuce (OLIVEIRA et al., 2011), broccoli (MACIEL; LOPES; MAURI, 2012), pumpkins and squashes (OLIVEIRA et al., 2014; SANTOS et al., 2018), beet (OLIVEIRA et al., 2015a), cabbage (OLIVEIRA et al., 2015b), melon (ARAÚJO et al., 2016), cucumber (ALBUQUERQUE et al., 2016) and bell pepper (SÁ et al., 2017).

With respect to the salinity tolerance index (STI), the pepper species began to reduce their relative yield from a salinity of 0.77 dS m^{-1}. Based on the classification of Fageria, Soares Filho and Gheyi (2010), in which plants classified as tolerant show yield reductions below 20%, the pepper species *C. annuum*, *C. frutescens* and *C. chinense* tolerate an EC_w of up to 1.78, 2.71 and 1.55 dS m^{-1}, respectively. *C. frutescens* stood out as the most tolerant species (Figure 3D).

CONCLUSIONS

An increase in irrigation water salinity reduces the emergence, growth and phytomass accumulation of the pepper species *C. annuum*, *C. frutescens* and *C. chinense*. These species tolerate an EC_w of up to 1.78, 2.71 and 1.55 dS m^{-1} respectively in their initial development stages; *C. frutescens* is the most tolerant and *C. chinense* is the most sensitive to salt stress among the species evaluated.

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