

## Emergence and phytomass accumulation of millet seedling under salt stress in semiarid conditions

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**ABSTRACT:** Millet is a crop of great agricultural importance, requires low investment, and it is able to develop rapidly in different environmental conditions, becoming an alternative for small producers in the Brazilian semi-arid region, mainly for forage production in times of water resources scarcity. The analysis of salinity effects on irrigation water in agricultural cultivation areas in semi-arid regions is extremely important for the elaboration, implementation, and execution of adequate management proposals. The effect of different salt concentrations on irrigation water during the emergence of millet seedlings (*Pennisetum glaucum* L. BR.) was studied. The experiment was set up in a greenhouse at the Federal Rural University of Pernambuco, Academic Unit of Serra Talhada, State of Pernambuco, Northeast region, Brazil, in the period from May 30 to June 11, 2018, using a completely randomized design with four treatments and five replicates, with 24 seeds in each plot. Treatments consisted of four saline concentrations (0 (distilled water), 4, 8, and 12 dS m<sup>-1</sup>) achieved by adding sodium chloride (NaCl) in the water. Variables assessed were seedling emergence percentage, emergence speed index, shoot length, root length, root dry mass, and dry shoot mass. Increasing salt concentrations in the irrigation water negatively affects the emergence of millet seedlings; thus, the maximum saline concentration recommended is 8 dS m<sup>-1</sup>.

**Key words:** forage; *Pennisetum glaucum*; salinity

## Emergência e acúmulo de fitomassa de plântulas de milheto sob condições de estresse salino

**RESUMO:** O milheto é uma cultura de grande importância agrícola já que consegue se desenvolver de forma rápida em diferentes condições ambientais, tornando-se uma alternativa para pequenos produtores do Semiárido brasileiro, principalmente na produção de forragem para os animais em épocas de escassez hídrica. A análise dos efeitos da salinidade na água de irrigação em áreas de cultivo agrícola em regiões semiáridas é de extrema importância para a elaboração, implantação e execução de propostas de manejo adequadas. Objetivou-se avaliar o efeito de diferentes concentrações salinas na água de irrigação sobre a emergência de plântulas de milheto (*Pennisetum glaucum* L. BR.), cv. IPA Bulk-1-BF. O ensaio foi conduzido em casa de vegetação na Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, PE, região Nordeste, Brasil, no período de 30 de maio a 11 de junho de 2018, sendo avaliado apenas a fase de plântula do primeiro ciclo, adotando-se o delineamento inteiramente casualizado, com quatro tratamentos e cinco repetições. Os tratamentos foram constituídos das concentrações salinas 0 (água destilada), 4; 8 e 12 dS m<sup>-1</sup>, obtidas através da adição de cloreto de sódio (NaCl) em água. As variáveis avaliadas foram: porcentagem de emergência de plântulas, velocidade de emergência, tempo médio de emergência, comprimento da parte aérea e do sistema radicular, porcentagem de sobrevivência e massa seca da parte aérea e do sistema radicular. O aumento da concentração de sais na água de irrigação afeta negativamente a emergência de plântulas de milheto, sendo recomendada água com concentração salina de até 8 dS m<sup>-1</sup>.

**Palavras-chave:** forragem; *Pennisetum glaucum*; salinidade



## Introduction

Irrigation is a practice to ensure agricultural production, especially in arid and semi-arid regions, where annual evaporative rate frequently exceeds rainfall in the same period. On the other hand, inadequate irrigation management and drainage deficiency, associated with characteristics of the soil source material and geomorphological and hydrological conditions, may cause soil salinization, reducing plant growth or development ([Bezerra et al., 2010](#)).

High concentrations of soluble salts in the soil solution, mainly NaCl but also other ions such as  $Mg^{2+}$ ,  $HCO_3^-$ , and  $SO_4^{2-}$ , causes plant growth inhibition due to decrease of water potential of the soil solution at levels below that required by plants to absorb water ([Andrade et al., 2021](#)).

Millet (*Pennisetum glaucum* (L.) R. BR.) is considered a moderately tolerant species to salinity; however, seed germination rate and emergence may be reduced significantly when seeds are subjected to high salt concentrations in the irrigation water ([Lucena et al., 2020](#)). Few studies are found in the literature on the effect of salinity on the seedling stage of millet. In such studies, an increase in the amount of salts in the soil due to use of poor-quality irrigation water brings adverse consequences on morphology, anatomy, and physiology of that crop ([Hussain et al., 2010](#); [Lucena, et al., 2019](#)). According to [Hussain et al. \(2010\)](#), the percentage of germination, height, grain development, and millet straw yield decreased with increasing salinity.

Given the above, the objective was to evaluate the effect of different salt concentrations in irrigation water on the emergence and phytomass of millet seedlings (*Pennisetum glaucum* L. BR. (Cv. IPA Bulk-1-BF)), with the purpose of establishing a critical level of irrigation water salinity in the initial growth stage of the crop.

## Materials and Methods

The experiment was conducted in a greenhouse at the Universidade Federal Rural de Pernambuco (UFRPE), Unidade Acadêmica de Serra Talhada (UAST) located in the semi-arid region of Pernambuco (7° 57'24.57" South; 38° 17'44.72" West, and 510 m of altitude) from 78 days, in the period from May 30 to June 11, 2018. The climate of this region is the BSw<sup>h</sup> type according to the Köppen classification, and annual averages of rainfall, temperature, and relative air humidity are 642 mm, 24.8 °C, and 62.5%, respectively ([Silva et al., 2015](#)).

The experimental design adopted was completely randomized, with four treatments (saline solutions of NaCl, calibrated for electrical conductivities in the irrigation water (CE<sub>ai</sub>) of 4.0; 8.0; 12.0 and 0 dS m<sup>-1</sup> (distilled water)) and five repetitions ([Rhoades et al., 1992](#)). Each plot consisted of 24 cells of Styrofoam trays (67.4 × 34 × 6.1 cm) made for production of vegetable seedlings. Each cell (67.4 × 34 × 6.1 cm) had a millet seed (cv. IPA Bulk 1 BF) and a substrate composed of washed sand and vermiculite at a ratio of 1:1. The seedling stage of the first millet cycle was evaluated.

Immediately after sowing, the substrate was moistened to field capacity with the solution for each level of salinity. During the conduction of the experiment, saline solutions were applied to the respective plots once a day to keep the humidity close to field capacity, making the substrate saturated.

To assess the effect of saline stress, the following variables were measured: Seedling emergence percentage (EP): determined up to the 12th day after sowing (DAS) based on daily observations of emergence; seedlings above the substrate surface were considered emerged seedlings.

Average emergency speed (AES): determined according to the methodology proposed by [Labouriau & Valadares \(1976\)](#), with results expressed in days.

Average emergence time (AET): determined by the daily count of seedlings emerged according to the methodology proposed by [Labouriau & Valadares \(1976\)](#), with results expressed in days.

Length of shoot and root system: shoot length was measured from the base of the plant stem to the apex of the rudimentary leaves of the seedling, and the root length was obtained from the base of the plant stem to the end of the larger root, with the aid of a ruler graduated in millimeters. Data were expressed in centimeters.

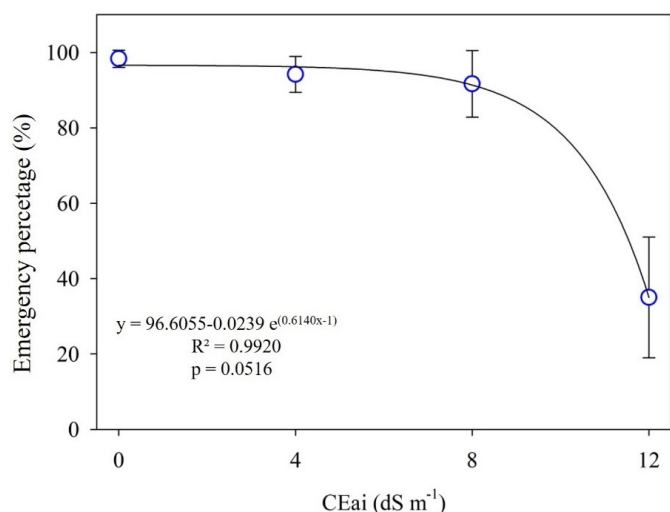
Survival percentage: determined at the end of the experiment (12 DAS), based on the number of live seedlings and the number of cells sown in each plot (survival percentage = [number of live seedlings/24] × 100).

Dry mass of the root system (SR) and dry mass of the aerial part (PA): after measuring the surviving seedlings, they were excised in the neck region, separating the aerial part from the root system. Subsequently, the seedlings were stored in paper bags and dried in a forced-air circulation oven at 65 °C for 72 hours to determine dry mass, following the methodology described by [Detmann et al. \(2012\)](#). After this period, samples were weighed on an analytical balance (0.0001 g) and the results of the dry mass of PA and SR were expressed in g seedling<sup>-1</sup>.

Except for the percentage of emergence and length of the aerial part, for which it was adopted the p-value ([R Core Team, 2018](#)), all results obtained were submitted to analysis of variance using the F test at 5% of probability. After the analysis of variance, means were submitted to regression analysis, with the exception of length of the root system. For this variable, means were compared by Tukey test at a probability level of 5%.

## Results and Discussion

The percentage of seedling emergence was influenced ( $p = 0.0516$ ) by increasing levels of salinity in the solution ([Figure 1](#)). An average of 35% was found in the largest EC<sub>iw</sub> (12 dS m<sup>-1</sup>), which in turn was lower than the other levels evaluated. As salinity of the solution increased, the percentage of emergence decreased, with the largest drop being observed from 8 to 12 dS m<sup>-1</sup>. The values of the percentage of emergence for each EC



**Figure 1.** Percentage of emergence (%) of millet seedlings, cv. IPA Bulk-1-BF, depending on salinity. ECiw = Electrical conductivity of irrigation water. Bars represent standard deviations. Open circles represent mean values ( $n = 5$ )  $\pm$  standard deviation.

level of the irrigation water, 0, 4, 8, 12 dS mL<sup>-1</sup>, were estimated for the equation, where the values were obtained: 96.57, 96.18, 91.58, and 38.00%, respectively.

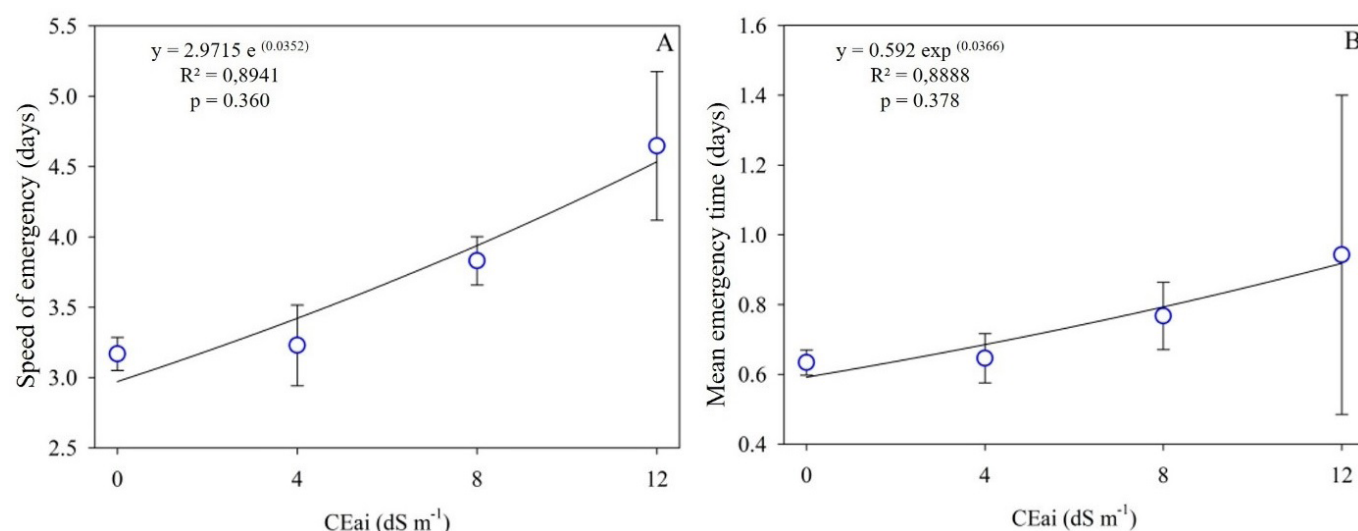
According to [Gomes et al. \(2021\)](#), water is absorbed through natural openings in the seed coat by diffusion and spreads through all tissues. The absorbed water induces cells to become turgid, which increases their size and makes the seed coat more permeable to oxygen, carbon dioxide, and salts. In this sense, the decrease in the percentage of seedling emergence at the 12 dS m<sup>-1</sup> level can be explained by the absorption of NaCl salts, which provokes dehydration (given their hygroscopic property) and leads to embryonic death of the seedling.

In addition, when high concentrations of salts are dissolved in the soil solution there is an increase in the osmotic potential, decreasing water availability. With greater osmotic

pressure, physiological drought may occur, and depending on the degree of salinity the plant may lose water rather than absorb it. According to [Pádua \(2019\)](#), water availability is pivotal to the germination, emergence, initial root growth, and elongation of plant tissues. Thus, the lower percentage of emergence observed with the treatment of 12 dS m<sup>-1</sup> ([Figure 1](#)) can also be attributed to an increase in osmotic pressure caused by excess of salts and, consequently, reduced water availability. [Vikrant & Roobavathi \(2020\)](#) found that rising levels of salinity impaired germination of millet seeds.

It was also found that salinity affected the emergence speed and the average emergence time in millet seedlings ( $p < 0.05$ ). The highest salinity level (12 dS m<sup>-1</sup>) promoted a 32% and 34% reduction in emergence speed and emergence time of the seedlings, respectively, when compared to the lowest salinity level (0 dS m<sup>-1</sup>) ([Figure 2B](#)). The emergency speed ranged from  $Y_{EST} = 4.53$  days for the highest electrical conductivity (12 dS m<sup>-1</sup>) to  $Y_{EST} = 2.97$  days for the lowest (0 dS m<sup>-1</sup>) ([Figure 2A](#)). The estimated values for speed of emergency of the equation in [Figure 2A](#) were 2.97; 3.42; 3.94, and 4.53 days, with EC of 0, 4, 8 and 12 dS m<sup>-1</sup>, respectively. For the equation of mean emergence time ([Figure 2B](#)) the estimated values were 0.59; 0.69; 0.79 and 0.92 days, for each EC of 0, 4, 8 and 12 dS m<sup>-1</sup>, respectively.

The delay in emergence speed and the increase in average emergence time shown in [Figure 2](#) occurs due to an impairment in seed vigor. A possible explanation is that depletion of reserves and induction of disturbances in cell membranes intensified with greater salinity. Thus, according to [Nguyen et al. \(2021\)](#), characteristics related to vigor show greater sensitivity to salinity than those related to germination. An increase in osmotic pressure caused by the salts in the substrate solution is another factor to consider; this effect compromised imbibition of the seed, influencing embryonic growth, limiting the seedling emergence process and phytomass production. The results of the present study are similar to those of [Sá et al. \(2013\)](#) have shown that



**Figure 2.** Speed (A) and mean emergence time (B) in millet seedlings, cv. IPA Bulk-1-BF, depending on salinity. ECiw = Electrical conductivity of irrigation water. Open circles represent mean values ( $n = 5$ )  $\pm$  standard deviation.

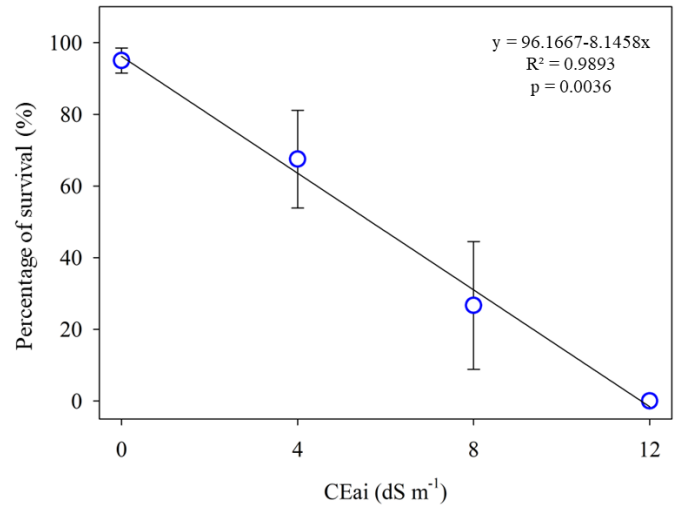
elevation of the sodium chloride concentration exert toxicity to seedlings, triggering ionic and hormonal changes in plant metabolism.

The length of the root system at 12 DAS was influenced ( $p < 0.05$ ) by salinity, where seedlings that emerged in an environment with a  $0 \text{ dS m}^{-1}$  solution presented, on average, 7 cm in root length whereas seedlings immersed in the substrate irrigated with the  $12 \text{ dS m}^{-1}$  solution did not develop their root system (Figure 3A). For the aerial part, the level  $0 \text{ dS m}^{-1}$  promoted an average of  $Y_{\text{EST}} = 5.53 \text{ cm}$ , whereas the  $12 \text{ dS m}^{-1}$  level supported no growth (Figure 3B). It is worth mentioning that, for the referred variables, only live seedlings at the end of the experiment were taken into account. The maximum estimated shoot growth was 5.90 cm, which corresponds to a level of  $2.41 \text{ dS m}^{-1}$  of electrical conductivity.

Corroborating the results observed in this trial, Sam et al. (2014) found that the length of the aerial part and fresh and dry masses of millet seedlings decreased with increasing concentrations of salts. Bukhat et al. (2020) and Nassar et al. (2020) pointed out a reduction in the dry mass of plant tissues associated with both the adaptation to salts and a reduction of photosynthetic rates per unit of leaf area.

Regarding the intermediate levels of salinity ( $4$  and  $8 \text{ dS m}^{-1}$ ), growth averages of  $5.8 \text{ cm}$  were observed for the root system. These data show that the growth of the root system did not change significantly at these salinity levels, but it was lower than that observed at the lowest salinity level ( $0 \text{ dS m}^{-1}$ ).

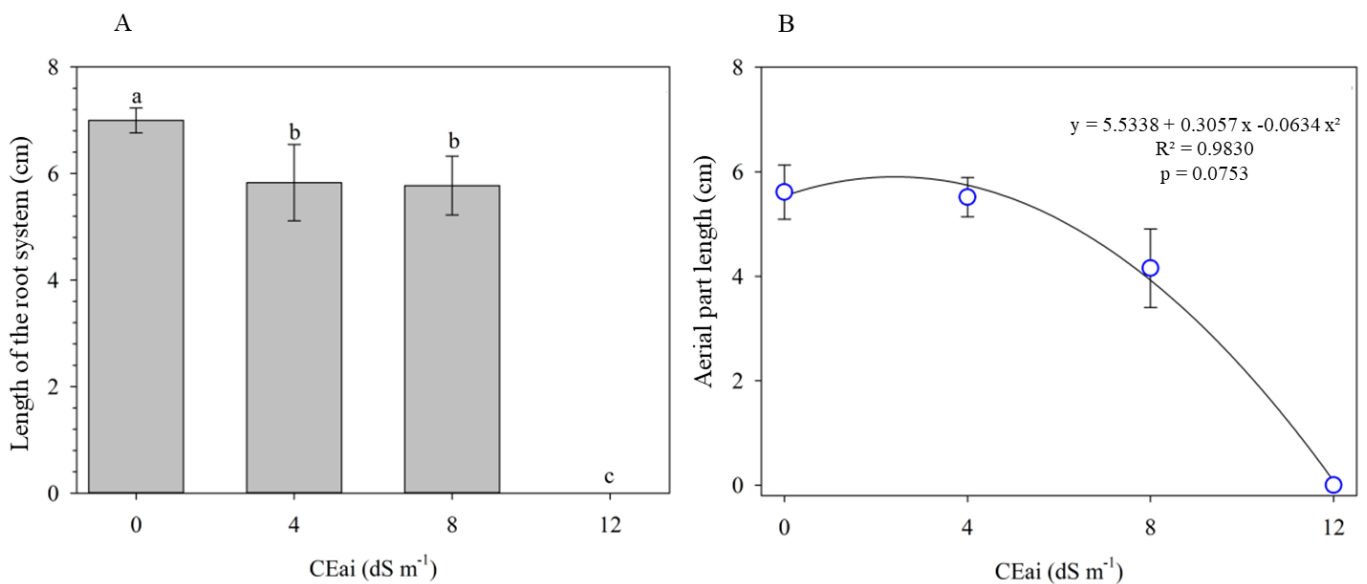
There was a reduction in the survival percentage of millet seedlings with the addition of salts in the irrigation water (Figure 4). For each unit increase in electrical conductivity, percentage of survival declined by an average of approximately ( $i = -8.146 \%$ ). The percentage of survival varied from  $0\%$ , at the highest concentration of NaCl in the irrigation water ( $12 \text{ dS m}^{-1}$ ), to  $95\%$  ( $0 \text{ dS m}^{-1}$ ) ( $i = -1.58 \%$ ). The estimated values



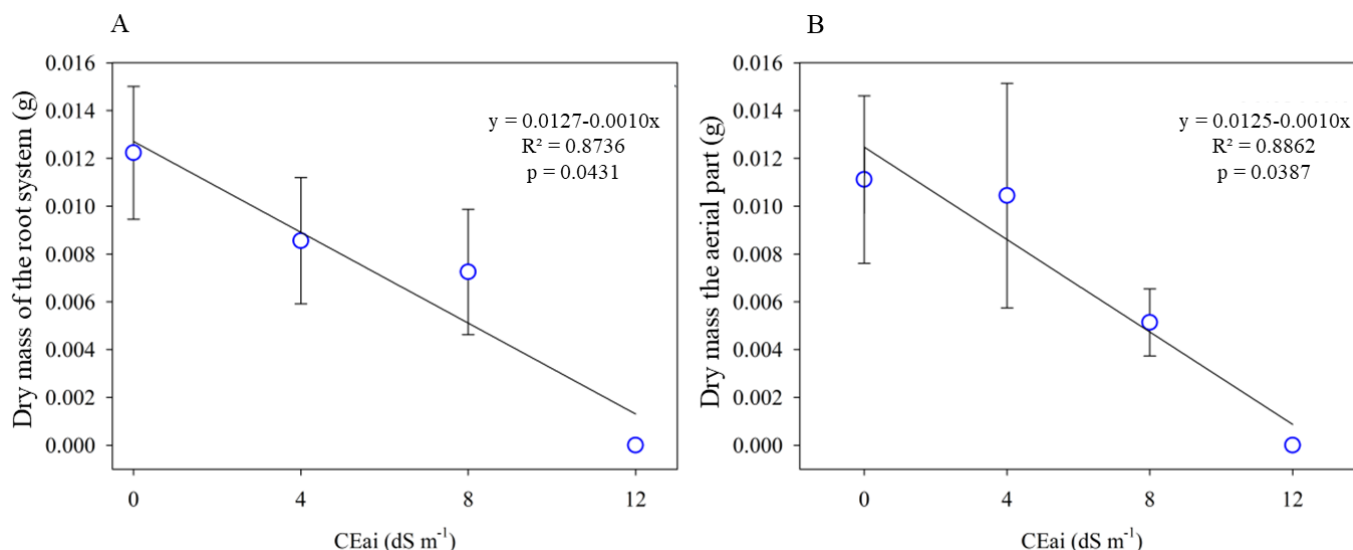
**Figure 4.** Percentage of survival (%) in millet seedlings, cv. IPA Bulk-1-BF, depending on salinity. ECi = Electrical conductivity of irrigation water. Open circles represent mean values ( $n = 5$ )  $\pm$  standard deviation.

for percentage of survival were 96.17, 63.59, 31.00, and  $-1.58\%$ , for EC levels of  $0$ ,  $4$ ,  $8$ , and  $12 \text{ dS m}^{-1}$ , respectively. For the YCE interval, the rate of decrease was  $32.58\%$ .

The seedling mortality (Figure 4) identified when seedlings were exposed to the salinity level of  $12 \text{ dS m}^{-1}$  is related to the deleterious effects of salinity on initial seedling growth. According to Hailu & Mehari (2021), excess salts can clog pores and alter physical and structural properties of the soil, such as hydraulic conductivity, infiltration, and aeration, and drastically reduce its productive capacity. Also, water deficiency is among the harmful factors of salinity on seedling emergence, since the presence of salts interferes with the water potential of the soil, diminishing the gradient potential between the soil and the seedling, which will negatively



**Figure 3.** Length (cm) of the root system (A) and aerial part (B) in millet seedlings, cv. IPA Bulk-1-BF, depending on salinity. ECi = Electrical conductivity of irrigation water. Equal letters do not differ statistically by Tukey test ( $p > 0.05$ ). Bars (A) and open circles (B) represent mean values ( $n = 5$ )  $\pm$  standard deviation.



**Figure 5.** Dry mass (g) of the root system (A) and aerial part (B) in millet seedlings, cv. IPA Bulk-1-BF, depending on salinity. ECiw = Electrical conductivity of the irrigation water. Open circles represent mean values ( $n = 5$ )  $\pm$  standard deviation.

impact water absorption and consequently, water availability (Li et al., 2019; Navaz et al., 2020).

The results shown in figure 4 corroborate those found by Oliveira & Gomes Filho (2011), who demonstrated reductions of 31.3 to 50% in the percentage of survival of sorghum seedlings when irrigated with saline water, under NaCl additions. According to Lopes & Guilherme (2007), taking into account the classification established by the U.S. Salinity Laboratory, soils with ECas of 4 dS m<sup>-1</sup> reduce growth and development of most crops by more than 50%. In this sense, it can be inferred that the  $Y_{EST} = 32.6$  decrease in the percentage of survival at the 4 dS m<sup>-1</sup> level is due to intrinsic tolerance of the millet culture to salt stress.

Rising levels of salinity in the irrigation water reduced dry mass values of the root system and the aerial part (Figure 5B). For each EC unit, there was a reduction of 7.87% and 8.00% in the dry mass of the root system and in the dry mass of the shoot, respectively. For root system dry mass, YES values of 0.0127, 0.0087, 0.0047, and 0.0007 g correspond to EC levels of 0, 4, 8, and 12 dS m<sup>-1</sup>, respectively. For shoot dry mass, YES values of 0.0125, 0.0085, 0.0045, and 0.0005 g correspond to EC levels of 0, 4, 8, and 12 dS m<sup>-1</sup>, respectively.

A linear reduction was observed, both in the dry mass values of the root system and of the aerial part, with the increase in salinity (Figure 5A). For the roots, the treatments of 0 and 4 dS m<sup>-1</sup> did not differ from each other, with average values of 0.0122 and 0.0086 g, respectively (Tukey,  $p < 0.05$ ). For the aerial part, there was a trend of reduction with the increase in salinity levels of the irrigation water. As in the roots, the aerial parts did not differ between the levels of 0 and 4 dS m<sup>-1</sup>, with average values of 0.0111 and 0.0104 g, respectively (Tukey,  $p < 0.05$ ).

These results corroborate those of Lima et al. (2015), who found a progressive reduction in length of the aerial part and length of the root system with the increase in salinity

in seedlings of *Albizia lebeck* (L.) Benth. The same authors pointed out that excess of salts in the root zone reduces dry mass accumulation in the seedling because of an extra supply of energy that needs to be triggered to promote water absorption from the substrate, since an excess of salts diminishes the water potential of the soil, in addition to reducing plant transpiration rates.

## Conclusion

The increase in the concentration of salts in the irrigation water promotes a reduction in the emergence and accumulation of phytomass in seedlings of *Pennisetum glaucum* L. BR., Cv. IPA Bulk-1-BF, with a level of 12 dS m<sup>-1</sup> being highly harmful to the species. However, this crop (considered moderately salinity tolerant) can support an electrical conductivity of irrigation water of up to 4 dS m<sup>-1</sup> in its molt phase.

## Compliance with Ethical Standards

**Author contributions:** Conceptualization: CWFB, VJLPS, JLPSI, MSS, VIT; Data curation: CWFB, VJLPS, JLPSI, MSS; Formal analysis: CWFB, VJLPS, JLPSI, MSS; Investigation: CWFB, VJLPS, JLPSI, MSS, MADSCP, VIT; Methodology: CWFB, MSS, MADSCP, VIT; Project administration: MADSCP, VIT; Resources: MADSCP, VIT; Supervision: VIT; Writing – original draft: CWB, VJLPS, JLPSI, MSS.

**Conflict of interest:** The authors declare that there is no conflict of interest.

**Financing source:** The authors thank the Fundação de Amparo à Pesquisa do Estado de Pernambuco (FACEPE) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brazil (CAPES) for the financial support - Finance Code 001.

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