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Agronomic characteristics of the *Pennisetum glaucum* submitted to water and saline stresses

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ABSTRACT. Agricultural production in semi-arid regions is limited due to water availability. In addition, the water quality available for irrigation is often compromised due to the high salt content present. Millet is a forage species considered tolerant to water deficit and moderately salt tolerant. In view of the above, the objective was to evaluate the growth of millet under water and saline stress associates. The experiment was carried out in a randomized block design, in a 4x3 factorial scheme, composed of four levels of water replacement, based on crop evapotranspiration (ETc): 25%.ETc, 50%.ETc, 75%.ETc and 100%.ETc and three levels of water salinity (0.03, 2.0 and 4.0 dS m^{-1}). With 25%.ETc independent of salinity, all morphological characteristics of millet were affected, occurring death of plants in the initial growth phase. In the absence of salt and greater availability of water, greater plant growth occurred. With respect to salinity, there was a reduction in the increment of all variables evaluated, with the highest reduction at the highest saline level (4.0 dS m^{-1}). Water and salt stresses, when associated, reduce the growth of millet, since concentrations above 2.0 dS m^{-1} and less than 50%.ETc compromise its full development, providing declines in yield.

Keywords: water availability; abiotic stresses; forage; millet; salinity.

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Introduction

Arid and semi-arid regions cover about a third of the world's territory and are inhabited by around 400 million people. In these areas, agricultural production is limited due to low water availability. In the Brazilian semiarid, this condition is caused due to the edaphoclimatic characteristics, where high variability can be observed in the spatiotemporal distribution of rainfall (Pereira Filho, Silva, & Cézar, 2013). In addition, the quality of water available for irrigation is often compromised due to the high salt content present. Several authors have pointed out water and salt stresses as the main factors affecting agricultural production in semiarid regions (Yahmed et al., 2016; El-Mageed, El-Samnoudi, Ibrahim, & Tawwab, 2018). In this way, the productivity losses caused due to irregular rainfall and the poor quality of water available for irrigation make agriculture a very vulnerable and risky activity in these regions.

One of the main economic activities in the Brazilian semiarid is the raising of ruminants. However, the low forage supply of native pasture in the dry period of the year makes it necessary to introduce forage plants to increase the availability of food for herds, making animal-raising activity competitive. Under this condition, the use of groundwater irrigation or rain catchment (Bezerra, Lacerda, Hernandez, Silva, & Gheyi, 2010) can be useful in order to make production feasible.

It is necessary to take into account that the groundwater in a large part of the Brazilian semiarid region has a high concentration of salts, and its use can contribute to the increase in salinized areas (Silva et al., 2017). Under these conditions, the use of species that are more resilient to environmental changes is necessary in order to increase productivity and ensure the availability of forage.

Millet (*Pennisetum glaucum* (L.) R. Br.) is a species of tropical origin with C4 metabolism, tolerant to moderate water deficit and high temperatures (Melo, Fernandes, & Galvão, 2015), being necessary, on average, 350 mm of water per cycle (Ullah, Ahmad, Khaliq, & Akhtar, 2017; Singh, Reddy, Reddy, & Reddy, 2015), which can be used as an alternative to pasture cultivated in semiarid regions, due to the high production of biomass and nutritional value (Ghatak et al., 2016).

Changes in the structural and morphogenic characteristics of millet can be indicative of stress, therefore, the evaluation of these growth characteristics becomes the most efficient way to identify these responses in the short term. According to Ullah et al. (2017), millet uses 70% of the water consumed by corn to produce the same amount of dry matter, proving to be a promising species for cultivation in semi-arid regions and under deficient irrigation conditions. In the Brazilian semi-arid region, studies on the effect of salinity on millet are scarce and necessary, as this crop has great productive potential for the region. Given the above, the objective was to evaluate the growth of millet subjected to water and saline stresses.

Material and methods

Research was carried out at the Forage Culture Sector of the Federal Rural University of Pernambuco (Serra Talhada County Campus), in the semiarid region of Pernambuco State, northeastern Brazil (07° 56' 15" S, 38° 18' 45" E, at an elevation of 429 meters). According to the Köppen classification, the climate is BSwh' type, called semi-arid, hot and dry, summer-autumn rains with average annual rainfall of 647 mm year^{-1} and average air temperatures above 25°C (Leite, Lucena , Sá Junior, & Cruz, 2017).

The experiment was carried out in randomized blocks, in a 4x3 factorial scheme, composed of four levels of water replacement, based on the culture evapotranspiration (ETc): 25%.ETc, 50%.ETc, 75%.ETc and 100%.ETc and three levels of water salinity (0.03; 2 and 4.0 dS m^{-1}), with four replications, totaling 48 experimental units. The water used in the control treatment (0.03 dS m^{-1}) is classified as C1, with no use restrictions; water of electrical conductivity (CEa) 2.0 dS m^{-1} is classified as C3, with a high risk of salinization; 4.0 dS m^{-1} CEa water is classified as C4, with a risk of salinization considered very high (Sales, Lopes, Meireles, Chaves, & Andrade, 2014). The salinity levels in the irrigation water were obtained by adding the sodium chloride salt (NaCl) corresponding to 1.168 g L^{-1} and 2.337 g L^{-1}, respectively for the levels of 2 and 4.0 dS m^{-1}.

An Cambisol (Cambissolo Háplico Eutrófico, Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2013) was used, collected at a depth of 0-20 cm, then homogenized and passed through a 2.0 mm sieve. Subsequently, about 10 kg of this soil was placed in plastic pots with a volume of 14.41 dm^3, drilled at the bottom and with a 2.0 cm layer of gravel to drain irrigation water. A sample composed of soil was collected for purposes of fertility analysis, where the following characterization was obtained: pH (water) = 6.80; P (Mehlich I extractor) = 40.0 mg dm^{-3}; K^+ = 0.45 cmol$_c$dm^{-3}; Ca^{2+} = 5.50 cmol$_c$dm^{-3}; Mg^{2+} = 1.6 cmol$_c$dm^{-3}; Al = 0.0 cmol$_c$dm^{-3}.

The millet cultivar (*Pennisetum glaucum* (L.) R. Br.) used in this experiment was IPA Bulk 1BF. Sowing took place on September 28, 2017 with four seeds per pot, 2.0 cm deep. Three days after sowing, 100% germination occurred. All pots were kept in the field capacity, aiming to establish the plants. Thinning occurred 10 days after emergence (DAE), removing the smaller and weaker plants, leaving only two plants per pot.

The blades used were determined from the culture evapotranspiration (ETc), obtained by the reference evapotranspiration product (ETo) and the culture coefficient (Kc). The Kcs used were 0.4; 0.68 and 1.14 for the initial growth, vegetative and flowering phases, respectively. Due to the absence of Kcs suitable for the millet culture, the Kcs of the sorghum culture were used, which presents morphophysiological characteristics similar to the studied culture.

To calculate ETo, the Penman-Monteith method standardized by FAO Bulletin 56 (Abreu, Silva, Teodoro, Holanda, & Sampaio Neto, 2013) was used, using meteorological data (Figure 1) obtained through a Meteorological Station Automatic, belonging to the National Meteorological Institute (INMET, www.inmet.gov.br), located 290 m from the experimental area.

The application of treatments started at 15 DAE, with water replenishment every three days. In total, the blades applied were: 9.11, 18.23, 27.34 and 35.74 L, for treatments of 25, 50, 75 and 100% ETc, respectively.

To evaluate the influence of treatments, biometric evaluations were carried out to obtain the following parameters: plant height, stem diameter, stem length, number of tillers, number of dead leaves, number of live leaves, width and leaf blade length. These started at 25 DAE and were spaced every 10 days, throughout the culture cycle. The plant height was measured with a millimeter measuring tape and included the distance from the base of the stem to the end of the curvature of the last fully expanded leaf; the stem length comprised the interval between the base and its apex; the stem diameter was determined at 5.0 cm from the ground level using a digital caliper; the leaf blade length was determined along the central rib, considering the insertion point of the ligula with the leaf blade up to its apex, in the older fully expanded leaf; leaf blade width was measured in the middle region of the oldest fully expanded leaf; number of live leaves, counting only the leaves that had more than 70% green color; number of dead leaves comprised all leaves with less than 70% green color; number of tillers, by accounting for all tillers of the plant.

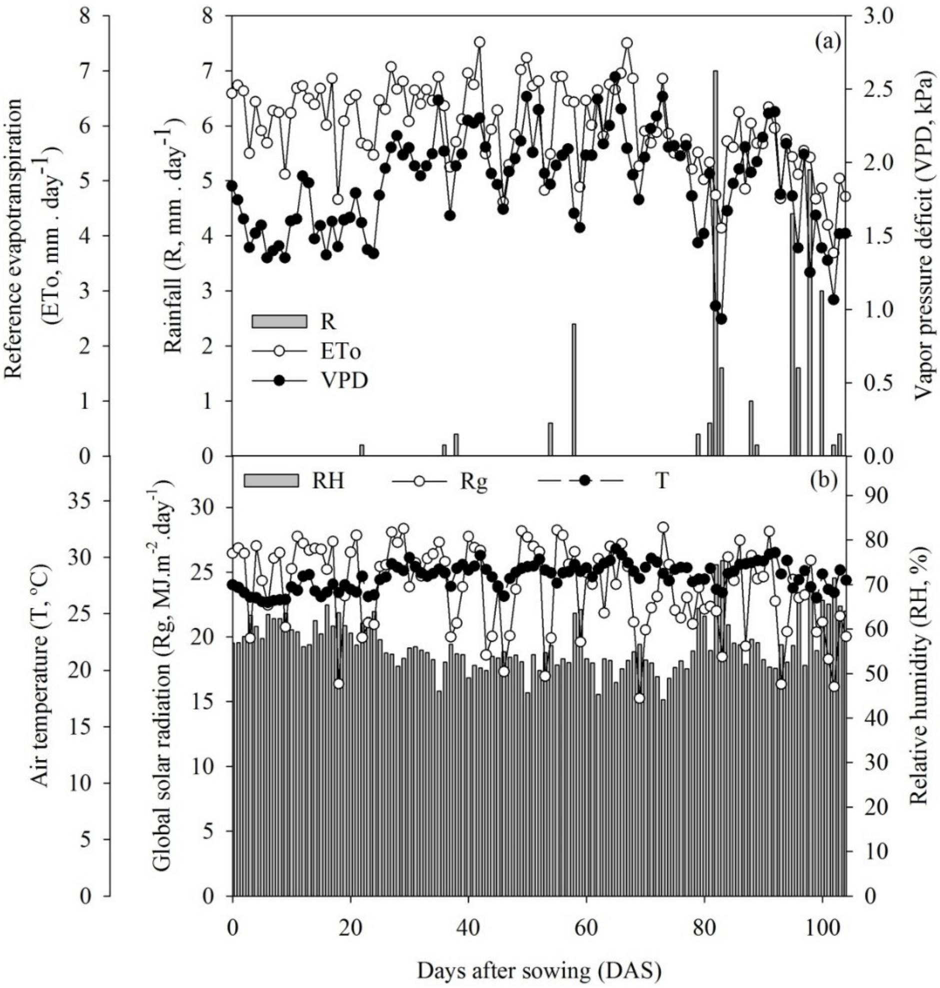

Figure 1. Variation of reference evapotranspiration, rainfall, vapor pressure deficit (a), air temperature, global solar radiation and relative humidity (b), in the experimental area between 02/10/2017 and 01/18/2018.

The models were chosen using the model determination coefficient (R^2) as a criterion. The level of 5% of probability for rejection of the null hypothesis was adopted, using software R - Project version 2.13.1. (R Core Team, 2019).

The results were submitted to the Kolmogorov – Smirnov normality test, analysis of variance and regression analysis. The regression models used to assess the variables under study were as follows:

Linear: $Y = b_0 + b_1x + \epsilon$

Quadratic: $Y = b_0 + b_1x + b_2x^2 + \epsilon$

Cubic: $Y = b_0 + b_1x + b_2x^2 + b_3x^3 + \epsilon$

Exponential: $Y = a * \exp(b_0 + b_1x)\epsilon$

Logistic: $Y = \frac{a}{1+\exp(b_0 + b_1x)} + \epsilon$

where, Y is the analyzed response variable; x is the variable days after emergence and ϵ is the random error, where ϵ follows normal distribution of mean 0 and constant variance $\sigma^2 > 0$. The unknowns a, b_0 and b_1 are the parameters to be estimated by the models.

The models were evaluated using the model determination coefficient (R^2), expressed by:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(Y_i - \widehat{Y}_i)^2}{\sum_{i=1}^{n}(Y_i - \overline{Y}_i)^2}$$

where, Y_i is the value of the i-th observation of the response variable; $\widehat{Y}_i$ the i-th value of the response variable after adjusting the model and $\overline{Y}_i$ is the average of the observed values of the response variable. The level of 5% of probability for rejection of the null hypothesis was adopted, using software R - Project version 2.13.1. (R Core Team, 2019).

Results and discussion

Note that the smallest stem diameter was observed in the lowest water availability (25%.ETc) with values between zero and 20 mm, regardless of salinity (Figure 2). The low availability of water together with the accumulation of salts in the soil caused the death of plants from treatments of 25%.ETc and, therefore, the behavior of the other variables was only observed until 35 days after emergence (DAE).

In treatments that received 50%.ETc, the largest stem diameters were observed when the water salinity was 0 dS m^{-1} (Figure 2b), with little variation over time. This result indicates that a 50% reduction in the need for water for millet does not affect the stem diameter. When the salinity of the irrigation water increased to 2.0 dS m^{-1} and 4.0 dS m^{-1} there was a gradual reduction in stem diameter from 35 DAE, with a reduction of up to 100% at the end of the cycle (Figure 2b).

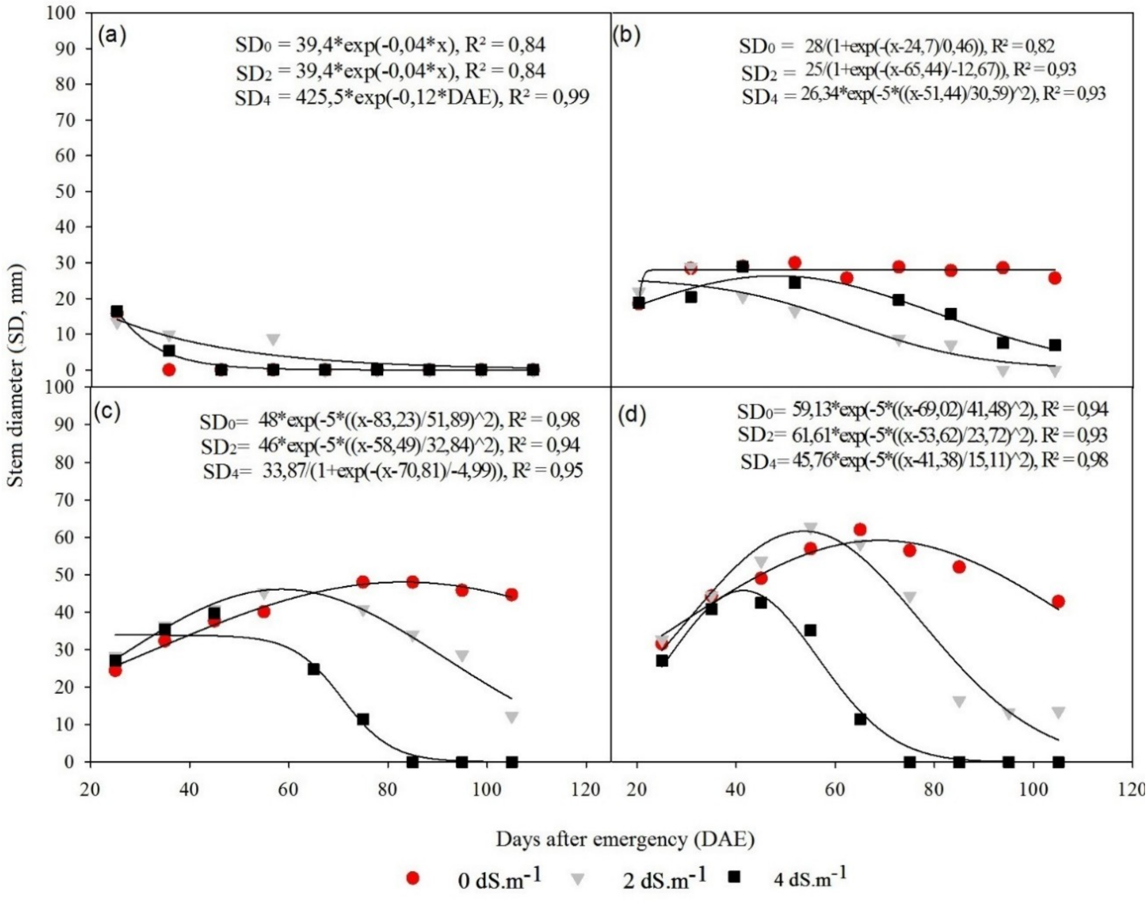

Figure 2. Stem diameter (mm) of millet under levels of 25% of the crop evapotranspiration (a), 50% of the crop evapotranspiration (b), 75% of the crop evapotranspiration (c) and 100% of the crop evapotranspiration (d) and three levels of salinity (0, 2.0 and 4.0 dS m^{-1}) as a function of the days after emergence (DAE).

The largest stem diameters occurred in the treatments irrigated with the blades of 75%.ETc and 100%.ETc, and both showed similar behavior throughout the cycle.

The largest stem diameters occurred in the treatments irrigated with the blades of 75%.ETc and 100%.ETc with salinity of 0 dS m^{-1} (45 and 60 mm, respectively), and both showed similar behavior throughout the cycle (Figure 2c and 2d). In these treatments there was an increase in stem diameter up to 70 DAE (100%.ETc) and 75 DAE (75%.ETc), respectively, with a reduction after that time, generating quadratic models (DC_0) for both. This reduction after 70 DAE may be related to the senescence of the plant, since the point of physiological maturity of millet is approximately at 70 DAE. Under irrigation with saline water, there was an anticipation of a decrease in the stem diameter of millet irrigated with both blades.

In the treatments that received 2.0 dS m^{-1} salinity water, the increase in stem diameter only occurred until 55 DAE in the two largest blades, with values close to the treatments without salt, with no statistical difference between treatments ($p < 0.05$) (Figures 2c and 2d).

Plants irrigated with 75%.ETc and salinity of 4.0 dS m^{-1} suffered a reduction in stem diameter from 45 DAE, with plant death at 82 DAE due to the accumulation of salts in the soil. In the treatments irrigated with 100%.ETc and salinity of 4.0 dSm^{-1} the growth peak occurred at 35 DAE, remained slightly constant and there was a decrease thereafter, until causing plant death at 78 DAE.

The stem length (Figure 3) showed a similar behavior to the stem diameter, in the treatment of 25%.ETc.

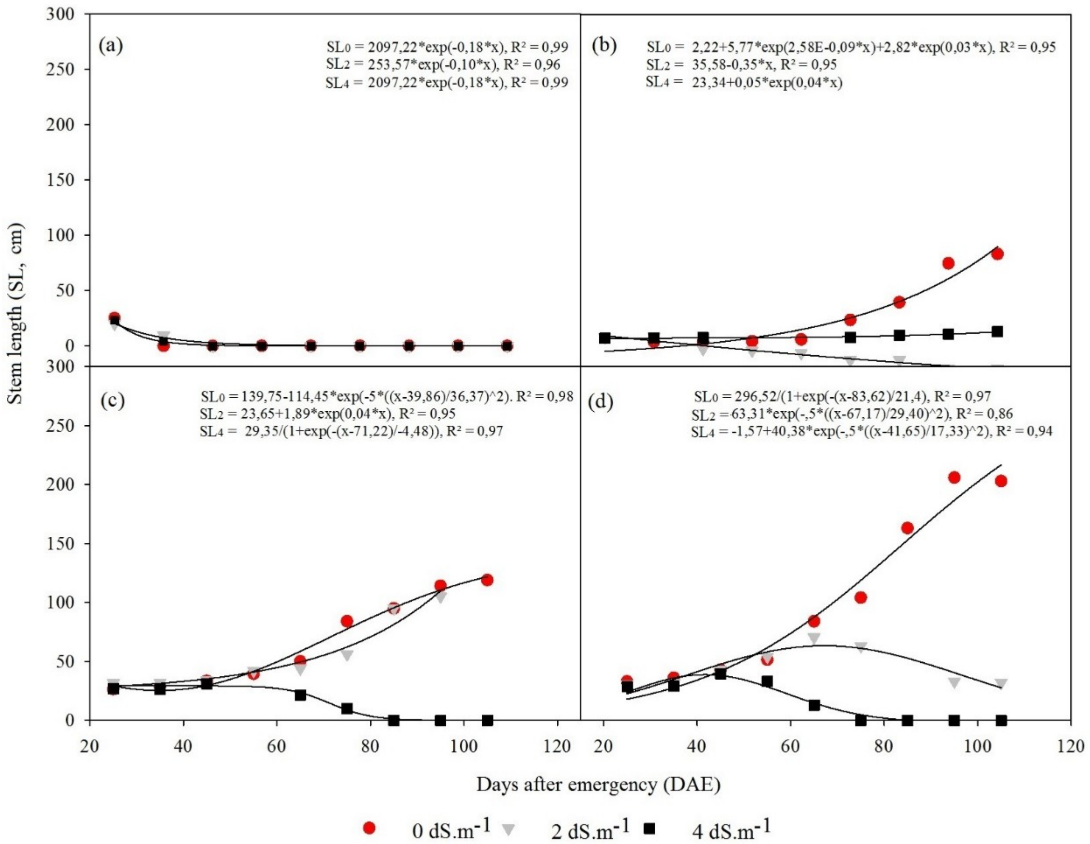

Figure 3. Millet stem length (cm) under irrigation of 25% of the crop evapotranspiration (a), 50% of the crop evapotranspiration (b), 75% of the crop evapotranspiration (c) and 100% of the crop evapotranspiration (d) with different levels of salinity (0, 2.0 and 4.0 dS m^{-1}) depending on the days after emergence (DAE).

When submitted to the blades of 50%, 75% and 100%.ETc and 0 dS m^{-1}, the stem length of the millet showed exponential growth as a function of time, this growth was all the greater the more water available (Figures 3b, 3c, 3d). When subjected to salinity of 2.0 dS m^{-1} there was a decrease in the length of the millet stalk when irrigated with 50%.ETc, however, an exponential growth of this variable under irrigation of 75%.ETc was observed, with this same level of salt. Therefore, observing these two behaviors, it is possible to state that under water deficit of 50%, millet did not show satisfactory growth and that under irrigation condition with 75%.ETc the salinity of 2.0 dS m^{-1} did not cause toxic effect on plant to the point of reducing its stem length. In the treatments that received water with salinity of 4.0 dS m^{-1}, there was a reduction in stem length regardless of the water layer applied.

The plant height of the millet also showed a difference between treatments ($p < 0.05$). In plants that received the treatment of 25%.ETc and 0 dS m^{-1} of salinity the maximum plant height was 64 cm (Figure 4a). The lower plant height in treatments with lower irrigation depth is due to water deficit, as it promotes less cell differentiation and fewer cells, compromising the expansion of plant tissues (Tardieu, Granier, & Muller, 2011).

With 50% available water and 0 dS m^{-1}, the plant height remained constant until 60 DAE, showing a slight increase thereafter (Figure 4b). In treatments with this same saline level and blades of 75%.ETc and 100%.ETc, the millet showed height of plant growing over time.

Under salinity of 2.0 dS m^{-1}, applying a blade of 75%.ETc the plants grew up to 65 DAE, reducing their height thereafter. Whereas in the 100%.ETc slide, growth only occurred until approximately 42 DAE (Figure 4c and 4d).

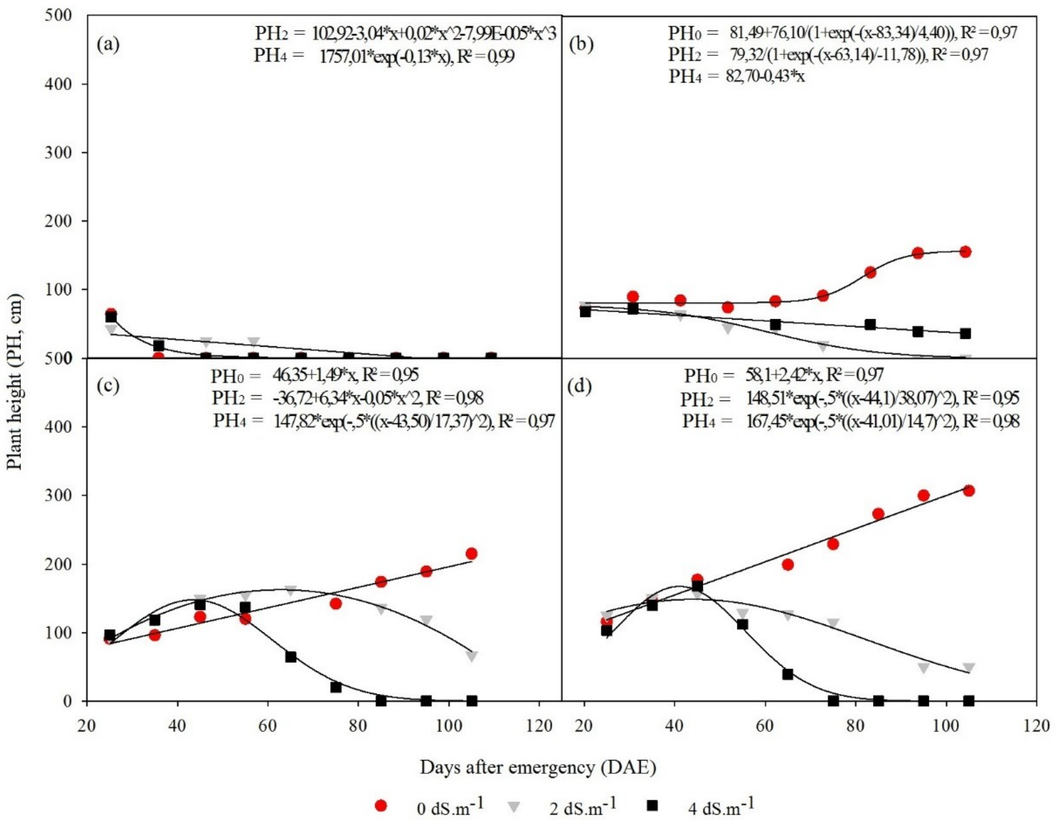

Figure 4. Millet plant height (cm) irrigated with 25% (a), 50% (b), 75% (c) and 100% (d) of the crop evapotranspiration (ETc) and salinity levels of 0, 2.0 and 4.0 dS m^{-1} depending on the days after the emergency (DAE).

The decrease in the plant height of millet in treatments that received the highest salt level (4.0 dS m^{-1}) is related to the accumulation of salts in the soil that can cause difficulty in water absorption, toxicity of specific ions in plants and by the interference of salts in the physiological processes (indirect effects), reducing the growth and development of the plant, which can result in death (Farias, Santos, Freire, & Silva, 2009; Neves, Lacerda, Teixeira, Costa, & Gheyi, 2010). The growth processes are particularly sensitive to the effects of salts, so the growth rate is a good criterion for assessing the degree of stress and the adaptive capacity of the plant to salt stress.

There was a variation in the number of live millet leaves under water and salt stress (Figure 5). As with the previous variables, the number of live leaves declined to 35 DAE in plants that received only 25%.ETc at the three levels of salinity (Figure 5a). On the other hand, there was no increase in the number of live leaves in the treatment plants of 50%.ETc and zero salt (Figure 5b). The treatments that received the highest salinity increased the number of live leaves over time.

As the plants that received 50%.ETc and 2.0 dS m^{-1} reduced their number of live leaves to zero at the end of the cycle and those with salinity of 4.0 dS m^{-1} reduced the number of live leaves but did not reach zero, it is consistent to state that under this blade, millet is able to keep its number of leaves alive despite the increase in the accumulation of salts. Probably the death of the leaves in the treatment of 2.0 dS m^{-1} occurred due to water stress and not to salt stress. Water stress triggers a series of physiological changes, such as stomata closure, reducing the entry of CO_2 into the mesophile, reducing the raw material for the photosynthetic process, which compromises the plant growth (Tardin et al., 2013).

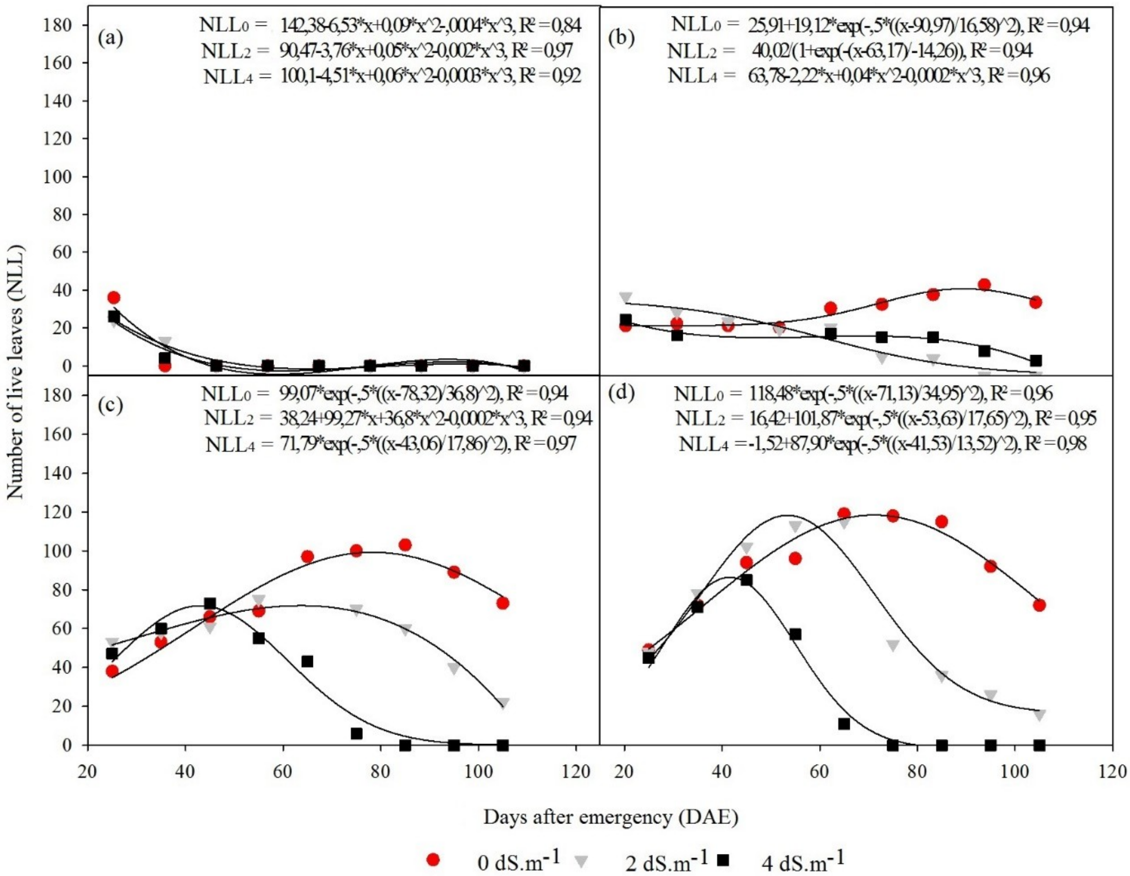

Figure 5. Number of live millet leaves irrigated with 25% of the crop evapotranspiration (a), 50% of the crop evapotranspiration (b), 75% of the crop evapotranspiration (c) and 100% of the evapotranspiration of culture (d) and water with three levels of salinity (0, 2.0 and 4.0 dS m^{-1}) as a function of days after emergence (DAE).

When the plants received slides of 75%.ETc (Figure 5c) and 100%.ETc (Figure 5d) with zero salinity, there was an increase in the number of live leaves up to 75 DAE and 65 DAE, respectively. But this decrease is related to the physiological maturity of the plant, which at this stage of growth redirects its energy to the filling of grain maturation. Receiving a blade of 75%.ETc and salinity of 2.0 dS m^{-1} (Figure 5c), millet reduced its number of live leaves after 55 DAE, which may be related to the anticipation of the life cycle as a way to deal with salt stress. The same occurred when the plants were irrigated with 4.0 dS m^{-1} salinity water, however the number of live leaves decreased after 45 DAE. This reduction in the number of live leaves at 45 DAE may be related to the toxic effect of salt. The same occurred in the treatments that received 100% water with salinity of 4.0 dS m^{-1}.

The excess of salts in the soil causes a series of changes in its properties, which will reduce the availability of water for plants due to the decline in their osmotic potential, decreasing the availability of water and nutrients. With the increase in osmotic pressure, physiological drought may occur, with a reduction in the number of leaves (Alves, Ferreira-Silva, Silveira, & Pereira, 2011). In addition, the accumulation of specific ions can cause toxicity at the cellular level, causing cellular plasmolysis and leaf senescence (Souza, Machado, Silveira, & Ribeiro, 2011).

The number of dead leaves increased over time on the three blades (50%.ETc, 75%.ETc and 100%.ETc) with zero salinity and was greater with the increase in available water (Figure 6). The increase in the number of dead leaves depending on availability and over time is explained by the plant senescence. However, under the condition of 50%.ETc and 2.0 dS m^{-1}, the number of dead leaves remains constant until 85 DAE, when plant death occurred in this treatment. This stability can be a defense mechanism for millet against salt stress. Under this same salinity, but with a blade of 75%.ETc the number of dead leaves remained constant until 85 DAE with a slight increase thereafter, period in which the number of live leaves started to decrease. In 75%.ETc, 100%.ETc and 4.0 dS m^{-1} there was no increase in the number of dead leaves.

The largest leaf blade length and the largest width in the smallest blade (25%.ETc) were observed in the treatment of zero salinity, 110 cm and 5 cm, respectively. On the other hand, under salinity of 2.0 dS m^{-1}, the largest leaf blade length and width were 70 cm and 4 cm, respectively (Figures 7 and 8). Whereas at the level of 4.0 dS m^{-1} of salt, the largest leaf blade length and width observed were 85 cm and 5 cm, not differing from the previous treatment ($p < 0.05$). As the highest values of length and width were observed at the intermediate level of salinity, it appears that this reduction is associated with an external factor, unrelated to the treatments applied.

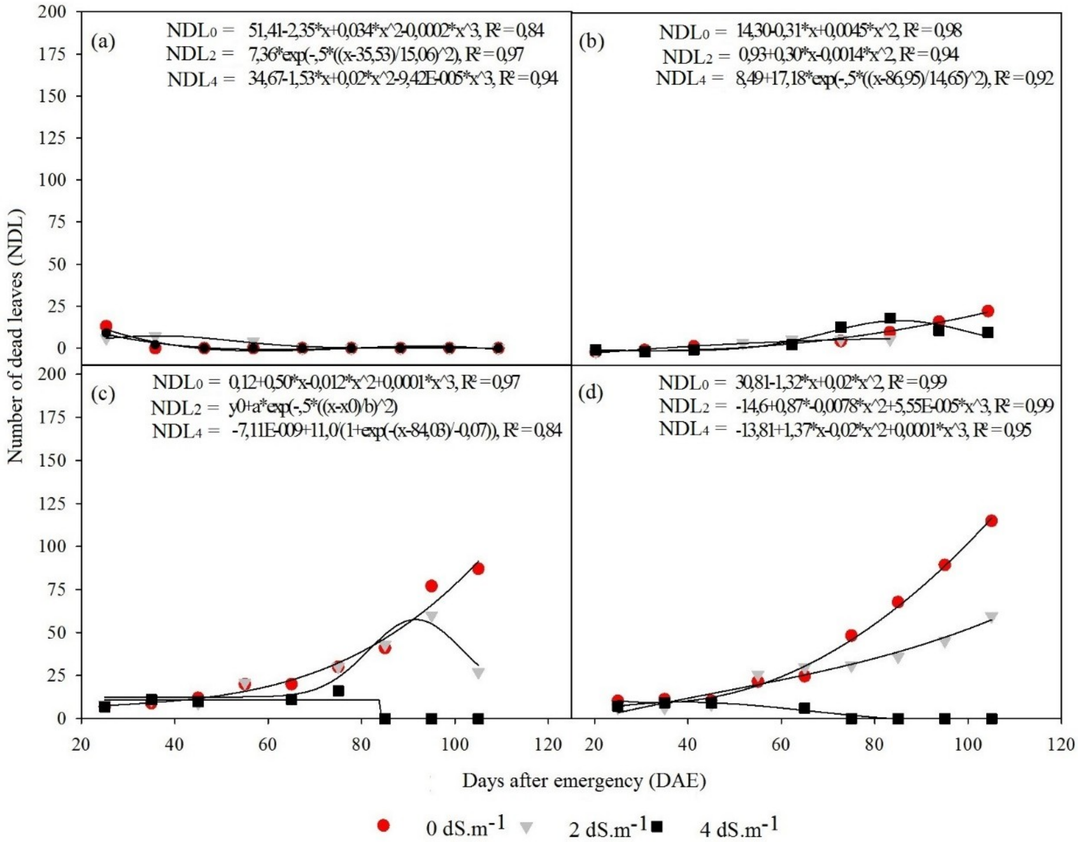

Figure 6. Number of dead leaves of millet irrigated with 25% (a), 50% (b), 75% (c) and 100% (d) of the crop evapotranspiration (ETc) and with water with salinity of 0, 2.0 and 4.0 dS m^{-1} depending on the days after the emergency (DAE).

The leaf blade length in the treatments of 50%.ETc, 75%.ETc and 100%.ETc with salinity of 0 dS m^{-1} showed similar results throughout the cycle (Figure 7b, 7c and 7d) showing linear growth or constant over time. However, in the 50% blade, both the leaf blade length and the leaf blade width were smaller compared to the larger slides (Figures 7 and 8). With less water available in the soil, millet tends to reduce the cellular expansion of its leaves. A lower water content at the cellular level substantially decreases leaf expansion.

The smaller leaf area (shorter in length and width) transpires less, effectively conserving a limited water supply in the soil for a longer period. Thus, the reduction of leaf area can be considered the first line of defense against drought (Gilbert, Zwieniecki, & Holbrook, 2011). An adjustment in the leaf area is an important change in the long term, benefiting the adaptation of the plant to a water limiting environment.

Still with 50% available water, when the salinity level was increased from zero to 2.0 dS m^{-1}, the two millet variables (leaf blade length and width) decreased throughout the cycle. Unlike the 75%.ETc and 100%.ETc blades that received water at this level of salt. In these two conditions, the leaf blade length and width increased until 45 and 55 DAE and after this time they started to decrease (Figures 7 and 8). In contrast, at the saline level of 4.0 dS m^{-1} and 50%.ETc there was a decrease in leaf blade length between 35 and 55 DAE (Figure 7b) and a reduction in leaf blade width after 40 DAE. Similar behavior to the 75%.ETc and 100%.ETc blades, referring to a tolerance in the initial growth to the accumulation of salts even with a greater contribution due to the larger blades. However, with the increase in the content of salts in the soil, there was a reduction of these variables, this reduction being more accentuated in the treatments of 75%.ETc and 100%.ETc.

The number of millet tillers showed the same behavior as the other variables in the availability of 25%.ETc (Figure 9a). In treatments with slides of 50%.ETc, the levels of salinity caused a reduction in the number of tillers (Figure 9b). In the treatment with a 75%.ETc blade and zero salt (Figure 9c), an exponential behavior is observed as a function of time, with an increase in the number of tillers up to 75 DAE, remaining constant thereafter. However, with 75%.ETc and 2.0 dS m^{-1} of salinity, the increase in the number of tillers only occurs until 55 DAE, the same occurred in the salt level of 4.0 dS m^{-1}, only this reduction started a little earlier.

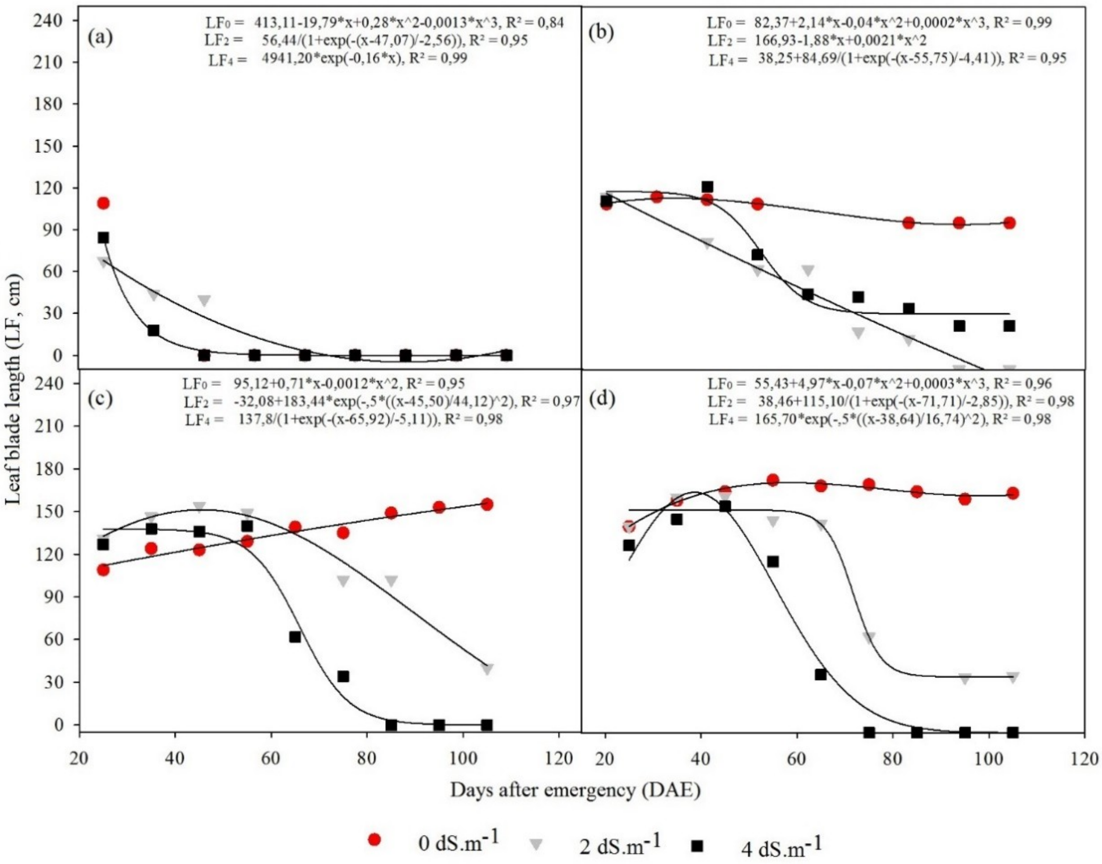

Figure 7. Leaf blade length (cm) of millet irrigated with 25% (a), 50% (b), 75% (c) and 100% of the crop evapotranspiration (d) and salt stress (0, 2.0 and 4.0 dS m^{-1}) as a function of the days after emergence (DAE).

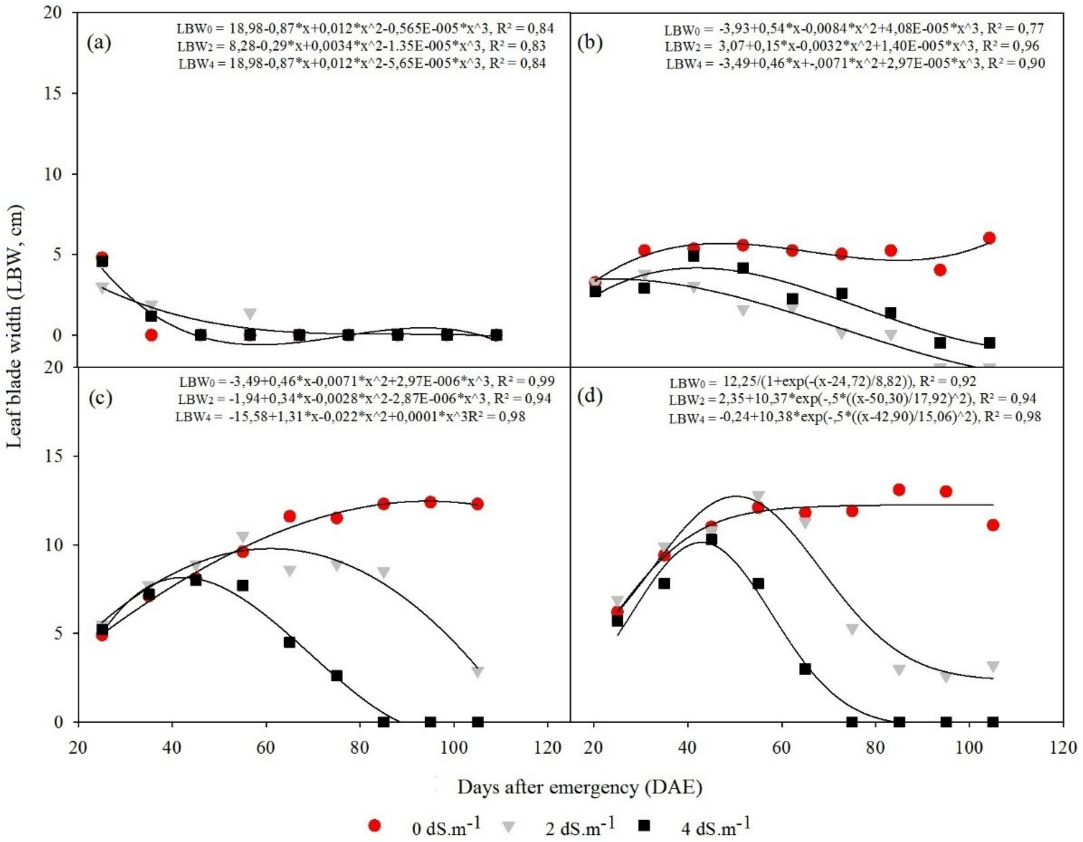

Figure 8. Leaf blade width (cm) of millet irrigated with 25% (a), 50% (b), 75% (c) and 100% (d) of the crop evapotranspiration (ETc) and different levels of water salinity (0, 2.0 and 4.0 dS m^{-1}) depending on the days after emergence (DAE).

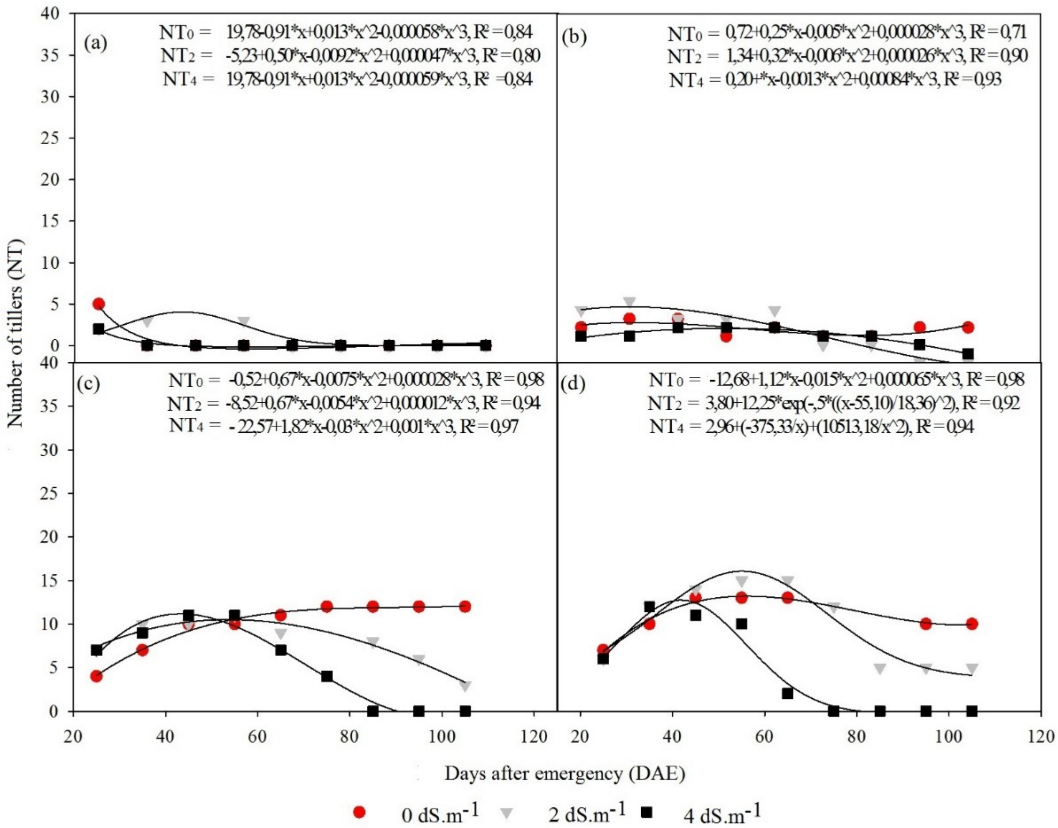

Figure 9. Number of millet tillers cultivated with different irrigation blades [25% (a), 50% (b), 75% (c) and 100% (d) of the crop evapotranspiration (ETc)] and salinity levels (0, 2.0 and 4.0 dS m^{-1}) as a function of the days after emergence (DAE).

The number of tillers in the plants subjected to 100%.ETc was higher in the salinity of 2.0 dS m^{-1} in relation to the control, which can be a strategy of the plant to dissipate the accumulation of salts by increasing the number of tillers. This causes a reduction in the toxic effects on the main tiller. Yet in the 100%.ETc blade, the number of tillers in the salinity treatment of 4.0 dS m^{-1} was the same as the treatment without added salt, which implies that up to 40 DAE the accumulation of salts does was not able to cause a reduction in the appearance of millet tillers.

These results show that the salt stress caused by the accumulation of salts causes a reduction in some variables of millet, in addition to the anticipation of its cycle. Therefore, in situations of use of saline water for millet production, it is possible to obtain satisfactory results taking into account the variables presented in this study, as long as the forage planning takes into account a decrease in the millet growth cycle in this condition.

Conclusion

Water and salt stresses, when associated, reduce the growth of millet, since concentrations above 2.0 dS m^{-1} and less than 50% of the crop evapotranspiration compromise its full development, providing declines in yield.

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