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Emergence of *Megathyrsus maximus* seedlings grown under salt stress

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ABSTRACT

The aim was to evaluate the effect of salt stress on the percentage and speed of emergence of seedlings in forage cultivars of the species *Megathyrsus maximus* (Jacq.). The study was conducted in a completely randomized design in a 3×4 factorial scheme, with three forage cultivars (Mombaça, Tanzânia and MG12-Paredão) and four salinity levels (0, 1.5, 3.0, and 4.5 dS/m), with six replications. Stress was induced by irrigating the pots with saline solution. The percentage of seedling emergence was evaluated at seven days after sowing (DAS), and the emergence speed index (ESI) until 15 DAS was evaluated. Analysis of variance for the percentage of emergence and ESI showed significant effect ($P<0.01$) for cultivars and salt stress and non-significant effect for the interaction between factors. The percentage of emergence was higher in the Mombaça cultivar (39.4%) and did not differ between the Tanzânia (30.5%) and MG12-Paredão (25.0%) cultivars. ESI was similar between the Mombaça and Tanzânia cultivars and higher than the MG12-Paredão cultivar. Salt stress reduces the percentage and speed of seedling emergence of the Mombaça, Tanzânia and MG12-Paredão cultivars of *Megathyrsus maximus*. The Mombaça, Tanzânia and MG12-Paredão cultivars show the same behavior regarding seedling emergence performance when subjected to different levels of salt stress.

Keywords: Emergence percentage, Emergence speed index, *Panicum maximum,* Salinity.

Emergência de plântulas de *Megathyrsus maximus* cultivadas sob estresse salino

RESUMO

Objetivou-se avaliar o efeito do estresse salino sobre a porcentagem e velocidade de emergência de plântulas de cultivares forrageiras da espécie *Megathyrsus maximus* (Jacq.). O estudo foi conduzido em delineamento inteiramente casualizado, em esquema fatorial 3x4, sendo três cultivares forrageiras (Mombaça, Tanzânia e MG12-Paredão) e quatro níveis de salinidade (0; 1,5; 3,0 e 4,5 dS/m), com seis repetições. O estresse foi induzido por meio da irrigação dos vasos com solução salina. Foi avaliada a porcentagem de emergência de plântulas aos sete dias após a semeadura (DAS) e o índice de velocidade de emergência (IVE) até o 15° DAS. A análise de variância para os caracteres porcentagem de emergência e o IVE evidenciou efeito significativo ($P<0,01$) para cultivares e estresse salino e não significativo para a interação entre os fatores. A porcentagem de emergência foi superior na cultivar Mombaça (39,4%) e não diferiu entre as cultivares Tanzânia (30,5%) e MG12-Paredão (25,0%). O IVE foi semelhante entre as cultivares Mombaça e Tanzânia e superiores a cultivar MG12-Paredão. O estresse salino reduz a porcentagem e a velocidade de emergência de plântulas das cultivares Mombaça, Tanzânia e MG12-Paredão de *Megathyrsus maximus*. As cultivares Mombaça, Tanzânia e MG12-Paredão apresentam o mesmo comportamento quanto ao desempenho na emergência de plântulas quando submetidas a diferentes níveis de estresse salino.

Palavras-chave: Índice de velocidade de emergência, *Panicum maximum,* Porcentagem de emergência, Salinidade.

1. Introduction

The use of irrigation in pastures has been increasing over the past years, especially in semiarid regions characterized by long periods of drought, which can contribute to increased forage and animal production (Silva et al., 2020). In these regions, the primary source of water for irrigation is groundwater, extracted from wells, as during drought periods, rivers and small streams cease to flow and reservoirs dry up or drastically reduce water levels. However, these groundwater and surface waters often have high salinity content, and their continuous use can lead to soil salinization (Silva et al., 2022).

Soil salinization is a concerning process in modern agriculture, especially in arid and semiarid regions, resulting from edaphoclimatic conditions and irrigated agriculture with water containing excess salts, associated with incorrect use of agricultural inputs (Silva et al., 2022). Excess salts can affect the biochemical and physiological functions of plants, causing osmotic stress, disturbances in water relations, alterations in the absorption and utilization of essential nutrients, and the accumulation of toxic ions (Amorim et al., 2010). These effects result in growth retardation and reduced crop production due to protein denaturation and membrane destabilization, leading to decreased hydration of macromolecules (Taiz et al., 2017).

Plant tolerance to salt stress depends on the efficiency of morphological and physiological mechanisms, which increase the plants' capacity to cope with increased salt concentration in the soil (Silva et al., 2022), potentially leading to a reduction in leaf area and consequently, lower absorption of water rich in salt ions (Silva et al., 2018). Despite the fact that the species *Megathyrsus maximus* (Jacq.) is a C4 species that tolerates high temperatures, cultivated in Brazil for several decades, and the numerous studies evaluating its adaptation to different regions and water regimes, little research has been conducted on the effect of salt stress on this forage grass. Lima et al. (2018) described *M. maximus* as the most productive tropical forage propagated by seeds, being used worldwide due to its high productivity potential and forage quality, thus making room for study under salt stress conditions. Praxedes et al. (2019), when evaluating the performance of the Tanzânia cultivar irrigated with saline water, did not observe tolerance of the cultivar to salinity above 2.8 dS/m, classifying it as moderately sensitive to irrigation water salinity. Salomón and Samudio (2015) found a reduction in germination speed and plant height of the Tanzânia and Mombaça cultivars with increasing salinity levels from 0.0 dS/m to 22.3 dS/m.

Therefore, the aim of the present study was to evaluate the effect of salt stress on the percentage and speed of emergence of seedlings in forage cultivars of the species *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs.

2. Material and Methods

The study was conducted in a greenhouse covered with low-density polyethylene and masonry benches with aphid-proof screens, located at the School Farm of the Federal University of Western Bahia (UFOB), in the municipality of Barra - BA (Latitude 11°05'20" S and Longitude 43°08'31" W). According to Köppen's classification, the region's climate is characterized as BSh (hot semiarid), with an average annual temperature and rainfall of 25.7 °C and 649 mm, respectively (INMET, 2022).

Different cultivars of *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs were evaluated, which were distributed in a completely randomized design, in a 3×4 factorial scheme, with three forage cultivars (Mombaça, Tanzânia, and MG12-Paredão) and four salinity levels (0, 1.5, 3.0 and 4.5 dS/m), with six replications, totaling 72 experimental units. Salt stress was induced by the daily irrigation of pots with saline solution according to different salinity levels. The amount of sodium chloride (NaCl) used to achieve salinity levels was determined considering the electrical conductivity of water (ECw) in dS/m at 28 °C. The solution for each salinity level was prepared every two days, and the conductivity of each solution was measured using a desktop electrical conductivity meter model ION 30107-03.

For the seedling emergence test, 50 seeds of each *M. maximus* cultivar were used in each plot, which were sown at a depth of 1 cm in a polyethylene pot with a capacity of 6 dm^3, filled with locally sieved and washed soil in distilled water. The soil used in the study was Quartzarenic Neosol, with 86.5% sand, 11% clay and 2.5% silt. After sowing, each pot received daily the solution corresponding to its treatment (0, 1.5, 3.0, 4.5 dS/m), until reaching pot capacity. The amount of solution used to reach pot capacity was determined by the weight difference of the pots, which were weighed upon reaching pot capacity and after 24 hours, always before the next irrigation.

With the onset of salt stress induction, after sowing, daily counting of emerged seedlings was performed until 15 DAS. A seedling was considered to have emerged when the first leaf was completely developed and exposed. With the number of emerged plants daily during the 15 days of evaluation, the emergence speed index was estimated, and with the number of emerged seedlings up to the 7th DAS, the percentage of seedling emergence at 7 DAS was estimated.

The emergence speed index (ESI) was calculated using the formula proposed by Maguire (1962):

$$ESI = E_1/N_1 + E_2/N_2 +... E_n/N_n$$

where: E_1, E_2... En is the number of emerged seedlings counted in the first count, second count and last count; and N_1, N_2...N_n is the number of days from sowing to the first, second, and last count.

The data were subjected to analysis of variance, according to the following statistical model:

$$Y_{ijk}: m + C_i + A_j + CA_{ij} + E_{ijk}$$

where:

Y_{ijk}: is the observed value of the combination between the i-th cultivar, with the j-th level of salt stress, in the k-th repetition.

m: overall mean.

C_i: is the effect of the i-th cultivar on the observed value Y_{ijk}.

A_j: is the effect of the j-th level of salt stress on the observed value Y_{ijk}.

CA_{ij}: is the interaction effect of the i-th cultivar with the j-th level of salt stress on the observed value Y_{ijk}.

E_{ijk}: is the error associated with observation Y_{ijk}.

Complementary procedures to the analysis of variance were adopted according to the significance of the main effect of each factor and their interaction. For the grouping of means of the cultivar factor, the Scott and Knott test was applied at 5% probability. For the salt stress factor, the analysis was performed through graphical analysis and regression model adjustment. Data analysis was performed using the Genes Program, a Computational application in genetics and statistics (Cruz, 2013).

3. Results and Discussion

The analysis of variance for the percentage of emergence at 7 DAS and emergence speed index up to 15 DAS showed significant effects ($P<0.01$) for the sources of variation cultivars and salt stress individually (Table 1). However, there was no significant effect ($P>0.05$) on the interaction between the two factors. This fact indicates that for the variable's percentage and speed of emergence, there is no differential expression by the cultivars concerning the different levels of salt stress to which they were subjected.

Thus, as in the present study, some studies in the literature have demonstrated the effect of salt stress on the development, germination, emergence and chemical composition of seedlings of different forage cultivars. Salomón and Samudio (2015) observed an interaction between cultivars and salinity levels, with a lower reduction in the germination percentage of the Tanzânia cultivar (20%) compared to the Mombaça cultivar (50%). Alvarez-Pizarro et al. (2019) observed a progressive reduction in the shoot/root ratio in the Mombaça cultivar of *M. maximus*, suggesting growth adjustment in both plant organs to promote salt tolerance by retaining toxic ions in the roots and reducing transpiration flow. Silva et al. (2020) found that the BRS Zuri cultivar of *M. maximus* is affected by increasing salinity levels and irrigation depths, showing higher dry matter content (228.57 and 242.55 g/kg) and neutral detergent fiber (622.71 and 631.18 g/kg) at salinity levels of 1.8 and 3.0 dS/m and a reduction in crude protein content (86.39 and 78.22 g/kg).

The percentage of emergence at 7 DAS was higher in the Mombaça cultivar (39.4%) and did not differ from the Tanzânia (30.5%) and MG12-Paredão (25.0%) cultivars (Figure 1). However, up to 15 DAS, the emergence speed index was similar to the Mombaça and Tanzânia cultivars, which were higher than the MG12-Paredão cultivar (Figure 1). These results indicate that there is genetic variability among cultivars for parameters related to seedling emergence. According to Shannon (1997), salt tolerance varies among species, genotypes of the same species and even among phenological stages of the same genotype. In the present study, statistically differentiated behavior among cultivars for parameters related to seedling emergence was not observed at the salinity levels employed.

Table 1. Summary of the analysis of variance for the seedling emergence percentage on the 7th day after sowing (DAS) and emergence speed index (ESI) up to 15 DAS in cultivars of *Megathyrsus maximus* under different levels of salt stress

Sources of variation	DF	Mean Square (1)	
		Seedling emergence percentage	ESI
Cultivar (C)	2	1,270.39**	539.90**
Salt stress (S)	3	7,866.72**	3,035.28**
Interaction C×S	6	122.17^{ns}	33.26^{ns}
Error	60	5,320.67	32.61
CV (%)		29.76	29.62
Mean		31.68	19.28

** and * significant at 1 and 5% probability, respectively, by the F test; ns non-significant by the F test. (2) DF: degrees of freedom; CV: coefficient of variation.

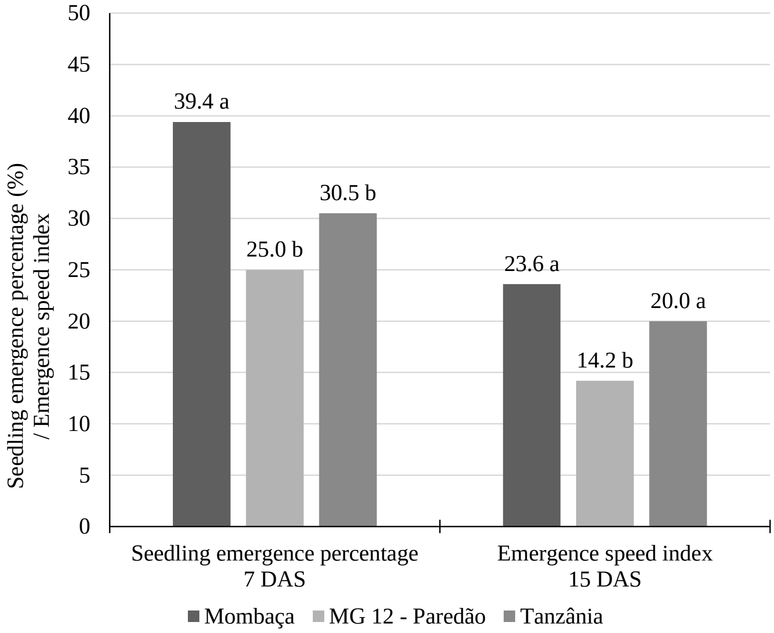

Figure 1. Grouping of means of *Megathyrsus maximus* cultivars, regardless of the level of salt stress, for the seedling emergence percentage at 7 days after sowing (DAS) and emergence speed index at 15 DAS. Cultivars with the same letter for the same variable belong to the same group by the Scott and Knott test at 5% probability.

Several studies have been conducted with other grass species, and the results show variation in the effect of salt stress among different species and cultivars (Coelho et al., 2014; Timm et al., 2015; Silva et al., 2020). Silva et al. (2020) observed that the chemical composition of the BRS Zuri cultivar of *Megathyrsus maximus* is negatively affected by increasing salinity levels, with a 9.46% reduction in crude protein (CP) content and a 1.34% increase in neutral detergent fiber (NDF).

Timm et al. (2015) observed a decrease of about 5.0% in seedling emergence percentage and 21.7% in the emergence speed index with increasing salt stress in all white oat (*Avena sativa* L.) cultivars, while in forage sorghum (*Sorghum bicolor* L. Moench), Coelho et al. (2014) found no differences in seedling emergence percentage among the cultivars evaluated. Although studies evaluating these parameters in *M. maximus* are scarce, it was observed that increasing irrigation with saline water causes damage to the percentage and speed of germination, as well as to the chemical composition of forage cultivars.

The cultivars behaved similarly at each level of salt stress. In Figure 2, one can observe the percentage of emergence of each cultivar at different salt stress levels throughout the evaluation period. The emergence curves for each level of salinity stress show the effect of salt stress on reducing seedling emergence capacity in the three cultivars evaluated. This effect can be verified for the cultivar average in Figure 3. The average percentage of emergence at 7 DAS decreased linearly with increasing levels of salt stress. For each increase of 1 dS/m in the salt stress level, a reduction of 10.69% in the percentage of emergence at 7 DAS was observed.

In addition to the percentage of seedling emergence, salt stress also affected the ESI of the cultivars (Figure 4). The average emergence speed index (ESI) for the three cultivars at 15 DAS decreased linearly with increasing levels of salt stress, with a reduction of 6.64 in the ESI for each increase of 1 dS/m in the salinity stress level at 15 DAS. These results indicate the sensitivity of the species *M. maximus*, represented here by the cultivars Mombaça, Tanzânia, and MG12-Paredão, to salinity, up to the level of 4.50 dS/m.

According to Lopes and Macedo (2008), the presence of salts interferes with the soil water potential, reducing the potential gradient between the soil and the seed surface, restricting water uptake by the seed, and causing a reduction in seed germination and emergence. In addition, restricted water absorption due to the osmotic gradient affects the development of the embryo, which depends on cell division and expansion processes (Klafke et al., 2012).

Under increasing salt levels, there is lower water potential, ionic toxicity and an imbalance in NaCl excretion. Higher levels of antioxidants indicate that they are actively involved in eliminating reactive oxygen species (ROS) generated by NaCl toxicity. ROS can affect biomolecules and cause lipid peroxidation of the cell membrane, resulting in plant damage (Alharbi et al., 2022). Similar results were found by Salomón and Samudio (2015), who observed a reduction of 15.0 and 10.0% in the germination percentage of the Mombaça and Tanzânia cultivars, respectively, under different levels of salt stress (ranging from 0.0 to 8.6 mS/cm) in irrigation water.

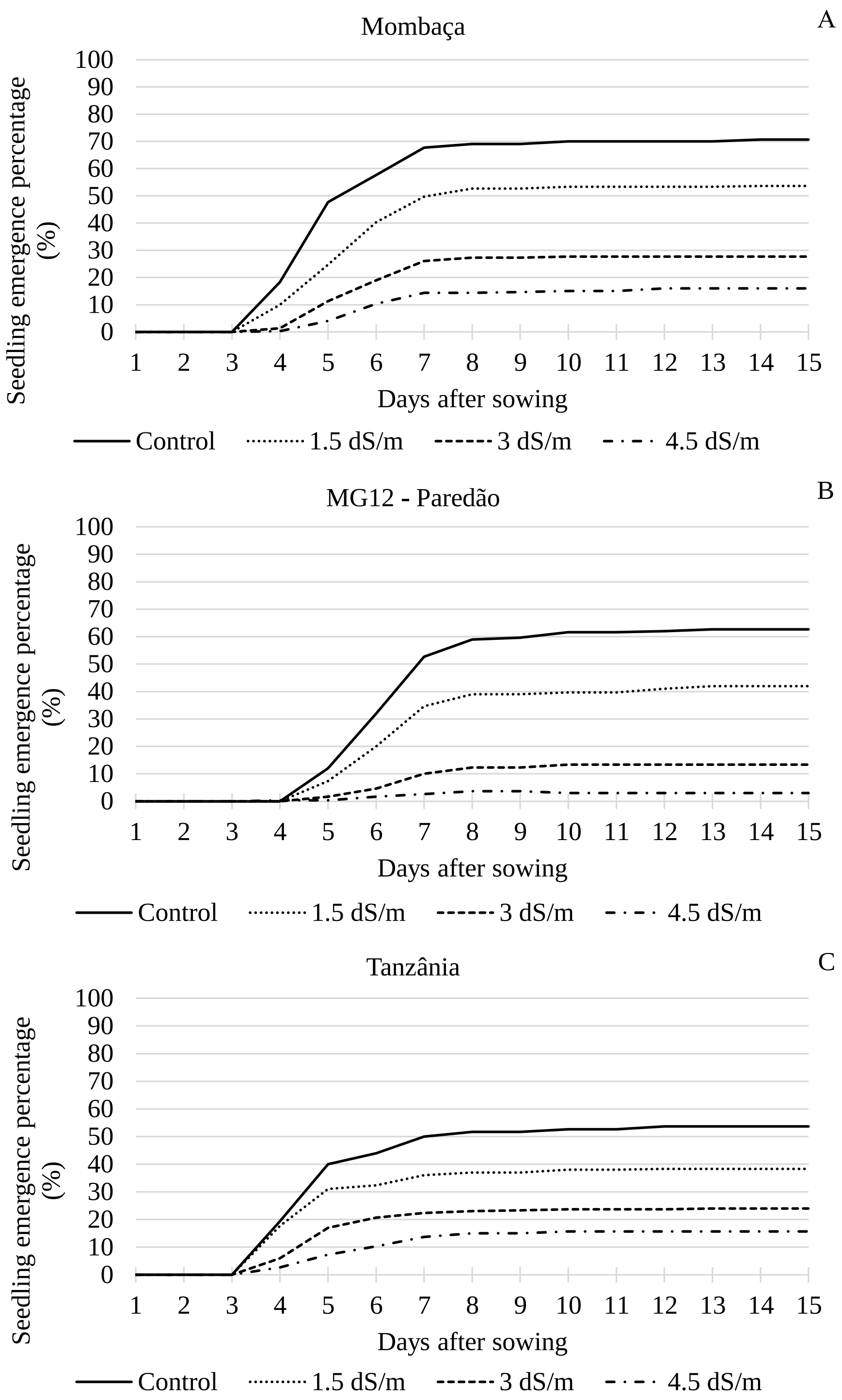

Figure 2. Percentage of emerged seedlings of the cultivars Mombaça (A), MG12-Paredão (B) and Tanzania (C) during 15 days after sowing at different levels of salt stress.

Praxedes et al. (2019) observed a decrease of 32% in the height of the Tanzânia cultivar for electrical conductivity of water (ECw) equal to 2.80 dS/m. The authors also observed a linear reduction in fresh mass with increasing salinity, at a ratio of 22.26 g for each unit increase in ECw, also classifying the cultivar as moderately sensitive to salinity. Amorim et al. (2015) observed a reduction in the percentage and speed of emergence of *Brachiaria brizantha* cultivars with increasing salinity of the water, showing behavior similar to that observed in this study.

According to Andreo-Souza et al. (2010), these effects are expected since germination speed is the first parameter affected by the reduction in water availability caused by the difference in osmotic potential due to salinity. Reductions or inhibitions in plant growth as they are subjected to increasing concentrations of salinity can be attributed to osmotic effects, toxicity due to excessive absorption of Na^+ and Cl^- ions, and nutritional imbalance caused by disruptions in the absorption of essential nutrients (Andrade et al., 2014).

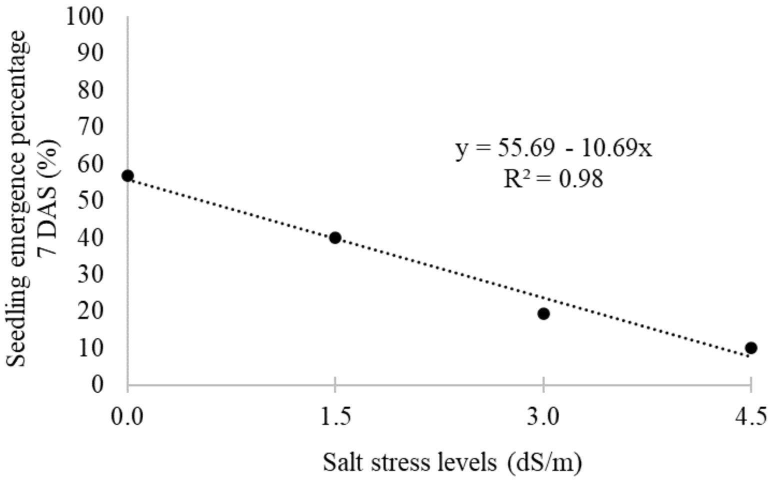

Figure 3. Average effect of salt stress on the seedling emergence percentage at 7 days after sowing (DAS) in cultivars of *Megathyrsus maximus*.

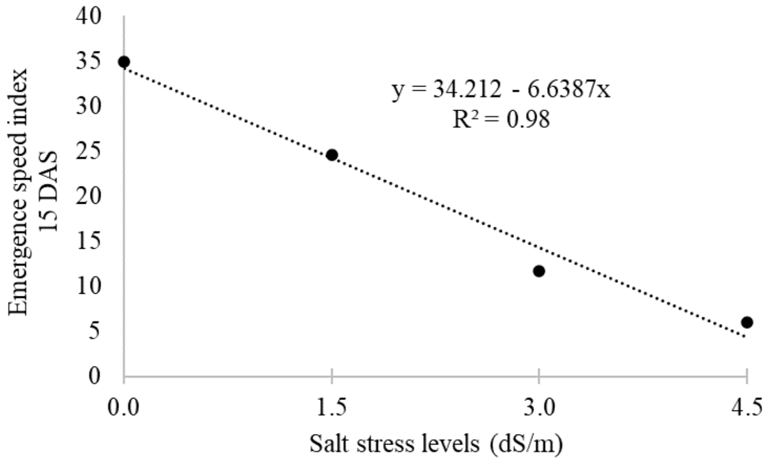

Figure 4. Average effect of salt stress on the emergence speed index 15 days after sowing (DAS) in cultivars of *Megathyrsus maximus*.

As the osmotic potential of the solution decreases, AuthorAs the osmotic potential of the solution decreases, becoming more negative due to increased salt concentration, there is a reduction in the percentage of seed germination as we can observe in Klafke et al. (2012).

4. Conclusions

The salinity of irrigation water linearly reduces the percentage and emergence speed of seedlings of the Mombaça, Tanzânia and MG12-Paredão cultivars of *Megathyrsus maximus*. The Mombaça, Tanzânia and MG12-Paredão cultivars exhibit the same behavior regarding seedling emergence performance when subjected to different levels of salt stress. The percentage of emergence at 7 DAS was higher in the Mombaça cultivar and did not differ between the Tanzânia and MG12-Paredão cultivars. The emergence speed index at 15 DAS was similar between the Mombaça and Tanzânia cultivars, and higher than the MG12-Paredão cultivar.

Authors' Contribution

Ueslei Figueiredo de Lima carried out the field experiment, collected and processed data, and wrote the manuscript. Laura Marina de Oliveira Castro assisted with field data collection and sample processing. Adérico Júnior Badaró Pimentel collaborated on the study planning and experimental design, conducted the statistical analysis, and revised the manuscript. Janaina de Lima Silva coordinated the planning and execution of the study, data evaluation and revision of the manuscript.

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