GERMINATION RATE OF *PENNISETUM GLAUCUM* SEEDLING IN FUCTION OF SALINE STRESS, GROWTH AND BIOMASS OF AERIAL PART AND ROOT

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- ABSTRACT: Millet is an annual cycle plant, with C4 metabolism, tolerant to water deficit, and it is cultivated in regions with scarcity of rain. The objective was to evaluate the germination of the millet seedling in function of saline stress, growth and biomass of the aerial part and the root system. The experiment was performed in a completely randomized design, with four treatments and five replicates, with the unit being represented by seeds of millet. The treatments were constituted by solutions with addition of NaCl corresponding to 2.337, 4.674 and 7.011 g/L, respectively for the electrical conductivities 4.0; 8.0; 12.0 dS/m and 0 dS/m (distilled water) as control. The millet cultivar used in this experiment was the IPA Bulk 1BF. The mean germination rate of seedlings in relation to control salinity level was higher than 90%, while for level of 12 $dS.m^{-1}$ salinity there was no germination. The fitted model presented a high coefficient of determination of model (pseudo-R^2= 90.96%) and low sum of squares of residuals (SSR=0.26). The beta regression model proved to be an appropriate option to analyse the millet seedling germination rate after 72 hours in function of salinity level, length of the root system and dry mass of the aerial part.

- KEYWORDS: Beta regression, millet, modelling, mortality

1 Introduction

Millet (*Pennisetum glaucum*) is an annual cycle plant, with C4 metabolism, and tolerant to water deficit (ULLAH *et al*., 2017; SINGH *et al*., 2015). It is cultivated in places with scarcity of rain, which is an important fact due to its low water demand (DAN *et al*., 2010). This plant is used as an alternative for grain production and pasture grown in semi-arid regions, due to the high production of biomass and nutritive value (SANTOS *et al*., 2017; GHATAK *et al*., 2016). Regarding salinity, millet presents moderate tolerance,

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with more than 70% reductions in seed germination and seedling growth when it is submitted to the concentration of 250 mM of NaCl (KRISHNAMURTHY *et al*., 2007).

The morphometric measurements of millet, such as the length of the aerial part and of the root system, are associated characteristics that directly influence the production of phytomass (LEITE *et al*., 2017). These characteristics are positively correlated with the productivity of forage plants (LEITE *et al*., 2017). However, several stages of millet development are affected by the salinity of irrigation water, which directly influences the productivity of this plant (HUSSAIN *et al.*, 2010).

High concentrations of soluble salts in the soil solution, mainly NaCl, and other ions, such as Mg^{2+}, HCO^{3-} and SO_4^{2-}, cause inhibition of plant growth, due to the decrease in the water potential of the soil solution at a level below the necessary for the absorption of water (SOARES *et al*., 2015). There are few studies on the effect of salinity in millet culture in the literature, and, in addition, it has been verified that the increase of salts in the soil due to use of low quality water for irrigation causes many adverse consequences in the morphology, anatomy and physiology of this culture (HUSSAIN et al., 2010). According to Hussain *et al*. (2008), the percentage of germination, height, grain and yield of millet straw decrease as salinity increases.

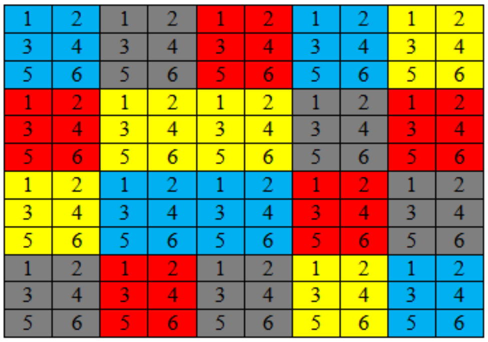
However, it must be considered that the groundwater of most of the planet's semi-arid regions has a high concentration of salts (SILVA *et al*., 2017). Thus, the use of species that are more resilient to environmental modifications is necessary in order to increase agricultural productivity. In view of this context, the objective was to evaluate the germination of the millet in function of saline stress, growth and biomass of the aerial part and the root system.

2 Material and methods

The research was carried in the Universidade Federal do Pernambuco, Academic Unit of Serra Talhada (UFRPE/UAST), Serra Talhada city, Pernambuco state, Brazil (elevation: 429 m, latitude: 7º 56' 15" S and longitude: 38º 18' 45" E). According to Koppen, the climate condition is a BSwh', which is denominated Semiarid, hot and dry, with rainy season during the summer, with average annual rainfall of 647 mm/year and average air temperatures greater than 25 ºC (LEITE *et al*., 2017).

The experiment was performed in a completely randomized design, with four treatments and five replicates, and the experimental unit was represented by a tray of 120 cells containing 24 seeds per treatment (Figure 1). The treatments were constituted by solutions with addition of NaCl corresponding to 2.337, 4.674 and 7.011 g/L, respectively for the electrical conductivities of (EC) 4.0; 8.0; 12.0 dS/m, and 0 dS/m (distilled water) as control.

The millet cultivar (Pennisetum glaucum) used in this experiment was the IPA Bulk 1BF. The seeding occurred on May 30, 2018, in styrofoam trays (67.4 × 34 × 6.1 cm), which had sand and vermiculite as the substrate in a 1:1 ratio. Initially, the substrate was humidified to field capacity, with water from each saline level evaluated and conducted in greenhouse. During the conduction of the experiment, the irrigations were carried once a day, in order to keep the humidity close to the field capacity, in order to leave the substrate in saturated conditions.



1	2	1	2	1	2	1	2	1	2
3	4	3	4	3	4	3	4	3	4
5	6	5	6	5	6	5	6	5	6
1	2	1	2	1	2	1	2	1	2
3	4	3	4	3	4	3	4	3	4
5	6	5	6	5	6	5	6	5	6
1	2	1	2	1	2	1	2	1	2
3	4	3	4	3	4	3	4	3	4
5	6	5	6	5	6	5	6	5	6
1	2	1	2	1	2	1	2	1	2
3	4	3	4	3	4	3	4	3	4
5	6	5	6	5	6	5	6	5	6

Figure 1 - Experimental design (blue - control treatment; red - treatment with 8 dS/m; gray - treatment with 4 dS/m and yellow - treatment with 12 dS/m).

To evaluate the effect of salt stress, we collected information of the length from the aerial part (LAP) and root (RSL), dry mass of the aerial part (DMAP) and of the root (DMRS). The results of these measures were compared by the Kruskal-Wallis test. The lengths were measured with the aid of millimetre ruler and expressed in centimetres. The aerial part was measured from the base of colon to the apex of the apical meristem of the seedling, and the root length was obtained from the measurement of the base of colon to the root end of the seedling. At the end of the experiment, we evaluated the germination rate of millet by counting the number of live seedlings divided by the number of seedlings seeded in each replicate. To determine the fresh mass of shoot and root, the collected material was weighed in precision balance (0.0001 g). To determine the dry mass, the parts of the plants were conditioned in paper bags and set to dry in drying oven with forced air circulation at 65°C, during 72 hours. After this period, the samples were weighed, and the results were expressed in g/seedling.

Regression analysis is a statistical technique used to model, based on a set of information, the relationship between the variable of interest and one or more explanatory variables. For situations where the response is continuous and restricted to the interval (0, 1), such as rates and proportions, Ferrari and Cribari-Neto (2004) have proposed the beta regression model.

The beta regression models are being used to evaluate the percentage of shrub cover (EKLESTON *et al*., 2011) and to model the diameter and height of trees (LI *et al*., 2002). Ferrari and Cribari-Neto (2004) proposed the beta regression model class, in which the response variable (Y) has a beta distribution.

Let Y be a random variable with Beta distribution (p, q), then define its probability density function by:

$$f(y; p, q) = \frac{\Gamma(p+q)}{\Gamma(p)\Gamma(q)} y^{p-1}(1-y)^{q-1}$$

where, y is the continuous variable in the interval (0,1), p> 0 and q> 0 are parameters of the probability density function and Γ (*) is the gamma function, which is defined by:

$$\Gamma(p) = \int_0^{\infty} y^{p-1} e^{-y} dy$$

Ferrari and Cribari-Neto (2004) proposed a different parameterization of the beta probability density function, by setting $\mu = p/(p+q)$ and $\emptyset = p + q$, this is, $p = \mu\emptyset$ and $q = (1-\mu)\emptyset$, so the density function is expressed by:

$$f(y; \mu, \emptyset) = \frac{\Gamma(\emptyset)}{\Gamma(\mu\emptyset)\Gamma((1-\mu)\emptyset)} y^{\mu\emptyset-1}(1-y)^{(1-\mu)\emptyset-1}$$

where, μ $(0 < \mu < 1)$ is the parameter associated with the mean of the response variable and $\emptyset > 0$ is the precision parameter. Its mean and variance are defined by, $E(Y) = \mu$ and $\mathrm{Var}(Y) = \frac{\mu(1-\mu)}{1+\emptyset}$.

Let $y = (y_1, y_2, \dots, y_n)$ be a vector of independent random variables, where each y_i $(i = 1,2,\dots,n)$ follows the density function Beta, this is, $y_i \sim B(\mu_i, \emptyset_i)$, such that the Beta regression model is defined by $f(y; \mu, \emptyset)$.

Then, the structure of the regression model is given by:

$$g(\mu_i) = \eta_i = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \cdots + \beta_k X_{ik}$$

where, η is the linear predictor, $\beta = (\beta_0, \beta_1, \beta_2, \dots, \beta_k)$ is the unknown parameter vector and $X_{i1}, X_{i2}, \dots, X_{ik}$ are the observations of k independent variables known (k<n) and i=1,2,...,n. In the beta regression model, you can choose different link functions, such as the logit function:

$$g(\mu) = \log\left(\frac{\mu}{1-\mu}\right)$$

where, the average of variable y_i can be written according to the explanatory variables by the following expression:

$$\mu_i = \frac{\exp^{(\beta_0+\beta_1 X_{i1}+\beta_2 X_{i2}+\cdots+\beta_k X_{ik})}}{1+\exp^{(\beta_0+\beta_1 X_{i1}+\beta_2 X_{i2}+\cdots+\beta_k X_{ik})}}$$

where, $0<\mu_i<1$. The estimation of the coefficients is obtained by maximizing the log-likelihood function, and the adjustments are evaluated by the pseudo coefficient of determination of the model (pseudo-R^2), by the square sum of residuals (SSR), Akaike information criteria (AIC) (AKAIKE, 1974) and Bayesian information criteria (BIC) (SCHWARZ, 1978). The contribution of each variable will be defined by $\exp^{(\beta_i)}$ (FERRARI and CRIBARI-NETO, 2004).

In this study, the response variable is defined as germination rate (GR) of millet seedlings, and the explanatory variables are refined by: salinity levels (SAL), length of the aerial part and root system, as well as the dry mass of aerial part and root system. The Beta regression model was initially proposed by:

$$\boldsymbol{GR = \frac{exp^{(\beta_0+\beta_1 SAL+\ \beta_2 LAP+\ \beta_2 RSL+\ \beta_3 DMAP+\beta_4 DMRS)}}{1+\ exp^{(\beta_0+\beta_1 SAL+\ \beta_2 LAP+\ \beta_2 RSL+\ \beta_3 DMAP+\beta_4 DMRS)}}}$$

where, the contribution of the variables salinity level in the germination rate is defined by $\boldsymbol{exp^{(\beta_1)}}$; the contribution of aerial part length is expressed by $\boldsymbol{exp^{(\beta_2)}}$; of root system length by $\boldsymbol{exp^{(\beta_3)}}$; dry mass of aerial part by $\boldsymbol{exp^{(\beta_4)}}$ and dry mass of root system by $\boldsymbol{exp^{(\beta_5)}}$. All analyses were made by the R software (R CORE TEAM, 2018) with the aid of the *betareg* package (CRIBARI-NETO and ZEILEIS, 2010).

3 Results and discussion

The mean germination rate of seedlings from the control salinity level was higher than 90%. For the level of 4 dS/m, the germination rate was around 67.5%, while at the level of 8 dS/m, the germination rate was less than 50%. Nonetheless, for the maximum level of salinity, the germination rate of seedlings was 0%. The Kruskal-Wallis test shows that the germination rate decreases with the increase of the salinity level (p-value<0.0001), Figure 2.

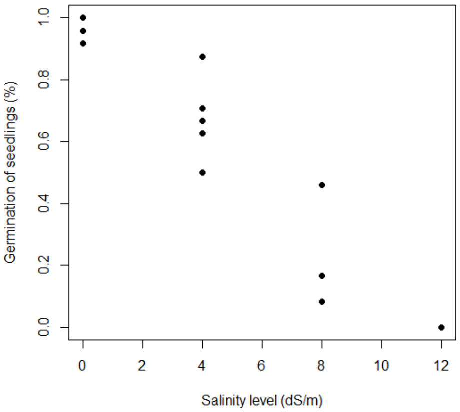


Figure 2 - Germination rate of millet seedlings in function of salinity level.

In a study aiming to identify millet strains [*Pennisetum glaucum* (L.) R. Br.] tolerant to salinity, Krishnamurthy *et al*. (2007) found divergent results, where at a salinity of approximately 25 dS/m, there was a 70% reduction of germination and seedling growth, whereas in the present study, the salinity of 12 dS/m was sufficient to cause total mortality of seedlings. Thus, it is suggested that the cultivar used in our research may have a lower tolerance to salinity when compared to other millet cultivars. However, this tolerance can be classified as moderate, since at the level of 4 dS/m, the plants had a reduction of 22.5% in the germination rate, when compared to the control plants.

When evaluating two millet strains, one of which was considered tolerant and another sensitive to saline stress, Hussain *et al*. (2008) observed that the differentiated growth between these two strains may have been due to differences in transfer rates and accumulation of ions in the aerial part of plants. For the tolerant lineage, the authors showed the lowest transfer rates of Na^+ and Cl^- under the saline stress, due to a better control of the absorption of these ions by the root system, as well as a greater retention of salt in roots. These same authors showed that the excess of Na^+ and Cl^- cause disturbances in relation to ionic balance, which reduces the transfer of ions K^+ and Ca^{2+} in relation Na^+. The highest content of ions Na^+ and Cl^- were found, by order, in stems, scabs, roots and leaves. This retention of potentially toxic ions in the stem and hem is considered as an example of a mechanism of tolerance of millet to salinity. This suggests that this species exports little Na^+ from the roots to leaf limbs, thus avoiding the excess of potentially toxic ions in photosynthetic tissues.

These answers evidenced the idea of a selective barrier between the roots and the aerial part, beyond the capacity in compartmentalize these ions in different tissues and cells, which is considered a quite common mechanism of tolerance to salinity in the majority of the glycophytes. According to Araújo *et al*. (2016), salinity affects plant growth at all stages of development, however, the germination, emergence and initial growth are the most affected phases, in most agricultural crops.

Table 1 reveals that, on average, the greatest length of seedlings, both in the aerial part and root system, were higher for the level of salinity control, with means of 5.6±0.52 cm and 7.00±0.23 cm, respectively. As observed by the Kruskal-Wallis test, the higher salinity level, the lower is the mean length of seedlings, both of the aerial part and root system, with p-value=0.0018 and 0.0009, respectively.

Table 1 - Descriptive measurements of length aerial part (LAP), of root system (RSL), dry mass of the aerial part (DMAP) and of root (DMRS), in function of salinity levels

	Salinity levels (dS/m) (Mean±SD)				p-value
	0	4	8	12	
LAP (cm)	5.60±0.52	5.50±0.36	4.16±0.77	0.00±0.00	0.0018
RSL (cm)	7.00±0.23	5.82±0.70	5.78±0.55	0.00±0.00	0.0009
DMAP (g/ seedling)	0.011±0.004	0.010±0.005	0.005±0.001	0.00±0.00	0.0011
DMRS (g/ seedling)	0.012±0.003	0.009±0.003	0.007±0.003	0.00±0.00	0.0018

SD-Standard deviation

In glycophytes, such as pearl millet, the tolerance to salinity is strongly linked to the plant's ability to avoid the accumulation of toxic ions such as Na^+ and Cl^- in aerial part. Conversely, sensitive species cannot avoid this accumulation or perform a compartmentalization of these ions in a specific tissue, and they interfere in cellular metabolism, which will impair the growth (HUSSAIN *et al*., 2010). This fact was verified in our research, where the lengths of aerial part and root system, as well as the dry mass of aerial part and root system were reduced with the increment of more than 4 dS / m of salinity.

The length of aerial part is a characteristic correlated with the productivity of phytomass, therefore, it is an important variable to be evaluated. The effect of salinity on plant height is related to reduction of soil water potential, which limits the absorption of water by roots, that interfere directly in processes of stretching, cell division and, consequently, in growth of plants (ALVES *et al*., 2011).

In relation to the dry mass of aerial part and root system, it was verified that the highest means were from the level of salinity control, with values of 0.0111±0.0035 g/seedling and 0.0122±0.0027 g/seedling, respectively (Table 1). As observed by the Kruskal-Wallis test, the mean dry mass of aerial part and root system decreased with the increase of level of salinity, with p-value=0.0011 and 0.0018, respectively. Aquino et al. (2007) also found that the dry root masses were affected when submitted solutions of up to 8 $dS.m^{-1}$.

The reduction of leaf area, with consequent decrease in the volume of cells, contributes to the osmotic adjustment, when it is assumed that the amount of solute absorbed is concentrated in a smaller volume of cellular juice. However, this reduction also represents changes in partition of photoassimilates, and a decrease in the area destined to the photosynthetic process, which may be related to reduction of dry mass production (ARAÚJO *et al*., 2010; GOMES *et al*., 2011).

After estimation of parameters, we verified that the variables length of aerial part, dry mass of the root system and the intercept were not significant, at the level of 5%, to explain the germination rate of millet seedlings (Table 2). The fitted model initially presented pseudo-R^2 of 83.94%, SSR of 0.478, AIC of -160.46 and BIC -154.49.

Table 2 - Estimation of initial model parameters to explain the germination of *Pennisetum glaucum*

	Adjusting of regression model			
Variables	Estimate	Standard deviation	Test statistic	p-value
Intercept	1.437	1.455	0.988	0.323
SAL	-0.508	0.115	-4.429	<0.0001
LAP	-0.352	0.302	-1.167	0.243
RSL	0.656	0.288	2.276	0.023
DMAP	186.19	80.51	2.313	0.021
DMRS	-267.686	132.32	-1.935	0.053
$\emptyset$	8.624	3.162	2.727	0.006

In order to find the model that best explains the germination rate of seedlings, new estimates of the parameters were performed. Thus, it was defined that the model that best explains germination rate has the level of salinity, the length of root system and the dry mass of aerial part as the explanatory variables, as showed in Table 3.

Table 3 - Estimation of the final model parameters to explain seedling germination of *Pennisetum glaucum*

		Adjusting of regression model		
Variables	Estimate	Standard deviation	Test statistic	p-value
SAL	-0.386	0.043	-8.895	<0.0001
RSL	0.210	0.097	2.169	0.0301
DMAP	126.86	59.016	2.150	0.0316
Ø	8.022	2.958	2.712	0.007

After estimating the parameters, the fitted model was defined by the following expression:

$$\boldsymbol{GR} = \frac{\boldsymbol{exp}^{(-\mathbf{0.386}\boldsymbol{SAL}+\mathbf{0.21}\boldsymbol{RSL}+\mathbf{126.86}\boldsymbol{DMAP})}}{\mathbf{1} + \boldsymbol{exp}^{(-\mathbf{0,386}\boldsymbol{SAL}+\mathbf{0,21}\boldsymbol{RSL}+\mathbf{126,86}\boldsymbol{DMAP})}}$$

The fitted model presented a high coefficient of determination of model (pseudo-R^2= 90.96%), low sum of squares of residuals (SSR=0.26), Akaike information criteria (AIC=-162.9) and Bayesian information criteria (BIC=-158.92), and such measures qualify the model as appropriate. Through the fitted model, we noticed that with each increment of salinity level, there is a decrease of 32% in the germination of seedling. Furthermore, at each increment of one centimeter in the length of root system, it is expected a mean increase of 23.37% in the germination of millet seedling, whereas with an increase of 0.01 grams per seedling in the aerial part dry mass, a mean increase of 26.87% in the germination of seedling is expected.

Figure 3 shows the observed values of the germination rate of the millet seedlings and those fitted by the model in function of each significant variable. For all the graphs, the values estimated by the model are very close to the observed values, which ensure a good quality of fit of the model.

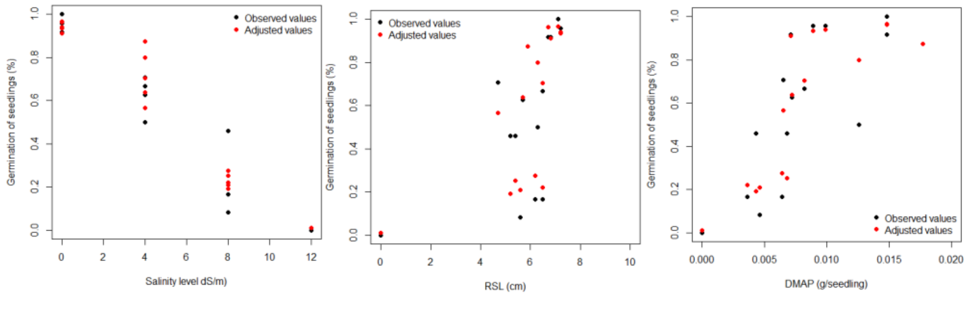


Figure 3 - Relation between the values observed and estimated by the model of germination rate of the millet seedlings in function of the variables: salinity level, root system length and dry mass of aerial part.

The Figure 4 shows different millet seedling germination scenario by setting a root system length of 5 cm, variable salinity level, from 0 to 15 dS/m, as well as the aerial dry mass, of 0.005 to 0.015 g/seedling. We verified that the highest germination rate for all saline levels is given for scenarios whose RSL is 5 cm and the dry mass of aerial part is 0.015 g/seedling, while the smaller values are given for scenarios with RSL of 5 cm and aerial part dry mass of 0.005 g/seedling. According to Nobre *et al*. (2013), the most sensitive organ of plants, regarding the effects of salts, in general, are the leaves. According to Cavalcante *et al*. (2010), the excess of potentially toxic ions such as Na^+ and Cl^- promote various physiological disturbances to the plant, which can cause their death. These results reinforce the hypothesis that, the tolerance to salt stress in initial development of millet seedlings, and its germination, is directly related to the capacity to avoid transfer and accumulation of ions Na^+ and Cl^- in the plant's aerial part.

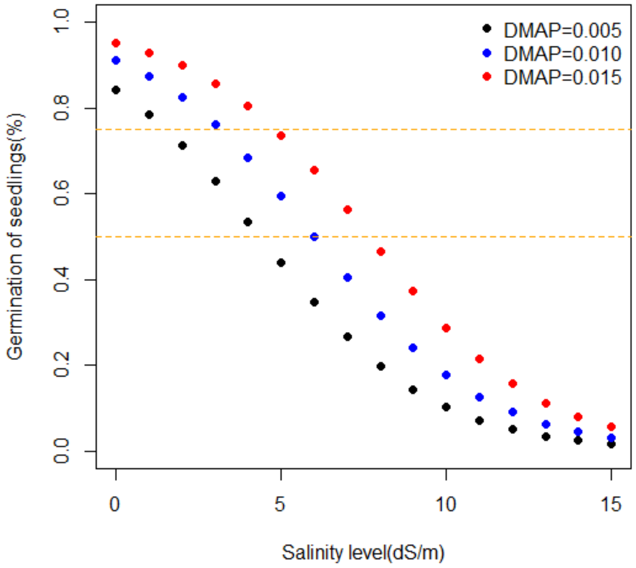


Figure 4 - Germination of millet seedlings for a 5 cm root system, with different levels of salinity and dry mass of aerial part.

As observed in Figure 4, 50% of the seedlings germination with a salinity degree of 4.36 dS/m for RSL of 5 cm and DMAP of 0.005 g/seedling. This same rate is obtained with 6 dS/m for RSL of 5 cm and DMAP of 0.010 g/seedling, and with 7.65 dS/m for RSL of 5 cm and DMAP of 0.015 g/seedling. 75% of the seedlings germination with the salinity degree of 1.51 dS/m for RSL of 5 cm and DMAP of 0.005 g/seedling, whereas for RSL of 5 cm and DMAP of 0.010 g/seedling, the degree of salinity tolerated is 3.16 dS/m.

Nonetheless, for seedlings with RSL of 5 cm and DMAP of 0.015 g/seedling, the degree of salinity tolerated is 4.8 dS/m.

These results evidenced the importance of mechanisms of salinity tolerance related to the retention of toxic ions in the roots. They also highlight the direct relation between the germination of millet seedlings and the preservation of photosynthetic tissues, since these are responsible for maintaining the metabolism and for the continuity of the development.

Conclusions

The beta regression model proved to be an appropriate option to analyse the millet seedling germination rate after 72 hours in function of salinity level, length of the root system and dry mass of the aerial part.

To each increment of one dS/m in the level of salinity, it is expected 32% of decrease in the seedling germination, on average.

To each increment of one centimetre in the length of root system, an increase of approximately 23.37% in the germination of millet seedling is expected.

To each increase of 0.01 grams in the dry mass aerial part per seedling, it is expected a mean increase of 26.87% in the seedling germination.

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- *RESUMO: O milheto é uma planta de ciclo anual com metabolismo C4 tolerante ao déficit hídrico e cultivado em locais com escassez de chuva. Objetivou-se avaliar a germinação de plântula do milheto em função do nível salino, crescimento e biomassa da parte aérea e do sistema radicular. O experimento foi instalado no delineamento inteiramente casualisados, com quatro tratamentos e cinco repetições, sendo a unidade experimental representada por uma bandeja de 120 células contendo 24 sementes por tratamento. Os tratamentos foram constituídos pelas soluções de NaCl correspondentes a 2,337, 4,674 e 7,011 g/L, respectivamente para as condutividades elétricas (CE) 4,0; 8,0; 12,0 dS.m^{-1} e 0 dS.m^{-1} como testemunha. A cultivar de milheto utilizada foi a IPA Bulk 1BF. A média da taxa de germinação das plântulas em relação ao nível de salinidade testemunha foi superior a 90% e para o nível máximo de salinidade foi 0%. O modelo proposto para explicar a taxa de germinação de plântula apresentou elevado poder de explicação (90,96%) e baixa soma de quadrados de resíduos (0,26). A taxa de germinação das plântulas pode ser explicada pelo modelo de regressão beta levando em consideração o nível de salinidade, o comprimento do sistema radicular e a massa seca da parte aérea.*

- PALAVRAS-CHAVE: *Milheto, modelagem, mortalidade, regressão beta*

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