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Full Length Research Paper

Effect of different NaCl levels in nutrient solution on the *Pennisetum* spp. genotypes

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The aim of this research was to evaluate the effect of three NaCl levels in nutrient solution on initial development and regrowth of nine *Pennisetum* spp. genotypes. The experiment was carried out in greenhouse conditions, using a completely randomized design, distributed in a 9 × 3 factorial arrangement [nine *Pennisetum* spp. genotypes (Cameroon, 'Mercker México', Mott, 'Elefante B', Taiwan 2.37, Taiwan 227, Taiwan 2.114, IRI 381, and the interspecific hybrid HV 241) and three NaCl levels (0, 60 and 100 mM)], with four replications. Traits associated with plant growth at 30, 60 and 90 days after planting (DAP) were evaluated. The results indicate a significant effect (P<0.05) of treatments on the vegetative traits in the initial stages development and regrowth, detaching decrease in tillers number, leaf and stem dry mass at 60 DAP; decrease in total mass (TM, leaf + stem) from 18.41 to 3.11 g, root mass (RM) from 6.52 to 0.38 g, and TM/RM ratio on the average of genotypes at 90 DAP. However, this reduction is lower for the Cameroon cultivar, showing to be a salt-tolerant cultivar.

Key words: Electrical conductivity, interspecific hybrid, *Pennisetum purpureum*, saline stress.

INTRODUCTION

The semi-arid regions account with, approximately, 70% of the total area of Northeastern Brazil, characterized by high water deficit, in around 2,000 mm/year (Alves, 2007). The production of ruminant feed in these regions is the biggest challenge faced by farmers due, mainly, by climatic variability. This fact has led farmers to seek alternatives for forage production in drought periods, being one of these the adoption of irrigation technique, using water coming artesian wells that have, commonly, significant levels of salt (Campos et al., 2009).

One of the most important aspects when it comes to the success of irrigation as agricultural practice, in respect to water quality, especially in arid and semi-arid regions, where can to exist an increase in the of salt concentration, causing problems in the soils and plants (Shannon, 1997). For Cushman (2001), the effects of salinity on growth and development of plants are found in three pathways: by saline stress, due to osmotic effect, restricting the water availability (water stress) and absorption of nutrients (antagonism); by toxicity, from specific ions accumulation; and by nutritional disorder, reflecting in morphological, structural and metabolic characters of the plants.

There are two research lines that address the essentiality of sodium (Na^+) in the mineral nutrition of plants (Schulin et al., 2010). Some authors affirm that this ion is beneficial or functional, by to replace in part, the functions of the K^+ ion (Malavolta, 2006). Another one, however, considers the Na^+ an essential micronutrient, especially in C_4 species (Brownell and Crossland, 1972), by to act in the regeneration of the enzyme

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phosphoenolpyruvate carboxylase (PEPcase) (Taiz and Zeiger, 2004), replace the K^+ ion and stimulate plant growth. However, both lines agree that in excess, Na^+ can cause physiological disorders (Shannon and Grieve, 1999; Munns and Tester, 2008), reflecting in the reduction of C_4 grasses yield (Wang et al., 2002; Muscolo et al., 2003; Amorim et al., 2005; Dantas et al., 2006; Santana et al., 2007). When non-salt-tolerant species (common bean, citrus) are subjected to soil salinity with electrical conductivity of saturation extract from 2.0 to 4.0 dS m^{-1}, the yield is affected, and above 8.0 dS m^{-1}, the yield of many crops of agricultural importance (soybean, corn, castor bean) is reduced (Pereira, 2008).

According to Munns and Tester (2008), in the world more than 800 million hectares of land are affected by salt. In Pernambuco State, about 7,819 km^2 or 8% of the total area are affected (Pereira, 2008). In these areas, the practice of irrigation with saline water is very common, with electrical conductivity of 5.0 dS m^{-1}, reaching 8.0 dS m^{-1}. However, the low tolerance of cultivars impedes investments, having as consequence the abandonment of lands after few years of cultivation.

In tropical regions, the use of elephant grass and their hybrids (*Pennisetum* spp.) as stocking piles under irrigation is promising and widespread practice, taking into consideration the potential forage accumulation, which varies according from genotype and environment where it's cultivate. When grown with satisfactory water availability and intensively managed, *Pennisetum* spp. genotypes can reach daily production at 200 kg dry matter ha^{-1} and crude protein concentration around 15% (Gomide, 1994). Research related to the use of elephant grass genotypes irrigated with saline water is scarce in the literature. In this sense, this research aims to evaluate the effect of three NaCl levels in nutrient solution in the initial development and regrowth of nine *Pennisetum* spp. genotypes.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse of the Department of Animal Science, Federal Rural University of Pernambuco, Brazil, from October 2009 to January 2010. The average temperature and relative humidity of the air during the experimental period was 29.7°C and 73.2%, respectively.

The experimental design adopted was completely randomized, with treatments distributed in 9×3 factorial arrangement, with nine *Pennisetum* spp. genotypes (Cameroon, 'Mercker México', Mott, 'Elefante B', Taiwan 2.37, Taiwan 227, Taiwan 2.114, IRI 381, and interspecific hybrid HV 241) and three NaCl levels in nutrient solution with four replications. Each experimental unit consist of one plant.

The nutrient solutions used as treatments were prepared to contain zero (control), 60 and 100 mM NaCl L^{-1} of Hoagland solution. These concentrations were equivalent to electrical conductivity, measured periodically with a portable conductivity, of 2.2 ± 0.2, 5.0 ± 0.2 and 8.0 ± 0.2 dS m^{-1}, corresponding, respectively, to non-saline treatment (control), slightly saline and moderately saline, according to Richards (1954). The experiment was conducted in transparent pots (PET, polyethylene ethyl), with capacity of 2.0 dm^{-3}, containing washed sand as substrate. The pots were enveloped by bags (black polyethylene) with the aim to prevent the proliferation of algae and the photo-oxidation of the auxin hormone by sunlight (Taiz and Zeiger, 2004), which could limit the development of roots.

At the time of planting, one stem with two nodes was used. The irrigations were daily, applying 0.1 L of the solutions in the first 30 days. After this period, with the growth of the genotypes, were performed irrigations twice/day (morning and afternoon), totaling 0.2 L pot^{-1}. In the pot' bottom was made perforations for drainage of the solution. However, the drained was not reused, prevent salts accumulation and, thereby, modify the planned treatments. Evaluations were performed at 30, 60 and 90 days after planting (DAP). At 30 DAP, the tillers number and its vigor, expanded leaf number, plant height and leaf chlorosis were evaluated. At 60 DAP, tillers number, plant height, expanded leaf number, leaf and stems mass, and leaf/stem ratio were evaluated. At 90 DAP the tillers number and its vigor, total mass (leaf + stem), root mass, and total mass/root mass ratio were evaluated.

The evaluation of tillers vigor at 30 and 90 DAP was performed by three observers, with scores of 0 (no tiller) to 9 (maximum development of tillers). To evaluate the leaf number (fully expanded), was considered the full development of the auricle (completely white color). Plant height was obtained with the aid of a tape (3.0 m), taking the base of the tiller most developed up to the inflection of the apical leaf (cm).

The harvest was performed by separation of the shoot (leaf + stem) of the roots, with the aid of one pruning shear. The roots were carefully washed to remove sand and then, the same way as acted with the shoot, they were placed in an oven with air circulation, set at 65°C, for subsequent dry mass determination. Evaluations of the leaf chlorosis (LCH) were performed, as of units SPAD (Soil Plant Analysis Development), with the aid of the SPAD-502 equipment (Konica Minolta®, Japan) in 20 points in the leaf fully expanded.

At the end of the experiment (when more than 50% of the plants died due to saline solutions), it was prepared an extract from the cultivation substrate, formed by 200 ml of substrate and 100 ml of distilled water. Then, the extract was filtered and measured the electrical conductivity (dS m^{-1}), taking the average of ten pots. Whereas the data of the variables evaluated do not present normal distribution, they were transformed by square root of 'x + 1' ($\sqrt{x+1}$), then, analysis of variance (ANOVA) carried out. The means were clustered by the Scott-Knott test ($P<0.05$).

RESULTS AND DISCUSSION

There were significant difference between genotypes ($P<0.05$) for all variables. This was not observed for the source of variation salinity, which showed significant difference ($P<0.05$) to ten variables and non-significant difference ($P>0.05$) to the other six, in the three periods of evaluation (Table 1). Even with the absence of significance, there was downward trend in the average values when the salt concentration increased.

The genotype × salinity interaction (G × S) was not significant for all the variable evaluates. The coefficient of variation (CV%) varied from 8.1% for leaf chlorosis at 30 DAP up to 36.2% for the total mass at 90 DAP. Those variables where the salinity factor was significant ($P<0.05$), the CV (%) was high, showing the influence of salts on the morpho-agronomics traits of the genotypes (Table 1). This effect could be more expressive if a clay

Table 1. Clustering average[1] by Scott-Knott test for the variables tillers number (TN), tillers vigor (TV), leaf number (LN), plants height (PH, in cm), leaf chlorosis (CHL, in SPAD units), leaf mass (LM, in g), stem mass (SM, in g), leaf/stem ratio (L/S), total mass (TM, in g), root mass (RM, in g), and TM/RM ratio of three NaCl levels (mM) in nutrient solution, evaluate at 30, 60 and 90 days after planting (DA P) (average of nine *Pennisetum* spp. genotypes.).

NaCl levels	Evaluation periods															
	30 DAP					60 DAP						90 DAP				
	TN	TV	LN	PH	LCH	TN	PH	LN	LM	SM	L/S	TN	TV	TM	RM	TM/RM
Control	2.33a[2]	7.03a	9.81a	84.92a	44.73a	3.44a	108.50a	8.94a	20.94a	107.61a	1.03a	6.81a	3.94a	18.41a	6.52a	2.71a
60.0	2.14a	6.36b	7.94b	78.42a	43.33a	3.02a	107.78a	8.86a	18.59b	79.89b	1.00a	4.78b	2.81b	14.66b	3.53b	2.99a
100.0	1.89a	5.92b	6.58b	74.00a	43.11a	2.64b	96.03a	7.72a	16.92b	65.5c	0.99a	1.0c	0.56c	3.11c	0.38c	0.53b
CV (%)	16.8	14.4	26.1	23.1	8.1	18.1	24.3	20.8	20.5	23.6	9.3	30.3	23.1	36.2	22.8	30.8

[1]Original average. [2]Average followed by same letters in columns belong to the cluster by Scott-Knott test (P>0.05).

soil was the substrate, since the Na^+ ions increase the cation exchange capacity of the soil, as observed by Santana et al. (2003), and this could cause dispersion of clay minerals, causing nutritional disequilibrium, because Na^+ is the ion in highest concentration in soil solution (Cushman, 2001).

The effect of saline solution was observed in both the traits, shoot and roots, reducing the tillers number at 30 DAP, being most expressive at 90 DAP. The leaf number reduced at 30 DAP and tended to decrease at 60 DAP, when the solution changed from non-saline to moderately saline solution (Table 1), confirming the results of Munns and Tester (2008). Reduction in the leaf number has direct effect in the reduction of leaf area, as observed by Oliveira et al. (2009) in *Zea mays* (L.), and in the production of energetic compounds (ATP and NADPH), reflecting in the decrease of activity of carboxylation enzymes, that will compromise all biochemical functions related to the growth and development of plants. Osmond and Greenway (1972), in the C_4 species *Z. mays* and *Atriplex* spp., observed decreasing in the concentration of the PEPcase enzyme with increasing salinity of the solution. Reduction of vegetative traits similar of this research was observed by Muscolo et al. (2003), in *P. clandestinum*, that reported a reduction in the leaf number when the saline concentration exceeded 8.0 dS m^{-1} at 15 DAP. Amorim et al. (2005) observed reduction of 35% in the plant height of *Z. mays* with increasing of salinity. Dantas et al. (2006), evaluating *P. purpureum* genotypes, observed reduction of up to 70% in the dry mass of shoots (6 and 81 genotypes) when the electrical conductivity increased from 0.69 to 15.0 dS m^{-1}. Reductions in the tillers number and dry matter of shoots and roots due to increased electrical conductivity of water (from 0.0 to 8.0 dS m^{-1}) were observed by Santana et al. (2007), in *Saccharum* spp.

Deleterious effects to the roots (MR) were observed with increasing of salinity at 90 DAP, occurring reduction from 6.52 to 0.38 g for solutions control and moderately saline, respectively (Table 1). Oliveira et al. (2009) obtained similar results. Muscolo et al. (2003) observed effect of salinity on the activity of important glycolytic enzymes present in the roots (glucose kinase, phosphoglycoisomerase and pyruvate kinase), resulting in accumulation of hexoses (by increasing the activity of the acid invertase enzyme) and reduction of biomass root, explaining, with this, the results obtained in this research.

Considering that after removing of shoot, the root elongation ceases (40 to 50% within 24 h) and the death of fine roots occur (Richards, 1993) to reduce the consumption of storage carbohydrate (responsible for regrowth in perennial grass), with increasing of saline concentration, plants can use part of these carbohydrates to expel Na^+ ions (by increase of respiration), compromising the regrowth after cut. This was observed in the reduction of the TM/RM ratio, indicating that the effect of salinity was most pronounced in shoots than in roots (Table 1). However, this reduction was lower for Cameroon and 'Elefante B' cultivars (Table 2), distinguishing itself as salt-tolerant species. Similar effects were reported by Lacerda et al. (2005) in *Sorghum bicolor* (L.) Moench. These authors affirmed that this phenomenon may be associated with an osmotic adjustment faster and a slower loss of turgor of roots compared to shoots.

Evaluating the genotypes at 30 DAP, the interspecific hybrid 241 HV was better in all traits (Table 2). In this evaluation, there was no effect of salinity on leaf chlorosis, represented by SPAD units. Considering that these units are highly correlated with chlorophyll levels of leaf (r = 0.87) in elephant grass (Cavalcante et al., 2010), concentrations of NaCl may not have inhibited the absorption of nitrogen and magnesium (nutrients constituent of the chlorophyll molecule). The differences found between genotypes for leaf chlorosis (due to genetic variations), allowed the formation of two groups by Scott-Knott test, with averages ranging from 36.0 SPAD units for Cameroon cultivar up to 50.1 SPAD units for interspecific hybrid HV 241 (Figure 1). Effect of salinity and lower values than this research were found by Wang et al. (2002), in *P. purpureum*, with averages varying from 34.9% to 1.5 dS m^{-1} up to 29.7% to 15.0 dS m^{-1}.

Table 2. Clustering average[1] by Scott-Knott test for the variables tillers number (TN), tillers vigor (TV), leaf number (LN), plants height (PH, in cm), leaf mass (LM, in g), stem mass (SM, in g), leaf/stem ratio (L/S), total mass (TM, in g), root mass (RM, in g), and TM/RM ratio of nine *Pennisetum* spp. genotypes evaluate at 30, 60 and 90 days after planting (DAP) (Average of three NaCl levels).

Genotypes	Evaluation periods													
	30 DAP				60 DAP						90 DAP			
	TN	TVP	LN	PH	TN	PH	LN	LM	SM	L/S	TN	TV	TM	TM/RM
Cameroon	2.2b[2]	7.9a	7.8a	105.5a	2.2c	136.1a	6.6c	23.3a	102.0b	1.2a	4.3a	3.9a	17.2a	3.2a
'M. México'	1.4b	3.5b	3.9b	59.0c	1.4c	57.8c	3.6d	9.9c	34.2d	0.7b	2.6b	1.8b	08.4c	2.0b
Mott	2.0b	7.3a	10.8a	78.7b	3.1b	96.5b	8.1c	20.1a	78.2b	1.2a	5.0a	2.6b	13.3b	1.9b
'Elefante B'	2.2b	6.8a	9.2a	94.1a	3.3b	135.3a	9.8b	21.9a	125.7a	0.9b	4.9a	1.8b	12.7b	2.9a
Taiwan 2.37	1.9b	6.6a	6.8a	76.9b	4.2a	110.6b	10.3b	18.6a	87.5b	0.9b	4.8a	2.7b	13.7b	2.2b
Taiwan 2.27	1.6b	5.8a	7.5a	74.6b	2.5c	70.8c	8.3c	14.6b	62.3c	0.8b	4.8a	2.5b	11.1b	1.6b
Taiwan 2.114	2.6a	6.0a	8.3a	79.9b	3.4b	106.5b	9.2b	20.1a	79.8b	1.1a	5.8a	2.4b	12.7b	2.1b
HV 241	3.3a	7.1a	11.0a	96.5a	4.5a	102.0b	13.8a	21.4a	91.7b	1.2a	3.3b	2.0b	08.9c	0.9b
IRI 381	1.9b	7.0a	7.6a	90.2a	2.8c	121.3a	7.1c	18.6a	97.7b	0.9b	2.3b	2.3b	10.5b	1.6b

[1]Original average. [2]Average followed by same letters in columns belong to the cluster by Scott-Knott test (P>0.05).

Table 3. Nutrient solution traits related to saline treatments, before and after the experiment.

Solutions[1]	Inclusion of NaCl (mM)	Electrical conductivity (dS m^{-1})[1]		Osmotic pressure (MPa)[2]	
		Before	After	Before	After
Control	0.0	2.2	4.04	-0.10	-0.19
Slightly saline	60.0	5.0	6,92	-0.23	-0.33
Moderately saline	100.0	8.0	10.96	-0.37	-0.52

[1]Obtained using a portable conductivity meter. [2]Calculed according to Klaus and Timm (2004).

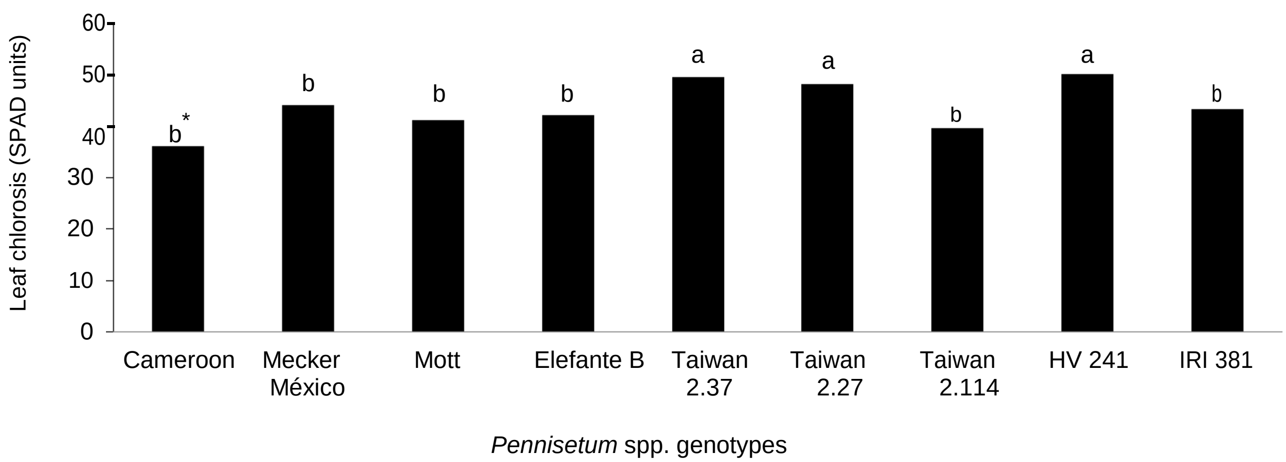

Figure 1. Leaf chlorosis (SPAD units) evaluated at 30 DAP, in nine *Pennisetum* spp. genotypes. Average of three NaCl levels (*average followed by the same letter belong the same cluster by Scott-Knott test, P>0.05).

At 60 DAP there was significant differences (P<0.05) between genotypes for all traits. The hybrid HV 241 stood out in relation to tillers number, leaf number, leaf mass, and leaf/stem ratio (Table 2). According to Dantas et al. (2006), the cross between elephant grass and pearl millet produces interspecific hybrids salt-tolerant, fact confirmed up to 60 DAP. Already at 90 DAP, the Cameroon cultivar stood out in the tiller number and its vigor, total mass and total mass/root mass ratio, confirming the performance of this type of stress (Table 2).

At the end of the experiment (At 90 DAP), the electrical conductivity of aqueous extract of the substrate was measured, and was observed to increase in the values of the solutions used and, therefore, reduction of the osmotic potential (Ψs) (Table 3), passing the solutions slightly and moderately saline to be classified as moderately and highly saline (Ayers and Westcot, 1991). This was due to the salt accumulation, since the drained solution was not sufficient to leach ions from soil solution. Added to this, the cut of the shoots realized at 60 DAP, which precluded the process of transpiration, being considered by Taiz and Zeiger (2004) an important mechanism for expulsion of salts in excess by plants. The increase of the electrical conductivity and the reduction of Ψs due to the salt accumulation (Table 3) may yet to have impaired the absorption of water by plants, reflecting in the turgescence and leaf expansion, its vigor and the total mass/root mass ratio, which passed from 3.94 to 0.56 and from 2.71 to 0.53 in control and moderately saline solutions, respectively, at 90 DAP (Table 1).

Conclusions

The NaCl levels exert influence on the morpho-agronomic traits of *Pennisetum* spp., in the initial stage development and regrowth. With increasing saline stress occurs reduction in the tillers number and its vigor, leaf number, total mass (TM, leaf + stem), root mass (RM), and TM/RM ratio. Among the genotypes, Cameroon is a salt-tolerant cultivar.

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