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Biomass production and chemical bromatological composition of jureminha submitted to increasing saline levels

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SUMMARY

Aiming at alternatives to minimize the problems generated by salinity, which is increasingly common in the world, it is necessary to understand plant growth and development in saline environments. The aim was to characterize the biomass production and determine the chemical bromatological composition of *Desmanthus pernambucanus* (L.) Thellung (jureminha) plants submitted to salinity. The trial was carried out in greenhouse through a completely randomized design with 5 treatments (0, 25, 50, 75, and 100 mM NaCl) and 6 replicates. In each 54 days were performed cuts separated in fractions as stem, leaf and pod. During October and November, plants submitted to 100 mM NaCl had 83 and 100% mortality, respectively, and only 50% of plants submitted to 75 mM survived until November. In August, as plants got grain-filling stage had the following changes: increase of pod biomass production (148%), fiber compounds (NDF: 11 and 9% for leaves and stems, respectively) and proline in stems (62%); reduction in leaf/stem ratio (33%), crude protein (16 and 14% for leaves and stems, respectively), and soluble carbohydrates (28 and 17% for leaves and stems, respectively). The grain-filling stage potentialized the harmful effects of salinity, wherein there is Na+ enhancing and K+ reduction in vegetable tissues. The salinity negatively affects the production and chemical bromatological composition of jureminha forage.

Palavras chave adicionais
Forragem.
Leguminosas.
Plantas nativas.
Tolerância ao sal.

Produção de biomassa e composição químico-bromatológica de jureminha submetida a crescentes níveis salinos

RESUMO

Visando alternativas para minimizar os problemas gerados pela salinidade, cada vez mais comum no mundo, é necessário entender o crescimento e o desenvolvimento das plantas em ambientes salinos. O objetivo foi caracterizar a produção de biomassa e determinar a composição químico-bromatológica de plantas de *Desmanthus pernambucanus* (L.) Thellung (jureminha) submetidas à salinidade. O experimento foi conduzido em casa de vegetação, em delineamento inteiramente casualizado, com 5 tratamentos (0, 25, 50, 75 e 100 mM NaCl) e 6 repetições. Em cada 54 dias foram realizados cortes separados em frações como caule, folha e vagem. Durante outubro e novembro, plantas submetidas a 100 mM de NaCl apresentaram 83 e 100% de mortalidade, respectivamente, e apenas 50% das plantas submetidas a 75 mM sobreviveram até novembro. Em agosto, as plantas na fase de enchimento de grãos apresentaram as seguintes alterações: aumento da produção de biomassa de vagens (148%), compostos fibrosos (FDN: 11 e 9% para folhas e caules, respectivamente) e prolina nos caules (62%); redução na relação folha/caule (33%), proteína bruta (16 e 14% para folhas e caules, respectivamente) e carboidratos solúveis (28 e 17% para folhas e caules, respectivamente). A fase de enchimento de grãos potencializou os efeitos nocivos da salinidade, em que houve aumento de Na+ e redução de K+ nos tecidos vegetais. A salinidade afeta negativamente a produção e composição químico-bromatológica da forragem de jureminha.

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INTRODUCTION

The salinity can be responsible by morphological, physiological, and biochemical changes in vegetables, which are due to nutritional, osmotic and/or ionic factors (Parida & Das 2005, p. 324; Hanumantharao et al. 2016, p. 3). The nutritional effects can occur due to reduction of nutrient availability to the plant, generated by competition in uptake and transport of ions within the plant, and/or changes in plasma membrane integrity (Gupta & Huang 2014, p. 1). Regarding the osmotic factors, the great amount of salts dissolved in soil solution decreases the osmotic potential of this solution, which reduces the absorption of water and nutrients

by plants (Hanumantharao et al. 2016, p. 3). The ionic effect is attributed to the ions absorbed by the plant, especially Na^+ and Cl^-, which in high concentrations can cause disturbances in ionic homeostasis of cells (Kim et al. 2014, p. 109; Hanumantharao et al. 2016, p. 3).

Therefore, the harmful effects of salinity are one of the main causes in reduction of crops yield, and affects not only agriculture, but also livestock, which has its productivity significantly affected by decreasing forage production. The practices to reduce salinity problems are usually expensive and often economically infeasible. The use of forage species adapted to saline conditions can optimize the use of salinized soils, as well as increase forage allowance.

The legume *Desmanthus pernambucanus* (L.) Thellung (jureminha) consists of plants erect to decumbent perennial shrubs, mostly 0.5–1.5 (–2.5) m tall, with yellow flowers, high seeds yield and leaves bipinnate, 4–11 cm long (**Figure 1**; Cook et al. 2005). This specie present great acceptability by a large diversity of animals and can be used in silages, hay, protein bank, mixed with grasses and other crops, and in green fertilization (Fontenele et al. 2009, p. 123). It is worth pointing out that forage legumes represent a low-cost protein source, which increases the quality of animal diet. In addition, it offers indirect benefits such as biological nitrogen fixation, which reduces the need for nitrogen fertilization and, consequently, expenses on these fertilizers.

Despite of jureminha forage potential, there is little information about its development under saline conditions. Thus, it was aimed to characterize the biomass production of jureminha submitted to increasing saline levels, as well as to determine its chemical and bromatological composition.

MATERIAL AND METHODS

The trial was carried out in a greenhouse with open sides, is set in Serra Talhada town, Brazil (07°59' S, 38°18' W, 435-m sea level). At 108 days after sowing, the seedlings were transplanted into the plots (plastic pots with 5 L capacity) filled with 3.5 kg of washed sand and vermiculite (1:1). Each plot was daily-irrigated 300 mL water in order to keep the substrate constantly moisturized. Fifteen days after seedlings transplant, 300 mL of nutritive solution, proposed by Hoagland & Arnon (1950, p. 29), was applied in 4 days interval in order to ensure the plants setting.

The design applied was completely randomized, 6 replicates, 5 treatments – different concentrations of NaCl (0, 25, 50, 75, and 100 mM) added to nutritive solution of Hoagland & Arnon (1950, p. 29). Fifty-eight days after transplanting was performed a uniformization cut up to 20 cm of intensity, as well as branches were removed, resting only the main stem. Then, 300 mL saline nutritive solution, was applied every four days regarding to treatments.

Variables were observed as plant mortality, floral buds, flowers, pods, and total of reproductive structures (buds + flowers + pods) at 54, 108, 162, and 216 days after set treatments on plots. Thus, after collecting reproductive structures, data were performed cuts similar to uniformization cut. The first cut, after plot treatments, was performed in June (18 th), second in August (11 th), third in October (04 th), and fourth in November (27 th) all of them in 2015. The plant material gathered was divided in fractions, as stem, leaf, and pod (with seeds), weighed in semi analytic digital scale to get the fresh weight of each fraction. After record the fresh weight, samples were placed in oven dryer at 55 ºC for 72 h, weighed in semi analytic scale, ground in micro knife mill Wiley. Then, samples were kept in identified flasks for chemical and bromatological composition analysis.

From plant material gathered was estimated individually the biomass, in dry matter basis, of leaf, stem, and pod fractions, as well as the leaf/stem ratio, total production of upper layer (sum of leaf and stem). The dry matter (DM), mineral matter (MM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed as described by Silva & Queiroz (2006, p. 23). Hemicellulose was calculated by difference between NDF and ADF.

Aqueous extract from samples were prepared to determine organic and inorganic solutes using about 0.1 g of each dried sample and placed in tubes identified. Then, 10 mL distillated water was added to tube, sealed, and incubated at 100 °C for 1 h through a water bath. The extracts were double filtered through cotton and kept in refrigerator for further analysis. Extracts were used to determine soluble carbohydrates according to Dubois et al. (1956, p. 350); proline according to Bates et al. (1973, p. 205); Na^+ and K^+ analyzed by flame photometry. All those concentration estimative were in g DM kg^{-1}. Through leaf and stem biomass, and Na^+ found in these fractions, was obtained the Na^+ amount extracted from substrate. It is noteworthy that the great plant mortality and low plant tissue available could not be analyzed in laboratory through fourth cut (November) and, in some cases, in third cut (October). Thus, analyses were performed just in the firsts cuts (June and August) for the following variables: pod biomass; NDF, ADF, and stems biomass; soluble carbohydrates and K^+ in leaves; mineral matter, crude protein, proline, and K^+/Na^+ ratio in leaves and stems.

Analysis of variance and the hypothesis testing (F-test) were performed for all variables responses. It was adopted as a criterion to affirm an effect of the classificatory variables (salt and cutting period) in isolation, or their interactions, the significance level for the F-test equivalent to $P<0.05$. To perform the F-test, it was considered the salt variable as a fixed factor in time and the cutting period as a repeated factor in time, so, it was carried out an analysis of repeated measurements in time (longitudinal data). When detecting the salt effect, it was performed a regression analysis while there being an effect of cutting period, the means were compared by Tukey test ($P<0.05$). For the analysis of repeated measurements in time and regression, Proc mixed procedures and Proc reg in the SAS (Statistical Analysis System, version 9.2) were used, respectively. For the comparison of means, it was used the LSMEANS command, PDIFF adjusted to Tukey.

Figure 1. *Desmanthus pernambucanus* **(L.) Thellung (jureminha) [(Cook et al. 2005)]** (*Desmanthus pernambucanus* (L.) Thellung (jureminha) [(Cook et al. 2005)]).

RESULTS

Plant mortality

During 216 days applying saline levels to plots, mortality was absented in 0, 25, 50 mM NaCl treatments. However, for 100 mM level, 83 e 100% of plants were dead in October, and in November, respectively. For 75 mM level, the mortality just occurred in November reaching 50%.

Reproductive structures

The interaction between salinity and cut period did not affect any variables related to reproductive structures (**Table I**). However, isolated treatment factors had simultaneous effect on floral bud, pods number, and

Table I. Number of floral buds, flowers, pods and total of reproductive structures per jureminha plant, on the 54th day of regrowth, as a function of salinity levels and the cut periods (Número de botões florais, flores, vagens e total de estruturas reprodutivas por planta de jureminha no 54º dia de rebrota, em função dos níveis de salinidade e dos ciclos de corte).

Salinity (mM)	Reproductive Structures			
	Floral buds	Flowers	Pods	Total
0	7.6 ± 1.0	0.6 ± 0.2	36.1 ± 1.6	44.3 ± 2.1
25	4.9 ± 1.0	0.4 ± 0.2	39.4 ± 1.6	42.3 ± 2.1
50	4.2 ± 1.0	0.6 ± 0.2	33.0 ± 1.6	37.7 ± 2.1
75	3.5 ± 1.0	0.8 ± 0.2	27.0 ± 1.6	31.3 ± 2.1
100	2.4 ± 1.6	0.7 ± 0.3	26.8 ± 4.2	29.9 ± 3.5
Y	6.89 – 0.0471x	0.7 ± 0.1	38.66 – 0.124x	45.04 – 0.1585x
R^2	0.91	.	0.78	0.96
P	0.0114	ns	0.0478	0.0032
Cut periods	Floral buds	Flowers	Pods	Total
June	7.8 ± 0.8a	1.1 ± 0.1a	25.6 ± 2.0b	34.5 ± 1.7b
August	3.2 ± 0.8b	0.6 ± 0.1b	49.6 ± 2.0a	53.3 ± 1.7a
October	2.6 ± 1.1b	0.2 ± 0.2c	20.7 ± 2.3b	23.5 ± 2.3b

Means ± standard error, followed by equal letters in the column, comparing the cut periods, did not differ among themselves by the Tukey test (P>0.05).

Table II. Biomass production (g DM plant^{-1}) of different fractions of jureminha plants and leaf/stem ratio, as a function of salinity levels and cut periods (Produção de biomassa (g MS planta^{-1}) de diferentes frações de plantas de jureminha e relação folha/caule, em função dos níveis de salinidade e dos ciclos de corte).

Salinity (mM)	Cut periods		
	June	August	October
	Leaves biomass		
0	5.1 ± 0.2a	3.5 ± 0.2b	2.8 ± 0.2c
25	5.3 ± 0.2a	2.8 ± 0.2b	1.7 ± 0.2c
50	4.4 ± 0.2a	2.5 ± 0.2b	1.7 ± 0.2c
75	4.5 ± 0.2a	2.3 ± 0.2b	1.0 ± 0.3c
100	4.2 ± 0.2a	2.1 ± 0.2b	0.0 ± 0.2c
Y	5.22 – 0.0104x	3.28 – 0.0128x	2.7 – 0.0252x
R^2	0.78	0.90	0.93
P	0.0487	0.0145	0.0074
	Stems biomass		
0	9.6 ± 0.4a	9.6 ± 0.5a	3.4 ± 0.4b
25	9.4 ± 0.4a	7.3 ± 0.5b	2.1 ± 0.4c
50	7.1 ± 0.4a	7.4 ± 0.4a	2.0 ± 0.4b
75	7.2 ± 0.4a	5.3 ± 0.4b	1.0 ± 0.4c
100	6.8 ± 0.4a	4.8 ± 0.4b	0.6 ± 1.1c
Y	9.58 – 0.0316x	9.2 – 0.0464x	3.16 – 0.0268x
R^2	0.82	0.92	0.94
P	0.0334	0.0103	0.0061
	Total above-ground biomass (leaves + stems)		
0	14.7 ± 0.6a	13.2 ± 0.6a	6.1 ± 0.6b
25	14.7 ± 0.6a	10.1 ± 0.7b	3.8 ± 0.6c
50	11.6 ± 0.6a	9.9 ± 0.6b	3.7 ± 0.6c
75	11.7 ± 0.6a	7.6 ± 0.6b	2.2 ± 0.7c
100	11.0 ± 0.6a	6.9 ± 0.6b	0.0 ± 0.6c
Y	14.82 – 0.0417x	12.53 – 0.06x	5.92 – 0.0552x
R^2	0.82	0.93	0.94
P	0.0342	0.0081	0.0063
Pods biomass	2.9 ± 0.3b	7.2 ± 0.3a	-
Leaf/stem ratio	0.59 ± 0.02a	0.44 ± 0.02b	0.64 ± 0.02a

Mean ± standard error, followed by equal letters in the row, for each fraction, did not differ by Tukey's test (P>0.05). (-) Insufficient plant material.

total reproductive structures. There was unique effect about cut period factor on flowers number per plant.

Biomass production

The interaction between cut periods and salinity levels had significant effect on leaf biomass production, stem biomass, and total above-ground biomass (leaves + stems) (**Table II**). However, the pods biomass production and leaf/stem ratio were affected only by cut periods.

Bromatological composition

The mineral amount in leaves was affected only by saline levels applied and the mineral amount in stems was affected only by cut periods (**Table III**). Just cut period affected crude protein for both fractions. There was interaction between cut periods and salinity on NDF, ADF, and hemicellulose in leaves. About the response between cut periods, there was increase in NDF and hemicellulose amount only in the greatest saline levels (75 and 100 mM), while ADF, increased only in the greatest saline level (100 mM). For the three variables, when analyzing the first and second cut (June and August), the data used in regression analysis were better adjusted for a linear model, decreasing in the first cut and increasing in the second. There was effect of cut period on NDF and ADF in stems, which the second cut was greater than first one. However, the hemicellulose amount in this tissue was not affected neither by salinity nor cut periods, presenting average 200.7 ± 4.1 g kg^{-1} DM.

Soluble carbohydrates and proline

In leaves, the soluble carbohydrates were affected only by cut periods, which were reduced in the second cut when compared to first (**Table IV**). In stems, this

Table III. Bromatological composition (g DM kg^{-1}) of the leaves and stems of jureminha plants as a function of salinity levels and cut periods (Composição bromatológica (g MS kg^{-1}) das folhas e caules de plantas de jureminha em função dos níveis de salinidade e dos ciclos de corte).

Cut periods	CP		MM	NDF	ADF
	Leaves	Stems	Stems	Stems	Stems
JUNE	260.7 ± 3.6A	83.5 ± 2.0A	28.1 ± 0.7B	498.3 ± 6.7B	711.2 ± 8.7B
AUGUST	218.8 ± 3.9B	73.9 ± 1.96B	33.8 ± 0.7A	543.1 ± 6.7A	744.4 ± 8.7A
			MM – Leaves		
Y		R^2	P		CV (%)
82.49 + 0.18x – 0.0031x²		0.99	0.0031		0.64
Salinity (mM)			Cut periods		
		JUNE	AUGUST		OCTOBER
			NDF – Leaves		
0		382.3 ± 25.2a	310.9 ± 27.6a		315.6 ± 25.2aA
25		333.3 ± 25.2a	297.5 ± 25.2a		297.9 ± 25.2aA
50		332.2 ± 25.2a	384.8 ± 30.8a		281.7 ± 25.2aA
75		302.0 ± 35.6b	397.8 ± 25.2a		-
100		283.2 ± 25.2b	419.5 ± 25.2a		-
Y		372.5 – 0.918x	298.6 + 1.27x		-
R^2		0.93	0.85		-
P		0.0081	0.0262		-
			ADF – Leaves		
0		216.6 ± 12.7a	192.2 ± 12.7a		210.9 ± 12.7aA
25		203.5 ± 12.7a	188.7 ± 12.7a		191.7 ± 12.7aA
50		202.6 ± 12.7a	220.8 ± 13.9a		213.6 ± 12.7aA
75		205.0 ± 17.9a	243.2 ± 12.7a		-
100		182.1 ± 12.7b	251.9 ± 12.7a		-
Y		24.84 – 0.1374x	184.58 + 0.6959x		-
R^2		0.73	0.92		-
P		0.0637	0.0109		-
			Hemicellulose – Leaves		
0		139.2 ± 15.0a	96.8 ± 16.8a		104.7 ± 13.7aA
25		129.8 ± 13.7a	108.8 ± 13.7a		106.3 ± 13.7aA
50		129.6 ± 13.7a	146.3 ± 16.8a		103.6 ± 19.4aA
75		97.0 ± 19.4b	154.6 ± 13.7a		-
100		101.1 ± 13.7b	179.5 ± 15.0a		-
Y		114.12 – 0.4357x	95 + 0.0444x		-
R^2		0.83	0.97		-
P		0.033	0.0026		-

CP: crude protein, MM: mineral matter, NDF: neutral detergent fiber, ADF: acid detergent fiber. Mean ± standard error, followed by equal letters, in the row (lower case) and column (upper case) for each fraction, do not differ by Tukey test (p>0.05). (-) Insufficient plant material.

variable had influence by the interaction between salinity and cut period.

Levels of proline in leaves had no influence by salinity and cut periods, which showed average and standard error 17.7 ± 1.1 g kg^{-1} DM and only cut periods had effect on proline of stems, which had increment from first cut (June) to second (August) (**Table IV**).

Na^+ and K^+ amount

For Na^+ amount, in both leaves and stems was observed interaction between salinity and cut periods (**Table V**). The amount of Na^+ in leaves and stems of plants submitted to the greatest salinity (100 mM) were 180 and 230%, respectively, greater than in leaves and stems of plants salinity free (0 mM) in June. In August, however, those values reached 321 and 427%. In October, samples were not enough to analyze the greatest saline levels, although in the data recorded are noticed a Na^+ increment of 428% in leaves of plants submitted to 75 mM NaCl than in plants without salinity (0 mM). Na^+ in stems, however, had an increase of 228% when compared plants submitted to 0 and 25 mM. For

Table IV. Proline content in stems and soluble carbohydrates (SC) (g DM kg^{-1}) in leaves and stems of jureminha plants, as a function of salinity levels and cut periods (Teor de prolina em caules e carboidratos solúveis (CS) (g MS kg^{-1}) em folhas e caules de plantas de jureminha em função dos níveis de salinidade e dos ciclos de corte).

	Cut periods		
	JUNE	AUGUST	OCTOBER
SC – Leaves	101.7 ± 2.6a	75.8 ± 2.7b	-
Proline – Stems	9.7 ± 1.5b	15.7 ± 1.5a	-
Salinity (mM)		SC – Stems	
0	80.3 ± 3.5a	65.2 ± 3.5b	67.0 ± 3.5bA
25	66.8 ± 3.8a	65.9 ± 3.8a	65.7 ± 3.8aA
50	66.0 ± 3.5a	60.6 ± 3.5a	-
75	71.1 ± 3.5a	54.2 ± 3.8b	-
100	73.2 ± 3.5a	50.0 ± 3.8b	-
Y	71.6 ± 1.7	67.63 – 0.1703x	-
R^2	.	0.92	-
P	ns	0.0097	-

Mean ± standard error, followed by equal letters, in the row (lower case) and in the column (upper case), do not differ by Tukey's test (P>0.05). (-) Insufficient plant material.

K^+ in leaves, only salinity promoted significant effect with negative linear response, which K^+ decreased 26% regarding to plants submitted to 0 and 100 mM NaCl (**Table V**). For K^+ in stems, cut periods and salinity had interaction. The interaction observed indicates linear reduction on K^+ amount in stems, where in this reduction, among cut periods, was featured in the greatest saline levels (75 and 100 mM). The K^+ in stems of plants submitted to 100 mM NaCl when compared to plants without salinity (0 mM) reduced 20 and 42% in June and August, respectively. In October, K^+ in stems had 24% reduction as compared to 0 and 25 mM NaCl.

For K^+/Na^+ ratio, in both fractions had effect of salinity and cut periods, but without significant interaction between these factors (**Figure 2**). This rate reduced as increasing saline levels and second cut.

DISCUSSION

Plant mortality

The *D. pernambucanus* can be considered as tolerant to high saline concentrations (up to 100 mM NaCl) for two periods. However, it is noteworthy that jureminha crop is focused on animal feed, and therefore, even if there is no mortality in the first two cuts, changes in biomass production and chemical composition of plants should be considered.

Reproductive structures

The number of flowers (1.6%of total) was not expressive as compared to floral buds (13.4% of total) and, mainly, pods (85% of total) (**Table I**). This suggests that there is a quick transition from flower to pod, that means a quick fertilization, probably caused by pollen and stigma proximity, as well as receptivity of this viable pollen. The number of pods was quite expressive, which reflected on increasing of total reproductive structures in the second cut (August), we can infer that plants were in grain-filling stage during second cut (August). The number of pods is a parameter directly related to persistence and nutritional value of a species. Generally, when occur a number of pods increasing, it is expected that the number of seeds increase too, which may allow the natural sowing and contribute to forage species persistence in pastures, a factor of fundamental importance in a grass-legume pasture mix, for example. However, as plants reach reproductive stage, forage tends to reduce the nutritive value, once there is usually fiber increase and crude protein reduction (Suksombat & Buakeeree 2006, p. 33). The reduction in number of floral buds and pods as salinity increasing (**Table I**) can be attributed to the fact that adverse conditions may negatively affect the photosynthesis and photoassimilates translocation, which consequently would limit the energy disposable for reproduction.

Biomass production

Regarding to cut periods effect, the reduction of leaves, stems, and total above-ground biomass production (**Table II**) can be explained by the fact that after removing the upper layer performed in each cut (remaining only 20 cm of main stem), plants can have ceased the photoassimilates production, once photosynthetic tissue was completely removed, and became dependent of reserve carbohydrates for regrowth. Therefore, high cut intensity can promote a lack of reserve carbohydrates, reduce the forage plant persistence, delay time for upper layer recovery. Responses observed for biomass production through cut periods differ as related by Calado et al. (2016, p. 748), which evaluated in field different genotypes of Desmanthus spp. under irrigation and had the greatest production in third cut. Thus, the biomass reduction applying 54 days cut frequency and intensity previously mentioned suppose that jureminha need more time for upper layer regrowth through cuts, which is essential to use larger intervals between cuts and/or lower intensity to enable plant recovery.

About salinity effect, the reduction of biomass production in our study (**Table II**), within each period

Table V. Na^+ and K^+ amount (g DM kg^{-1}) in leaves and stems of jureminha plants, as a function of salinity levels and cut periods (Conteúdo de Na^+ e K^+ (g MS kg^{-1}) em folhas e caules de plantas de jureminha em função dos níveis de salinidade e dos ciclos de corte).

Salinity (mM)	Cut periods		
	JUNE	AUGUST	OCTOBER
Na^+ – Leaves			
0	1.0 ± 0.4b	1.4 ± 0.3b	2.1 ± 0.4a
25	1.4 ± 0.4b	1.9 ± 0.3b	7.6 ± 0.4a
50	2.2 ± 0.3c	3.5 ± 0.3b	8.6 ± 0.5a
75	3.0 ± 0.3c	5.3 ± 0.3b	11.1 ± 0.7a
100	2.8 ± 0.4b	5.9 ± 0.4a	-
Y	1.04 + 0.0208x	1.12 + 0.0496x	3.15 + 0.112x
R^2	0.90	0.97	0.91
P	0.0141	0.0027	0.048
Na^+ – Stems			
0	1.0 ± 0.4b	1.5 ± 0.4b	3.2 ± 0.4aB
25	1.7 ± 0.4c	3.4 ± 0.5b	10.5 ± 0.5aA
50	2.2 ± 0.5b	3.8 ± 0.5a	-
75	2.6 ± 0.4b	6.1 ± 0.5a	-
100	3.3 ± 0.5b	7.9 ± 0.4a	-
Y	1.06 + 0.022x	1.44 + 0.062x	-
R^2	0.99	0.97	-
P	0.0004	0.0024	-
K^+ – Stems			
0	13.7 ± 0.9b	13.3 ± 0.9b	16.7 ± 0.9aA
25	12.9 ± 0.9a	14.9 ± 0.9a	12.7 ± 1.0aB
50	12.2 ± 0.9a	10.5 ± 0.9a	-
75	12.0 ± 0.9a	8.3 ± 0.9b	-
100	10.9 ± 0.9a	7.7 ± 0.9b	-
Y	13.64 – 0.026x	14.5 – 0.0712x	-
R^2	0.97	0.81	-
P	0.0026	0.0361	-
K^+ – Leaves			
Y	R^2	*P*	CV (%)
24.77 – 0.0563x	0.83	0.0304	5.23

Mean ± standard error, followed by equal letters, in the row (lower case) and in the column (upper case), do not differ by Tukey's test (P>0.05); (-) Insufficient plant material.

may be a result of NaCl toxic effect, caused specifically by Na^+ and Cl^- ions, which when absorbed in excess can promote ionic toxicity and nutritional imbalance (Mahesh & Sathyanarayana 2015, p. 187). For example, large amount of Na^+ in soil solution could promote Ca^{2+} reduction in plant tissues due to Na^+ displaces Ca^{2+} from root plasma membrane, which promotes integrity loss and ionic imbalance, as well as efflux cellular of organic and inorganic solutes (Zhang et al. 2010, p. 48; Wu & Wang 2012, p. 125). These changes in the root membrane can potentiate plant sensitivity to saline conditions, which affect the reduction of root growth, and consequently, upper layer development. It is also noteworthy about water potential reduction of soil solution promoted by salt osmotic effect, which reduces water and nutrients uptake by plant. This reduction in water uptake also decreases the cells turgor pressure, which directly affects cell division, protein and cell wall synthesis, stomatal closure, and thus, inhibiting photosynthesis (Kaushal & Wani 2016, p. 69). The damage in cell turgidity promotes lower tissue growth, and consequently, lower biomass production.

For pods biomass production, however, the increase observed in August (**Table II**) is strictly related to reproductive phenological stage of plants during this cut period, wherein there was an expressive increase in number of pods (**Table I**).

There was greater reduction in leaf production (44%) than stems production (14%), which decreased the leaf/stem ratio during the first (June) and second cut (August). The reduction leaf/stem ratio (**Table II**) can be due to full reproduction stage (grain-filling) of plants in second cut (August). Generally, shrub plants

have low leaf/stem ratio, however, in case of forage plants, it is important occur a greater leaves amount than stems, which can increase the animal forage intake, in view of their selectivity for plant fractions with greater digestibility and nutritional value such as leaves.

Bromatological composition

The initial increase of minerals in leaves (**Table III**) can be explained by salt accumulation in plant tissue as an osmotic adjustment attempt. From 29 mM level, the water and nutrients uptake by plant can have reduced due to toxicity of Na^+ and Cl^- ions absorbed in excess and osmotic effects promoted by the salt, which possibly decreased the mineral matter amount in leaves. It is noteworthy that leaves are more susceptible to NaCl toxic effects than roots and stems, once leaves store greater amounts of Na^+ and Cl^- ions, transported by xylem through transpiration and stored in leaf tissue as water is transpired by plant (Hasegawa 2013, p. 21). Thus, in leaves may occur sodium increase, but this will result in lower absorption and transport of other minerals by roots to leaves, and lower mineral matter amount. Furthermore, mineral amount changes according to phenological stage of plant. Therefore, it is noteworthy that plants got the reproductive stage in second cut (August), then the reproductive structures demand large amount of mineral nutrients for growth (mainly in grain-filling stage), which may suggest that these nutrients were supplied by leaves. Stems performs mineral carriage from leaves to reproductive structures, which probably resulted in mineral matter increase in this tissue noticed in August.

The reduction in CP for both leaves and stems (**Table III**) is possibly related to full reproductive stage (grain-filling) of plants during the second cut (August). Generally, the protein amount of forage species is greater through vegetative stage than when reaches the reproductive period (Suksombat & Buakeeree 2006, p. 31). Despite the reduction noticed in August, the leaves and stems protein amount was 22% and 7%, respectively, thus supporting the level of 7% recommended by NRC (2001, p. 44). Therefore, the jureminha plants submitted to increasing saline levels had enough protein amount to ensure the minimum required by ruminants, which stimulates forage voluntary intake and supply in N as a necessary substrate for bacteria reproduction responsible for rumen fermentation, allowing a greater utilization and digestibility of forage ingested. It is noteworthy that N from CP will not be completely available for animals, while that element can be linked to plant cell wall as neutral detergent insoluble in nitrogen (NIDN) and acid detergent insoluble in nitrogen (ADIN). Furthermore, great proportion (up 4%) of condensed tannins (CT) can reduce forage intake and acceptability by animals because these compounds have protein-bound promoting antinutritional effect (Cruz et al. 2007, p. 1041). However, Cruz et al. (2007, p. 1040) evaluated bromatological composition of jureminha and CT characterization, reported low NIDA, CT (0.5% and 2.4%, respectively), and astringency (factor that determine the capacity of tannin to bind proteins). This suggest that CT amount in jureminha can avoid timpanism and provide bypass protein (not degraded) to small intestine, which improve their use.

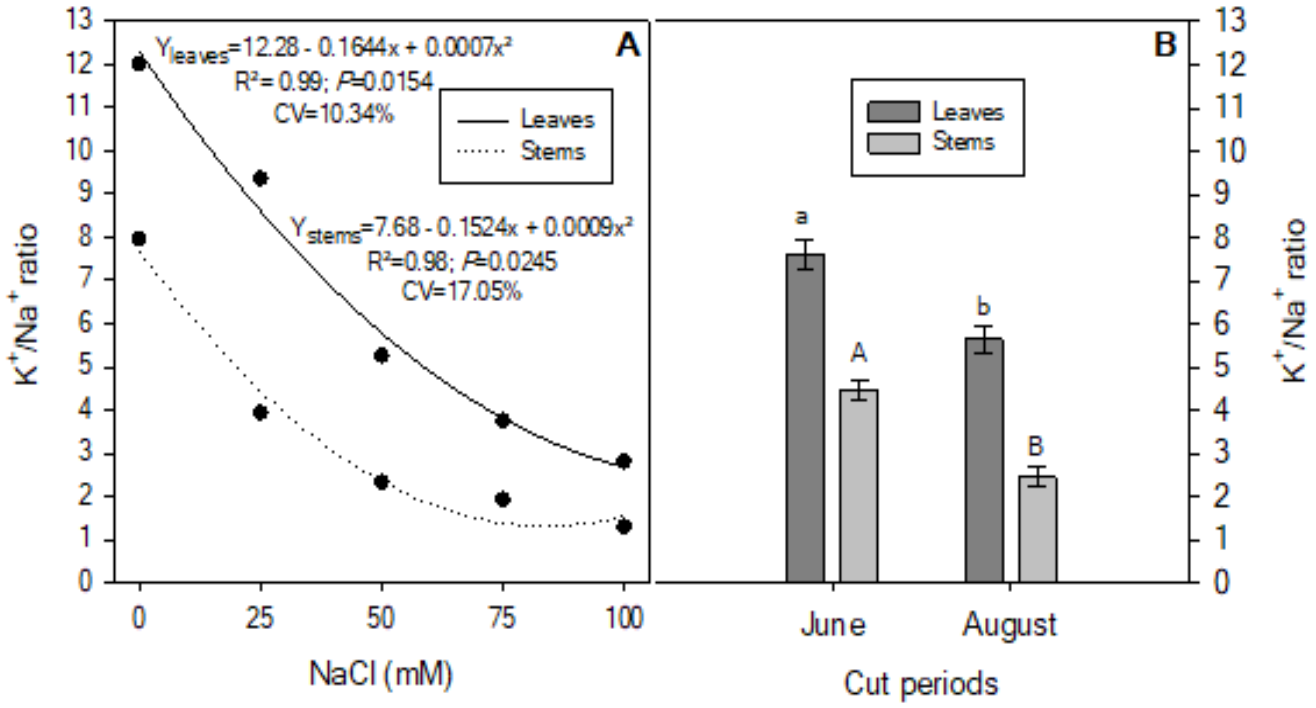

Figure 2. K+/Na+ ratio in leaves (A) and stems (B) of jureminha plants as a function of salinity levels. (– –) Value considered as minimum necessary to maintain nutritional balance of plants (Maathuis & Amtmann 1999, p. 124) (Relação K+/Na+ em folhas (A) e caules (B) de plantas de jureminha em função dos níveis de salinidade. (– –) Valor considerado como mínimo necessário para manutenção do balanço nutricional das plantas (Maathuis & Amtmann 1999, p. 124)).

Fiber (NDF, ADF and hemicellulose) in leaves reduced in June as increased the salinity (Table III), probably occurred due to the osmotic effects promoted by salinity, which decrease water and nutrients uptake by plants, and consequently affect several physiological and biochemical processes as photosynthesis essential for biomass production. Thus, the photosynthesis may be affected by changes in opening and closing dynamics of stomata, thereby reducing CO_2 fixation and consequently the carbon skeletons synthesis required for structural compounds production. In August, despite the osmotic effects possibly caused by salinity, which decreased the water and nutrients uptake, plants were in full reproductive stage (grain-filling phase), and thereby, demanded a large amount of photoassimilates. Thus, these compounds could have been partially carried to grain-filling through leaves (translocation), inferring that occurred a greater foliar tissues development destined to such transport (conduction vessels), which could have promoted fibers accumulation as saline increase.

The increment on NDF and ADF in stems (**Table III**) can be attributed to the greater conduction vessels tissues development of this plant part, which transport water and nutrients uptaken by roots, as well as photoassimilates translocated from storage structures (stem and root) in order to promote growth and development of reproductive tissues.

Soluble carbohydrates

During the second cut (August), there was a reduction in leaf/stem ratio (Table II), which may have resulted in a lower photosynthetic rate when compared to first cut (June), and consequently lower photoassimilates as soluble carbohydrates. It is noteworthy that the complete reproductive stage (grain-filling) in August, because at this stage the soluble carbohydrates produced in leaves are preferentially placed in plants reproductive structures. The reduction pattern response of soluble carbohydrate in stems, observed in each new cut period (Table IV), is probably related to phenological phase, although plants got grain-filling phase in August. In June and October, the soluble carbohydrates of stems were not influenced by saline levels applied (Table IV). However, as salinity increment in August, that organic compound had decreasing linear response. Thus, in August, the effects of full reproductive stage (grains-filling) on soluble carbohydrate (cited above), are potentiated by osmotic and toxic effects of salinity, which negatively affect the photoassimilates synthesis, which compromises the synthesis and storage of soluble carbohydrates necessary to maintain plant growth (Silva et al. 2011, p. 63). The reduction observed in that solute in plants submitted to salinity was not expected, since several authors (Silveira et al. 2009, p. 1) affirm that soluble carbohydrates accumulation is a common response to the osmotic adjustment in plants submitted to saline stress, which can assume that this organic solute did not perform the adjustment function in jureminha, or that the cut management applied (frequency and intensity) did not allow this compound to perform such function. This fact can be indicated by observing the soluble carbohydrate in stems of plants that had no NaCl (0 mM), wherein this compound decreased in August and was not restored in October.

Proline

These results diverged from results reported by Mahesh & Sathyanarayana (2015, p. 185). The proline did not perform functions of osmotic adjustment in the tissues sampled as to salt stress. Thus, this compound could not be considered as an effective biochemical and physiological indicator of salinity effects jureminha access 235C. The greatest proline amount in stems during second cut period (**Table IV**) suggest that the full reproductive phenological stage (grain-filling) of plants promoted that increment. That is attributed to the fact that proline can act as a carbon and nitrogen source (Silveira et al. 2009, p. 7), therefore, as the nutritional requirements of plants in full reproductive stage (grain-filling) are greater, proline could have been carried through stems from roots and leaves to reproductive structures.

Na^+ and K^+ amount

The level of 0 mM NaCl also had Na^+ increase (Table V), which can be associated to constant application of nutritive solution with sodium molybdate in this case. The Na^+ increment in both tissue analyzed is due to the saline levels increase added to nutritive solution and probably low selectivity in nutrient uptake by jureminha roots. This promote great salt concentration in soil solution (particularly NaCl in this case), which can reduce the water potential, as well as water and nutrients availability to plants, which absorb ions as Na^+ and Cl^- in order to reduce their internal water potential and less ions in soil, and to allow the water influx via osmotic gradient (Kosová et al. 2013, p. 6764). After uptake, plants can store salt ions in vacuole, but if these were taken in large amounts can exceed their store capacity and promoting efflux from vacuole to cytoplasm (Hasegawa 2013, p. 22). Large Na^+ amount in cytoplasm are toxic, which can bind in K^+ site (due to chemical similarity) and stop K^+ functions promoting changes in enzymatic activities and cell metabolism (Kabała & Janicka-Russak 2012, p. 377).

The complete nutritive solution applied provided an adequate K^+ amount to substrate, however, according to Gupta & Huang (2014, p. 1) great Na^+ amount can reduce K^+ absorption rate. The reduction of K^+ absorption by root occurs because although K^+ transporters are largely selective in the root zone, they can absorb Na^+ (element with physicochemical, hydrated ionic radius and electric charge similar to K^+) when its concentrations are greater than K^+ (Apse & Blumwald 2007, p. 2247). In addition, the Na^+ excess can cause Ca^{2+} removal from root cell membrane, which promotes the integrity loss and ionic absorption imbalance, as well as reduces its selectivity of K^+ ions by roots and activate channels related to K^+ efflux of cell (Shabala 2003, p. 632). Therefore, the K^+ reduction is a common response in plants submitted to salinity.

The K^+ increment in stems observed in 0 mM treatment (Table V) can be attributed to constant application of nutritive solution (every four days), providing that

nutrient in adequate levels and the absent of NaCl application.

The K^+ reduction observed in our work (Table V) can lead to changes in osmotic potential; cellular turgidity regulation; ion balance; stomata opening and closing, consequently, decreases CO_2 input, and negatively influences photosynthesis (Parida & Das 2005, p. 324). Despite K^+ does not make part of any plant organic compound, it acts on several enzymes activation, which promotes Rubisco synthesis (that generally represents more than 50% of leaves protein in C3 physiology plants), which make part of CO_2 fixation mechanism. Besides, plants poor in K^+ may have lower accumulation of amino acids, starch, nitrate, and protein synthesis. At the same time, these plants may have a reduction in growth and biomass production caused by the negative influence of K^+ amount reduction in photosynthesis.

The reduction of K^+/Na^+ ratio found in our study (**Figure 1**) show greater Na^+ uptake and translocation than K^+ as increasing saline levels, which suggest the jureminha sensibility to saline levels applied. Such rate, as reach values lower than 1.0 indicates nutritional imbalance (Maathuis & Amtmann 1999, p. 124), fact that did not occur until 108 days after applying treatments. It suggests that, in spite of jureminha had saline sensibility, even plants submitted to 100 mM NaCl, kept during two cut periods (108 days) a K^+/Na^+ ratio close to values considered necessary to preserve the metabolic functions fulfilled by K^+ and resistance to greater Na^+ levels.

CONCLUSIONS

Our findings showed that salinity up 75 mM associated to cut management promote the jureminha mortality. The salinity increase associated to cut management promote reduction in forage production, negatively affecting the chemical and bromatological composition of jureminha, increasing the fiber content, reduce the content of mineral matter, crude protein and leaf/stem ratio, which consequently decreases the nutritive value of the forage of Jureminha. Besides that, those effects are potentialized by grain-filling phenological stage. Thus, in this study, we show that 235C jureminha access tolerates salinities close to 50 mM NaCl.

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