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Mombaça Grass Responds to Partial Replacement of K^+ by Na^+ with Supplemental Ca^{2+} Addition in Low Fertility Soil

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Abstract

Partial replacement of potassium by sodium may be an alternative to reduce the cost of pasture fertilization and reduce the dependence on imported potassium sources. The objective of this study was to evaluate different sources and doses of calcium as enhancers of sodium effect on the partial replacement of potassium by sodium. Here, Mombaça grass (*Megathyrsus maximus*) was grown on low fertility soil. The experiment was conducted in a factorial (3 × 5) based on a completely randomized design with 4 replications as follow: three sources of Ca^{2+} (dolomitic limestone, agricultural gypsum and calcium chloride), five doses of Ca^{2+} (0, 10, 20, 30 and 40 mg dm^{-3}) and two additional treatments (fertilization with 100% of K^+ without application of Ca^{2+} and one control without any fertilization). Potassium was partially replaced (25%) by Na^+ prior to Ca^{2+} additions. Plant height, growth rate, dry weight, Na^+, K^+, K^+/Na^+ and shoot proline contents were evaluated as well as Na^+ levels and the electrical conductivity of the soil. The results show that the addition of Ca^{2+} provided better plant development when K^+ was partially replaced by Na^+ and that the supply of Ca^{2+} reduced the absorption of sodium by plants. The partial replacement of K^+ by Na^+ did not increase soil salinity or caused stress to the plants.

Keywords: *Megathyrsus maximus*, pastures, fertilization, proline

1. Introduction

Brazil is among the largest fertilizer consumers in the world. In 2016, the country consumed nearly 34,084 thousand tons with a total of 72% of imported products. Potassium (K^+), which is the main inorganic component of living cells (White, 2013; Pi et al., 2016; Liu et al., 2017), accounted for approximately 44.7% of these imports (CONAB, 2017). The high dependence on importation along with the deficiency in Brazilian soils and the demand for products containing K^+, show the importance of developing research focusing on feasible alternatives to replace potassium that has been obtained from imported sources (Andrade et al., 2014).

Although Na^+ is responsible for nutritional imbalances in plants, studies show that Na^+ and K^+ share physiological functions and physicochemical similarities that are beneficial to plants. Sodium can also eliminate the symptoms of deficiency in a limited condition of K^+ (Subbarao et al., 2003; Wakeel et al., 2011; Benito et al., 2014; Pi et al., 2016; Liu et al., 2017).

The presence of Na^+ in the environment and its uptake by plants can reduce the amount of K^+ required to meet basic metabolic requirements (Benito et al., 2014), since Na^+ can partially replace K^+ in some non-specific functions (Wakeel et al., 2010, 2011, 2013; Silva et al., 2014; Krishnasamy et al., 2014) such as: enzymatic activation of ATPase, osmoregulation, macronutrient uptake, cell permeability, carbohydrate synthesis, conversion of fructose to glucose, stomatal opening and closing, plant vigor and carbon dioxide transport (CO_2) for C4 plant cells (Inocencio et al., 2014; Krishnasamy et al., 2014), especially when plants have the ability to absorb, translocate and compartmentalize Na^+ in their vacuoles (Krishnasamy et al., 2014).

Partial replacement of K^+ by Na^+ in addition to reducing problems related to import dependence and high cost in fertilization, would also reduce the problems related to low concentrations of Na^+ in pastures, which can cause hypomagnesemia in animals due to the low availability of magnesium caused by the low Na^+/K^+ in animal saliva (Wakeel et al., 2011). In a study carried out with the partial replacement of K^+ by Na^+ in *Megathyrsus maximus* cv. Mombaça, Andrade et al. (2014) verified that there was no significant reduction in forage yield when the addition of up to 25% Na^+ was used instead of K^+. Furthermore, Krishnasamy et al. (2014) found that the addition of 25 to 50 mg Na^+ kg^{-1} reduced the symptoms of K^+ deficiencies in wheat plants, but did not significantly affect the dry mass production of the plant area. Based on these results, we believe that the use of minimizers of the negative effect of Na^+ on soil and plant may allow even greater substitution of K^+ for Na^+ in grasses fertilization.

An alternative to increase plant tolerance to substitution of Na^+ by K^+ is the increase in Ca^{2+} supply to soil, which act as a Na^+ stress minimizer. According to Benito et al. (2014) and Pi et al. (2016) under a limited K^+ condition, Na^+ (together with Mg^{2+} and Ca^{2+}) can replace K^+ in the vacuole acting as an alternative ion in osmosis processes, which alleviates K^+ deficiency. Therefore, it is believed that exogenous applications of Ca^{2+} to the root environment may reduce the effects of salinity on plant growth and development as indicated by Lahaye and Epstein (1971) and Guimarães et al. (2011), since supplemental calcium in saline soils reduces Na^+ absorption and maintains K^+ levels and other metabolites in the root tissue (Silva et al., 2003). According to Melloni et al. (2000), the externally applied calcium decreases saline stress by means of an unknown function that preserves the K^+/Na^+ selectivity and inhibits K^+ absorption sites, which can reduce Na^+ influx mediated by low K^+ affinity.

Considering that K^+ fertilization is a common practice for the cultivation of several crops in the Brazilian Cerrado biome, and that Na^+ response to K^+ has been observed in *Megathyrsus maximus* cv. Mombaça (Andrade et al., 2014), the possibility of using Ca^{2+} applied as an alternative to minimize the effect of Na^+ on plants is important. In this scenario, the aim of this study was to evaluate the application of different calcium sources and doses as enhancers of the effect of sodium on partial replacement of potassium in the fertilization of *Megathyrsus maximus* cv. Mombaça.

2. Material and Methods

2.1 Experimental Conditions

This work was conducted in the experimental site of the Federal University of Tocantins (UFT), Campus Gurupi. According to the Köppen (1948) climate classification system the area where soil was collected is B1wA'a'. The research was carried out in a greenhouse (4 m width × 20 m length) covered with transparent plastic of 150 microns and with the presence of a darker shade on the sides, with retention capacity of 50% of incident solar radiation. The experimental units consisted of plastic pots with a capacity of 5.0 dm^3, using 4.0 dm^3 of sandy-loamy dystrophic soils (Santos et al., 2014) (Table 1).

Table 1. Characterization of the dystrophic Red-Yellow Latosol of clay-sandy texture

Organic matter (dag kg^{-1})	0.11	Exchangeable aluminum (Al^{3+}) ($cmol_c\ dm^{-3}$)	0.00
pH ($CaCl_2$)	5.30	Potential acidity (H+Al) ($cmol_c\ dm^{-3}$)	1.80
Calcium (Ca^{2+}) ($cmol_c\ dm^{-3}$)	0.40	Sum of bases (SB) ($cmol_c\ dm^{-3}$)	0.68
Magnesium (Mg^{2+}) ($cmol_c\ dm^{-3}$)	0.20	Cation exchange capacity (CEC) ($cmol_c\ dm^{-3}$)	2.48
Potassium (K^+) (mg dm^{-3})	23.0	Sand (g kg^{-1})	465
Phosphorus (P) (mg dm^{-3})	0.50	Silt (g kg^{-1})	63.0
Sodium (Na^+) ($cmol_c\ dm^{-3}$)	0.02	Clay (g kg^{-1})	270

2.2 Experimental Design and Data Collection

The experimental design was completely randomized with four replicates. The treatments were obtained in a 3 × 5 + 2 factorial scheme. The first factor consisted of three sources of Ca^{2+} ($CaMg(CO_3)_2$ – 30% CaO and 18% MgO (100% PRNT); $CaSO_4 \cdot 2H_2O$ – 20% Ca, 15% S and $CaCl_2$); and the second factor by five doses of Ca^{2+} (0, 10, 20, 30 and 40 mg dm^{-3}). These doses were applied to soil along with the partial replacement of K^+ (25%) by Na^+, resulting in the application of 45 kg ha^{-1} of K_2O and 15 kg ha^{-1} Na^+. The two additional treatments were standard fertilization (Sf.), in which the application of 100% of the recommended potassium (60 kg ha^{-1} K_2O) was applied without the application of Ca^{2+} and a control (Ct.) with no fertilization.

The recommendation of the establishment fertilization was performed according to Ribeiro et al. (1999) for the medium level of technology. Fertilization was carried out using urea as a source of nitrogen (50 kg of N ha^{-1}), simple superphosphate as a source of phosphorus (120 kg of P_2O_5 ha^{-1}), potassium chloride as the source of K_2O (60 kg of K_2O ha^{-1}) and sodium chloride PA (15 kg Na^+ ha^{-1}) as the sodium source.

The forage used was *Megathyrsus maximus* cv. Mombaça, which is one of the most important cultivars of *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs (syn. *Panicum maximum* Jacq.) due to its rapid growth in the region in recent years. After 10 and 20 days after emergence of plants, a total of seven and five plants well distributed were left in each pot, respectively. After the emergence of plants (48 days) a uniformity cut was performed at 20 cm height from the soil surface. In addition to the standardization cut, three more cuts were performed in every 21 days for evaluation purposes at a height of 20 cm from the soil.

For the evaluation of the treatments, the following characteristics of the plants were measured in the forage cuts: plant height, growth rate and dry weight. Contents of Na^+ and K^+ were determined as well as the Na^+/ K^+ ratio of the shoot of the plants according to Malavolta et al. (1997). The determination of proline levels accumulated in shoot was carried out according to Bates et al. (1973). The available Na^+ and the electrical conductivity (EC) of the soil at the end of the experiment were performed according to Embrapa's (1997) methodology.

In order to determine the dry weight and Na^+, K^+ and proline contents plants were dried at 55 ºC with forced air circulation in the greenhouse. For Na^+ and K^+ nutrient levels, a nitric-perchloric digestion was carried out followed by atomic absorption spectrophotometry. The determination of the electrical conductivity (EC) was performed using a digital conductivity meter and the soil Na^+ contents were extracted in Mehlich-1 solution and determined by atomic absorption spectrophotometry. Proline levels were determined using a standard curve after reading in a spectrophotometer at 520 nm.

2.3 Statistical Analysis

The results were submitted to analysis of variance and regression. The regression models were chosen based on the significance of the coefficients of the regression equation (β), adopting 5% of probability.

3. Results and Discussions

3.1 Development and Production

Table 2 shows the values of F and significance levels for the variables plant height (PH), growth rate (GR) and shoot dry weight (SDW). The partial replacement of K^+ by Na^+ did not reduce the development and production of *Megathyrsus maximus*.

Table 2. Values of F, level of significance and results of plant height (PH), growth rate (GR) and shoot dry mass (SDW) as a function of sources and doses of Ca^{2+} in the partial replacement of K^+ by Na^+ in the fertilization of *Megathyrsus maximus* cv. Mombaça

FV		PH	GR	SDW	Doses	TC -- cm day^{-1} --	MSPA ------------ g pot^{-1} ------------	
						$CaSO_4{\cdot}2H_2O$	$CaMg(CO_3)_2$	$CaSO_4{\cdot}2H_2O$
Sources (S)		1.17^{ns}	3.65^{*}	6.33^{**}	0	4.83±0.25	12.15±0.91	12.15±0.91
Doses (D)		6.92^{a**}	3.62^{**}	2.42^{ns}	10	4.98±0.21	12.42±0.77	12.59±0.86
Int.[1] S × D		2.19^{b*}	1.22^{ns}	2.31^{*}	20	5.08±0.25	12.66±0.78	12.97±0.72
Fac.[1] × Sf. + Ct.		403.19^{**}	346.72^{**}	357.41^{**}	30	5.08±0.27	12.96±0.80	12.89±0.66
Sf.[1] × Ct.[1]		270.00^{**}	199.45^{**}	619.75^{**}	40	4.97±0.30	13.05±0.70	12.57±0.78
C.V. (%)[1]		4.75	5.41	7.08	Mean	4.99±0,11 a	12.65±0.37 a	12.64±0.33 a
	F1[e]	L^{c**}	L^{**}	ns	Sf.	4.57±0.25 a	14.25±0.72 a	14.25±0.72 a
Regression model	F2	L^{**}	ns	ns	Ct.	2.23±0.29 b	0.61±0.34 b	0.61±0.34 b
	F3	Q^{d**}	Q^{**}	L^{**}				

Note. [a]** significant to F test and regression analysis at 1% level ($p < 0.01$), [b]* at 5% level ($p < 0.05$), and [ns]: not significant; [c]L: linear; [d]Q: quadratic; [e]F1: $CaMg(CO_3)_2$; F2: $CaSO_4{\cdot}2H_2O$; F3: $CaCl_2$ P.A. [1]Sf.: Standard fertilization; Ct.: Control; C.V.: coefficient of variation; Int.: interaction; Fac.: Factorial. Means followed by the same lowercase letter in the column did not differ from each other by the Tukey test ($p < 0.05$).

Plant height (PH) and growth rate (GR) of *Megathyrsus maximus* cv. Mombaça in response to the addition of increasing doses of Ca^{2+} via different sources presented adjustments to the linear and quadratic regression models (Figures 1A and 1B). Plant height and growth rate presented linear increases as a function of Ca^{2+} doses when dolomitic limestone ($CaMg(CO_3)_2$) was used. For the addition of Ca^{2+} with the use of agricultural gypsum ($CaSO_4{\cdot}2H_2O$) plant height presented a linear increase, whereas growth rate was not significant (Table 2). With the use of calcium chloride as a source ($CaCl_2$), the variables PH and GR presented an adjustment to the quadratic model.

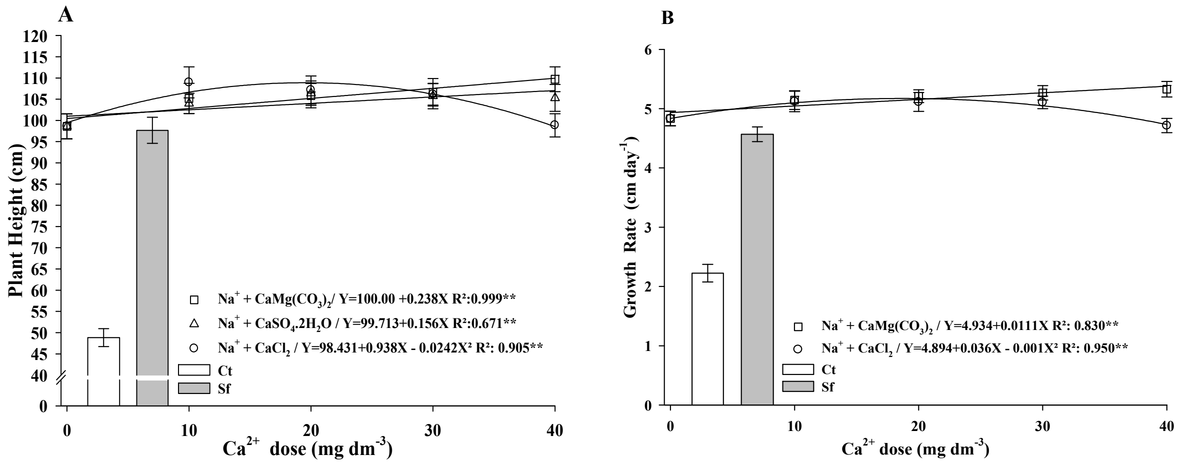

Figure 1. (A) Plant Height (PH) and (B) Growth Rate (GR) of *Megathyrsus maximus* cv. Mombaça as a function of doses and sources of Ca^{2+} in the Na^+ - K^+ partial replacement

The use of $CaMg(CO_3)_2$ as a source of Ca^{2+} in the highest dose promoted an increase in PH of 9.48 and 14.13% in relation to dose 0 of Ca^{2+} and to standard fertilization (100% of recommended K^+), respectively. For this source, plants reached a maximum height of 109.48 cm, which represents an increase of 0.24 cm^{-1} mg^{-1} of Ca^{2+} added. The source $CaSO_4{\cdot}2H_2O$ promoted a 0.15 cm increase in supplementary Ca^{2+}. On the other hand, the source $CaCl_2$ presented the highest response in the dose of 19.38 mg dm^{-3} Ca^{2+}, reaching a height of 107.52 cm.

Similar to PH, growth rate (GR) presented a linearly increasing response when $CaMg(CO_3)_2$ was used as the Ca^{2+} source. Plants had a maximum GR of 5.38 cm day^{-1} and there was an increase of 0.01 cm day^{-1} mg^{-1} of Ca^{2+}. With the addition of 40 mg dm^{-3} of Ca^{2+} as $CaMg(CO_3)_2$, there was an increase of 11.17 and 17.59 % in relation to the dose 0 mg dm^{-3} of Ca^{2+} and to standard fertilization, respectively.

The use of increasing doses of Ca^{2+} as $CaSO_4{\cdot}2H_2O$ did not present significance to the F test. For this source, the rate growth (RG) presented a mean of 4.99 cm day^{-1}. With the use of $CaCl_2$ the RG showed a quadratic behavior with maximum RG at dose 18.00 mg dm^{-3} Ca^{2+} with approximately 5.22 cm day^{-1}.

Plant height and growth rate did not differ from the standard fertilization when 25% K^+ was replaced by Na^+, regardless of the used Ca^{2+} source. The control plants (cultivated in the soil without fertilization) were inferior to all other plants. When Ca^{2+} was added the forage development increased significantly when compared to no Ca^{2+} application.

Shoot dry weight (SDW) did not show significant differences with increasing Ca^{2+} doses for $CaMg(CO_3)_2$ and $CaSO_4{\cdot}2H_2O$ sources (Table 2). Using $CaCl_2$ as a source of Ca^{2+} promoted a linear increase in SDW, reaching 14.71 g pot^{-1} (Figure 2).

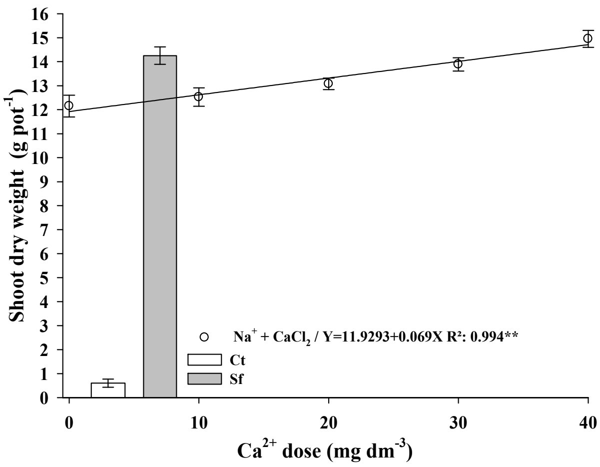

Figure 2. Shoot dry weight (SDW) of *Megathyrsus maximus* cv. Mombaça as a function of doses and sources of Ca^{2+} in the Na^+ - K^+ partial replacement

When $CaCl_2$ was used as the source of Ca^{2+} there was an increase of 0.07 g of SDW for each milligram of Ca^{2+} applied. This increase corresponded to an increase of 23.30% in SDW in relation to dose 0 mg dm^{-3} of Ca^{2+} with 25% of K^+ replaced by Na^+. For $CaMg(CO_3)_2$ and $CaSO_4.2H_2O$ SDW did not differ between doses, presenting a mean of 12.65 and 12.64 g pot^{-1}, respectively. Shoot dry weight did not differ from the standard fertilizer plants when 25% of K^+ was replaced by Na^+ regardless of the used Ca^{2+} source used. The control plants (cultivated in the soil without fertilization) were inferior to all other plants.

The responses of the plants exposed to Na^+ by the application of external Ca^{2+} results in an increase in the degree of resistance due to the excess of cations (Alves et al., 2011). Lacerda et al. (2004) evaluated the influence of Ca^{2+} on the growth of sorghum seedlings under a saline environment and verified that the increase of Ca^{2+} in the solution favored the development of sorghum seedlings. These authors explained that such effect occurs as a function of the reduction of Na^+ concentration in the leaves of plants, which corroborates with the results of the present work, in which the use of $CaMg(CO_3)_2$ and $CaCl_2$ reduced the Na^+ concentration in the leaves (Table 3 and Figure 3).

Krishnasamy et al. (2014) found that when K^+ supply is low in the soil, the addition of low doses of Na^+ (25 and 50 mg kg^{-1}) eliminates the symptoms of K^+ deficiency in old leaves, but has no significant negative effects on shoot dry weight as well as plant (wheat) development. Therefore, with adequate K^+ supply, the addition of 25-50 mg Na^+ kg^{-1} had no effect on shoot dry weight. Krishnasamy et al. (2014) verified that the Na^+ effect varied with K^+ efficiency of wheat cultivars, being more responsive to low to moderate levels of Na^+ in the soil and low K^+. According to Krishnasamy et al. (2014) a possible explanation for Na^+ stimulation in wheat growth is that Na^+ increases the supply of K^+ to the shoot, which in turn stimulates photosynthesis and therefore, the greater supply of photoassimilates allows greater root growth.

3.2 Levels of Na^+ and K^+ and K^+/Na^+ Ratio in Shoots

Table 3 shows the values of F and levels of significance for Na^+ and K^+ contents in plants (*Megathyrsus maximus* cv. Mombaça). The partial replacement of K^+ by Na^+ did not alter the Na^+ and K^+ uptake by plants regardless of the Ca^{2+} source used and when compared to standard fertilization. Sodium content presented significance to the F test and regression analysis as a function of increasing doses of Ca^{2+} for the sources $CaMg(CO_3)_2$ and $CaCl_2$ (Figure 3). However, K^+ content did not differ as a function of the doses and sources of Ca^{2+} used (Table 3).

Contents of Na^+ in leaves decreased linearly as a function of increasing Ca^{2+} doses when both $CaMg(CO_3)_2$ and $CaCl_2$ were used (Figure 3). However, for K^+ contents, there was no difference when the sources were used for the addition of increasing doses of Ca^{2+}.

Table 3. Values of F and level of significance of Na^+ and K^+ foliar content (*Megathyrsus maximus* cv. Mombaça) as a function of sources and doses of Ca^{2+} in the Na^+ - K^+ partial replacement

FV		Na^+	K^+	Doses	Na^+ --- g kg^{-1} ---	K^+ --------------------- dag kg^{-1} --------------------		
					$CaSO_4{\cdot}2H_2O$	$CaMg(CO_3)_2$	$CaSO_4{\cdot}2H_2O$	$CaCl_2$
Sources (S)		0.89^{ns}	0.54^{ns}	0	3.18±0.11	6.02±0.32	6.02±0.32	6.02±0.32
Doses (D)		5.63^{a**}	0.91^{ns}	10	3.12±0.08	6.25±0.60	6.19±0.47	6.60±0.25
Int.[1] S × D		1.02^{ns}	0.37^{ns}	20	3.05±0.21	6.54±0.16	6.30±0.91	6.66±0.53
Fac.[1] × Sf. + Ct.		446.94^{**}	127.23^{**}	30	3.00±0.12	6.64±0.45	6.40±0.65	6.48±0.28
Sf.[1] × Ct.[1]		476.26^{**}	181.12^{**}	40	2.94±0.16	6.82±0.33	6.58±0.40	6.11±0.21
C.V. (%)[1]		6.75	13.76	Mean	3.05±0.10 a	6.45±0.32 b	6.30±0.21 b	6.37±0.29 b
	F1[c]	L^{b**}	ns	Sf.[1]	2.95±0.26 a	5.81±1.18 b	5.81±1.18 b	5.81±1.18 b
Regression model	F2	ns	ns	Ct.[1]	0.31±0.02 b	13.91±2.06 a	13.91±2.06 a	13.91±2.06 a
	F3	L^{**}	ns					

Note. [a]** significant to F test and regression analysis at 1% level ($p < 0.01$), [b]* 5% level ($p < 0.05$), [ns]: not significant, and L: Linear; [c]F1: $CaMg(CO_3)_2$; F2: $CaSO_4{\cdot}2H_2O$; F3: $CaCl_2$ P.A. [1]Sf.: Standard fertilization; Ct.: Control; C.V.: coefficient of variation; Int.: interaction; Fac.: Factorial. Means followed by the same lowercase letter in the column did not differ from each other by the Tukey test ($p < 0.05$).

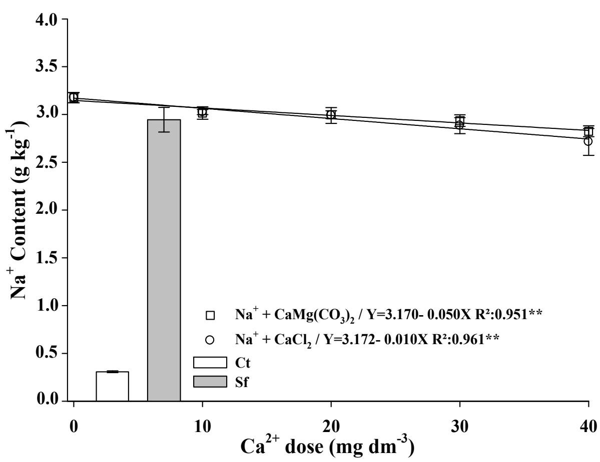

Figure 3. Sodium content (Na^+) in leaves of *Megathyrsus maximus* cv. Mombaça as a function of doses and sources of Ca^{2+} in the Na^+ - K^+ partial replacement

Leaf Na^+ contents reduced about 0.05 and 0.01 g kg^{-1} mg^{-1} Ca^{2+} for $CaMg(CO_3)_2$ and $CaCl_2$, respectively. When using $CaSO_4{\cdot}2H_2O$ as a source, the mean Na^+ leaf content was 3.05 g kg^{-1}. For K^+, the mean was 6.45, 6.30 and 6.37 dag kg^{-1} for $CaMg(CO_3)_2$, $CaSO_4{\cdot}2H_2O$ and $CaCl_2$ sources, respectively.

The addition of supplemental Ca^{2+} reduced the uptake of Na^+ by plants. The control plants showed the highest levels of K^+ in leaves due to their lower development and biomass production. Leaf contents of Na^+ and K^+ were above the recommended critical levels, 0.326 g kg^{-1} and 2.1 dag kg^{-1}, respectively (Malavolta, 2006). Although Na^+ extraction occurred much higher, up to ten times higher than the critical level, there was no significant reduction in forage yield when compared to standard fertilization.

According to Alves et al. (2011) one of the mechanisms of resistance to excess of Na^+ in plants is to maintain adequate potassium nutrition in plant tissues, which occurred in the present work, where K^+ contents were above the critical level. The selectivity of the root system for K^+ over Na^+ should be sufficient to satisfy K^+ contents required for the metabolic processes, ion transport regulation and osmotic adjustment (Munns & Tester, 2008). One of the beneficial effects of the addition of calcium in the root environment of plants exposed to Na^+ excess is associated with the maintenance of the integrity of the membranes of the cells that favors the best control in the absorption and maintenance of K^+ (Lacerda et al., 2004; Alves et al., 2011).

The K^+/Na^+ ratio was not altered as a function of the doses and sources of Ca^{2+} applied (Table 4).

Table 4. Values of F and level of significance of Na^+/K^+ foliar content (*Megathyrsus maximus* cv. Mombaça) as a function of sources and doses of Ca^{2+} in the Na^+ - K^+ partial replacement

FV		K^+/Na^+	Doses	K^+/Na^+		
				$CaMg(CO_3)_2$	$CaSO_4{\cdot}2H_2O$	$CaCl_2$
Sources (S)		0.03^{ns}	0	18.97±1.20	18.97±1.20	18.97±1.20
Doses (D)		0.22^{ns}	10	20.67±2.90	20.42±1.64	22.07±1.04
Int.[1] S × D		0.01^{ns}	20	22.12±1.05	21.37±4.00	22.38±2.74
Fac.[1] × Sf. + Ct.		3164.75^{a**}	30	21.56±2.02	21.40±2.60	21.86±2.56
Sf.[1] × Ct.[1]		3635.71^{**}	40	22.62±2.57	21.99±1.92	22.36±2.84
C.V. (%)[1]		23.99	Mean	21.18±1.43 b	20.83±1.18 b	21.53±1.45 b
Regression model	F1[b]	ns	Sf.[1]	19.77±4.64 b	19.77±4.64 b	19.77±4.64 b
	F2	ns	Ct.[1]	435.92±43.88 a	435.92±43.88 a	435.92±43.88 a
	F3	ns				

Note. [a]** significant to F test and regression analysis at 1% level ($p < 0.01$), and [ns] not significant to F; [b]F1: $CaMg(CO_3)_2$; F2: $CaSO_4{\cdot}2H_2O$; F3: $CaCl_2$ P.A. [1]Sf.: Standard fertilization; Ct.: Control; C.V.: coefficient of variation; Int.: interaction; Fac.: Factorial. Means followed by the same lowercase letter in the column did not differ from each other by the Tukey test ($p < 0.05$).

The K^+/Na^+ ratio of the plants under the influence of the partial replacement of K^+ by Na^+ did not differ from the standard fertilization. The control plants presented the highest K^+/Na^+ ratio due to the high K^+ content in their tissue by the concentration effect.

According to Benito et al. (2014) in order to maintain K^+/Na^+ ratio appropriately high in the leaves, excess of Na^+ needs to be excluded from photosynthetically active tissues and transported through the phloem to roots. This fact explains the Na^+ accumulation in roots as it can be observed in several studies (Lacerda et al., 2004, Alves et al., 2011; Krishnasamy et al., 2014). Krishnasamy et al. (2014) found a reduction in the K^+/Na^+ ratio in wheat plants of different cultivars when Na^+ was added in the soil. These authors also verified that in the presence of insufficient K^+, Na^+ stimulates a better development of plants, eliminating the symptoms of K^+ deficiency. Alves et al. (2011) also observed a reduction in the K^+/Na^+ ratio in cashew plants in the presence of Na^+, however when external Ca^{2+} was applied this relation did not decrease significantly when compared to control plants.

According to Alves et al. (2011) the beneficial effect of Ca^{2+} supplementation on the culture medium is related to the increase of K^+/Na^+ ratio, which is also confirmed by Lacerda et al. (2004). These authors further mention that calcium supplementation in the root environment increases the absorption and transport of K^+, mainly to the photosynthetic organisms, in addition to reducing Na^+ uptake and transport. This corroborates with Melloni et al. (2000) who verified an increase in Na^+ contents in leaves and stems when they were submitted to doses of NaCl in the presence of Ca^{2+} in the culture medium when using external Ca^{2+} application to alleviate the effects of Na^+ on mineral nutrition.

Ebert et al. (2002) studied the effects of external Ca^{2+} concentrations on nutrient uptake in guava seedlings submitted to NaCl and verified an increase in K^+ concentration in the aerial part with the increase of Ca^{2+} levels in the culture medium. Lacerda et al. (2004) verified a reduction of Na^+ in leaves of sorghum plants due to the addition of Ca^{2+}, which corroborated with the results obtained in the present study. For K^+, the results of this work contradict those of Lacerda et al. (2004) and those of Ebert et al. (2002) who verified that the addition of Ca^{2+} increases K uptake in sorghum and guava plants respectively, since K^+ in *Megathyrsus maximus* cv. Mombaça was not altered as a function of Ca^{2+} doses.

3.3 Proline Production

Proline production by *Megathyrsus maximus* cv. Mombaça plants did not differ as a function of the Ca^{2+} doses. There was a difference only in function of the sources, in which $CaCl_2$ presented the lowest proline production (Table 5).

Table 5. Values of F and level of significance of foliar proline content (*Megathyrsus maximus* cv. Mombaça) as a function of sources and doses of Ca^{2+} in the Na^+ - K^+ partial replacement

FV		Proline	Doses	Proline µmol g^{-1}		
				$CaMg(CO_3)_2$	$CaSO_4{\cdot}2H_2O$	$CaCl_2$
Sources (S)		8.89^{**a}	0	2.41±0.60	2.41±0.60	2.41±0.60
Doses (D)		1.23^{ns}	10	2.58±0.57	2.25±0.17	1.76±0.91
Int.[1] S × D		0.58^{ns}	20	2.59±1.08	2.30±0.22	1.64±0.18
Fac.[1] × Sf. + Ct.		8.34**	30	2.45±0.10	2.45±0.67	1.63±0.23
Sf.[1] × Ct.[1]		4.00^{ns}	40	2.31±0.27	2.04±0.18	1.50±0.30
C.V. (%)[1]		21.48	Mean	2.47±0.12 aA	2.29±0.16 aA	1.79±0.36 aB
	F1[b]	ns	Sf.[1]	1.23±0.29 b	1.23±0.29 a	1.23±0.29 a
Regression model	F2	ns	Ct.[1]	1.97±0.55 b	1.97±0.55 a	1.97±0.55 a
	F3	ns				

Note. [a]**significant to F test and regression analysis at 1% level ($p < 0.01$) and [ns]not significant; [b]F1: $CaMg(CO_3)_2$; F2: $CaSO_4{\cdot}2H_2O$; F3: $CaCl_2$ P.A. [1]Sf.: Standard fertilization; Ct.: Control; C.V.: coefficient of variation; Int.: interaction; Fac.: Factorial. Means followed by the same lowercase letter in the column did not differ from each other by the Tukey test ($p < 0.05$).

Plants submitted to saline stress can accumulate proline. However, so far it is not clear to what extent this accumulation actually contributes to resistance to stress or if it is a symptom of metabolic disorder (Willadino & Camara, 2010). When testing the accumulation of proline in herbaceous cotton, beans and sorghum that were irrigated with water with electrical conductivity of up to 8.0 dS m^{-1}, Sousa et al. (2010) verified that sorghum presented the lowest accumulation of proline (1.18 µmol g^{-1}) and did not vary according to the level of electrical conductivity (salinity) of the water used in irrigation. The accumulation of proline in plants under saline stress conditions has also been reported in other crops, such as maize (Turan et al., 2009), rice (Lima et al., 2004), sorghum (Oliveira et al., 2006) and beans (Souza et al., 2011).

In this study Ca^{2+} doses did not influence proline production of plants. Similar results were found by Lacerda et al. (2004) who evaluated the accumulation of proline in sorghum plants under saline stress and supplemental Ca^{2+} and observed the accumulation of this amino acid only in the leaves, mainly in the sensitive genotype, independently of Ca^{2+} levels. Sousa et al. (2010) also verified that the level of salinity does not influence the accumulation of proline in sorghum plants.

According to Lacerda et al. (2004), although the increase in percentage of proline with saline stress is high, the contents of this organic solute, however, remained always very low compared to other ions, showing the inexistence of effective participation of this solute in the mechanisms of tolerance to salinity. The same is observed in the present study, since there was no change in proline production with K^+ - Na^+ substitution, and with the addition of increasing doses of Ca^{2+}.

Along with results of the present work, Lacerda et al. (2004) and Sousa et al. (2010) also working with grasses showed that these plants do not present alteration in proline content as a function of salinity or presence of Na^+. According to Ashraf and Foolad (2007) the accumulation of this amino acid is correlated with stress tolerance.

3.4 Electrical Conductivity of Soil and Na^+ Contents in Soil

The electrical conductivity (EC) in the soil at the end of the experiment did not present significance to the F test and in the regression analysis as a function of the increasing doses of Ca^{2+} (Table 6). Soil EC with partial replacement of K^+ by Na^+ did not differ from the standard fertilization. The soil of the control plants presented the lowest EC. The conductivity presented a mean of 0.408, 0.405 and 0.397 dS m^{-1}, respectively for the sources $CaMg(CO_3)_2$, $CaSO_4{\cdot}2H_2O$ and $CaCl_2$.

Table 6. Values of F and level of significance of soil electrical conductivity (EC) as a function of sources and doses of Ca^{2+} in the Na^+ - K^+ partial replacement

FV		CE	Ca^{2+} -- mg dm^{-3} --	EC ---- dS m^{-1} ----		
				$CaMg(CO_3)_2$	$CaSO_4 \cdot 2H_2O$	$CaCl_2$
Sources (S)		0.20^{ns}	0	0.37±0.04	0.37±0.04	0.37±0.04
Doses (D)		1.65^{ns}	10	0.38±0.02	0.38±0.02	0.38±0.02
Int.[1] S × D		0.86^{ns}	20	0.44±0.05	0.39±0.07	0.41±0.04
Fac.[1] × Sf. + Ct.		44.64^{a**}	30	0.43±0.10	0.42±0.09	0.40±0.08
Sf.[1] × Ct.[1]		40.29^{**}	40	0.44±0.02	0.45±0.06	0.38±0.02
C.V. (%)[1]		17.52	Mean	0.41±0.03 a	0.40±0.03 a	0.39±0.02 a
	F1[b]	ns	Sf.[1]	0.37±0.04 a	0.37±0.04 a	0.37±0.04 a
Regression model	F2	ns	Ct.[1]	0.12±0.02 b	0.12±0.02 b	0.12±0.02 b
	F3	ns				

Note. [a]** significant to F test and regression analysis at 1% level ($p < 0.01$), and [ns] not significant; [b]F1: $CaMg(CO_3)_2$; F2: $CaSO_4 \cdot 2H_2O$; F3: $CaCl_2$ P.A. [1]Sf.: Standard fertilization; Ct.: Control; C.V.: coefficient of variation; Int.: interaction; Fac.: Factorial. Means followed by the same lowercase letter in the column did not differ from each other by the Tukey test ($p < 0.05$).

The lowest soil EC of the control treatment occurred due to low soil fertility, which presented low levels of basic cations (salts) such as Ca^{2+}, Mg^{2+} and K^+. The EC results show that the partial replacement of K^+ by Na^+ in low fertility soil does not cause salinity. Therefore, in the condition of the present work, Na^+ did not present deleterious effect on the plants, acting more as a supporter of K^+ deficiency, which may have participated in some unspecific functions, guaranteeing the development of plants and eliminating possible symptoms of K^+ deficiency.

In order to define soil salinity, the electrical conductivity value (EC) of 4 dS m^{-1} is used as the dividing line between saline and non-saline soils. However, reductions in crop yield can be observed in soils with EC between 2 and 4 dS m^{-1} (Fernandes et al., 2010). It was observed that salinity was not a problem for the development of Mombaça grass (*Megathyrsus maximus* cv. Mombaça), since the maximum EC obtained was 0.44 dS m^{-1} (Table 6). Regarding Na^+ available levels in soil, it was verified that there was no presence of this element available to plants, possibly because plants extracted a part of Na^+ for the aerial part and a part remained in the roots.

The substitution of K^+ by Na^+ did not cause a significant reduction in forage production, especially when supplemental Ca^{2+} was used. However, care should be taken to replace this nutrient due to the possible problems that can be caused to soil by the excess of Na^+. Therefore, the monitoring of soil characteristics should be carried out with greater frequency, aiming at the appropriate management and maintenance of soil quality. This work was carried out to evaluate the effect of this substitution in soils with low natural fertility. Therefore, future studies should be carried out in high fertility soils to verify the effect of this substitution on the development of this forage. Research also ought to be performed in soils with correction of acidity through the application of limestone.

4. Conclusions

The addition of calcium as a conditioner of the effect of sodium in the partial replacement of K^+ by Na^+ caused greater development of Mombaça grass (*Megathyrsus maximus* cv. Mombaça).

The addition of calcium reduces the absorption of sodium by *Megathyrsus maximus* cv. Mombaça.

Partial replacement of potassium by sodium does not cause salinity in the soil.

Partial replacement of potassium by sodium does not have a toxic effect on *Megathyrsus maximus* cv. Mombaça.

The accumulation of proline in plants does not change due to the substitution of potassium by sodium and the doses of supplemental calcium.

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