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Growth of cowpea plants inoculated with Rhizobium in a saline-sodic soil after application of gypsum[1]

Crescimento de plantas de caupi inoculado com rizóbio em solo salino-sódico após aplicação de gesso

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ABSTRACT - Two experiments were carried out with the aim of evaluating the growth of cowpea cultivated in saline-sodic soils corrected with gypsum: one experiment in the laboratory, to identify the best level of gypsum for the correction of the saline-sodic soils of the state of Pernambuco, Brazil; and the other in a greenhouse, after correction of the soils. As the test plant, the cowpea cultivar *pele de moça*, inoculated with Rhizobium strain BR3267 was used. The experiments were arranged in a randomised block design in a 2 x 5 factorial arrangement, two soils and five levels of the gypsum requirement (GR), equivalent to 50, 100, 150, 200 and 250% of the GR of the soil, as determined by the Schoonover M-1 method, with five replications. The following were evaluated: electrical conductivity of the soil saturation extract (EC), soil exchangeable sodium and percentage of soil exchangeable sodium (ESP), number of nodules (NN), nodule dry weight (NDW), shoot dry weight (SDW), shoot height (PH) and nitrogen concentration (N) in the shoots. Application of 100% of the GR, followed by the enough water for leaching, was effective for the correction of soil sodicity. The application of increasing levels of soil GR resulted in an increase in the number of nodules, dry weight of the nodules and shoots, and the height and levels of N absorbed by the plants in soil S2. In soil S1, the use of levels of 200 and 250% of soil the GR caused a decrease in all the variables under study.

Key words: *Vigna unguiculata* L.. Exchangeable sodium. Gypsum requirement.

RESUMO - Com o objetivo de avaliar o crescimento do feijão-caupi, cultivado em solos salino-sódicos corrigidos com gesso, realizou-se dois experimentos: um em laboratório, para identificar o melhor nível de gesso para a correção de solos salino-sódico de Pernambuco. E outro em casa de vegetação, depois da correção dos solos, utilizando como planta teste o feijão-caupi, cultivar *pele de moça*, inoculada com Rizóbio, estirpe BR3267. Os experimentos foram dispostos no delineamento em blocos casualizados, com arranjo fatorial 2 x 5, dois solos e cinco níveis da necessidade de gesso (NG), equivalentes a 50; 100; 150; 200 e 250% da NG do solo, determinado pelo método Schoonover M-1, com cinco repetições. Foram avaliados: condutividade elétrica do extrato de saturação do solo (CE), sódio trocável e percentagem de sódio trocável (PST) do solo, número de nódulos (NN), massa seca dos nódulos (MSN), massa seca da parte aérea (MSPA), altura da parte aérea (APA) e teores de nitrogênio (N) na parte aérea. A aplicação do nível de 100% da NG, seguida de lâmina de lixiviação, foi eficaz para correção da sodicidade dos solos. A aplicação de níveis crescentes da NG do solo resultou em incremento no número de nódulos, massa seca dos nódulos e da parte aérea, altura e teor de N absorvido pelas plantas no solo S2. A utilização dos níveis de 200 e 250% da NG no solo S1 ocasionou decréscimo em todas variáveis estudadas.

Palavras-chaves: *Vigna unguiculata L.*. Sódio trocável. Necessidade de gesso.

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INTRODUCTION

The process of soil salinisation has been an issue that deserves attention, because it is causing limitations on productivity, particularly in irrigated areas of arid and semi-arid regions, thereby reducing the areas under cultivation. The semi-arid northeast is characterised by low and irregular rainfall and high evapotranspiration. Under these conditions, the main problems are the scarcity of water together with the salinisation and sodification of soils through natural processes, but mainly as the result of inadequate manaogement of the soil and water (COELHO, 2012).

The high salinity of the soil is a limiting factor in its productivity, creating serious economic problems, since the soils become unfit for agriculture, and are thus dropped from the production system. The correction of soils affected by salt requires effective and viable techniques of removing the excess salts and exchangeable sodium, enabling these soils to be included, once again, in the production process (BARROS *et al*., 2005).

To remove soluble salts, leaching is the most effective method for recovery. In regions with low rainfall, the removal of salts should be carried out as a way of reducing soil salinity to a level which is not harmful to the crop to be planted (ANDRADE *et al*., 2004). The use of water should be in sufficient quantity to dissolve and then transport the soluble salts to the drainage system. The depth of recovery depends on the type of crop that will be used (FERREIRA *et al*., 2002).

The correction of saline-sodic and sodic, soils requires the excess of exchangeable sodium to be replaced with calcium and the product of that reaction to be removed from the root zone by applying water for leaching. Several corrective agents can be used in the recuperation of soil with excess exchangeable sodium, with gypsum being the product which is most used, due to its low cost and being found in abundance in most of the world (ARAÚJO *et al*., 2011; MELO *et al*., 2008).

The gypsum requirement for the recuperation of saline-sodic and sodic soils can be calculated by formulas or tables, using the percentage of exchangeable sodium to be replaced, as well as the cation exchange capacity of the soil and the depth of soil to be reclaimed, or it can be ascertained through laboratory methods (BARROS; SANTOS; TERRAZ, 2006).

The cowpea, also known as the string bean, and locally as *feijão macassar* or *feijão fradinho*, represents the main subsistence crop in the north and northeast regions of Brazil and is a legume of great socioeconomic importance, where it constitutes a dietary component of high nutritional value, also generating employment and income in rural and urban areas (LIMA *et al*., 2007; LOPES *et al*., 2011; ROCHA *et al*., 2009; TEÓFILO *et al*., 2008). It is a legume which is able to benefit from biological nitrogen fixation (BNF) when associated with bacteria known as rhizobia; BNF has advantages such as low cost, the absence of environmental problems and the abundance of nitrogen in the atmosphere (NASCIMENTO *et al*., 2010).

Results of research into the effects of gypsum on the growth of the cowpea cultivated in saline-sodic soils newly-corrected with gypsum are scarce, so the aim of this study was to evaluate the efficiency of the application of levels of gypsum requirement (LGR) used in the correction of saline-sodic soils, on growth and nodulation in the cowpea.

MATERIAL AND METHODS

This research was carried out at the Federal Rural University of Pernambuco, where it was divided into two stages, one in the laboratory to correct the sodicity and salinity of the soil, and the other in a greenhouse to evaluate growth in the cowpea.

Two soil samples (S1 and S2) were collected at a depth of 0-40 cm in the Ibimirim Irrigation Area, located in the semi-arid zone of the state of Pernambuco, Brazil. Soil characterisation was performed on air-dried samples, broken up and sieved through a 2 mm mesh, after first removing larger clods, in order to obtain the soil density.

In the physical characterisation, which complied with the Empresa Brasileira de Pesquisa Agropecuária (1997) procedure, the following were determined: hydraulic conductivity (K0); soil density (Ds), by the paraffin clod method; particle density (Dp), using the volumetric balloon method; granulometric composition, using the densimeter method**,** after washing the soil with 60% ethanol until the chlorides were completely eliminated (Table 1). The saturation extract was obtained following the method described by Richards (1954), and the electrical conductivity (EC) of the saturated paste extract determined. By also applying the methodology suggested by Richards (1954), soil pH was determined in the soil to water ratio (1:2.5), the exchangeable cations (CEC) were extracted in 1 mol/L ammonium acetate, with calcium and magnesium being quantified by atomic absorption, and sodium and potassium by flame photometry. The exchangeable sodium percentage (ESP) was obtained from the CEC and exchangeable sodium data. The results of the chemical characteristics of the soil are shown in Table 2.

For soil correction, levels of gypsum requirement (GR) were applied equivalent to 50, 100, 150, 200 and

Table 1 - Physical characteristics of soil samples

Soil	Granulometric Analysis			Textural Classification	K_o	D_p	D_s
	Sand	Silt	Clay				
	-------------------g kg^{-1}-----------------------				cm h^{-1}	Kg dm^{-3}	
S1	13.90	56.20	29.90	Loamy-Silt-Clay	0.00	2.52	1.36
S2	34.20	41.60	24.20	Loamy	0.00	2.45	1.45

K_0 - Hydraulic Conductivity; D_p - Particle Density; Ds - Soil Density

Table 2 - Chemical characteristics of soil samples

Soils	Exchangeable Cations				GR	CTC	ESP	pH	CE
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}					
	--------------------$cmol_c$ dm^{-3}-----------------------						%		dS m^{-1}
S1	3.28	1.28	8.13	0.85	9.38	13.50	60.04	7.18	60.61
S2	2.89	1.16	4.75	0.82	5.98	9.60	49.38	7.10	36.16

GR - Needed Gypsum; CEC - Cation Exchange Capacity; pH - Ratio1:2,5; ESP - Exchangeable Sodium Percentage; EC- Electrical Conductivity

250% of the GR determined by the Schoonover M-1 method, as described by Barros and Magalhães (1989).

In the two experiments, the treatments were arranged in a randomised block design, with a 2 x 5 factorial array: two soil textures and five levels of gypsum requirement (LGR), with five replications, giving a total of 50 experimental units.

Analyses of soil fertility (Table 3) were carried out following the methodology described by Empresa Brasileira de Pesquisa Agropecuária (1997). Applying fertiliser becomes unnecessary; observing the natural fertility of the soils, it can be noted that they are rich in phosphorus and potassium, and only require inoculation with Rhizobium to provide nitrogen.

The experimental units consisted of PVC pipes, 10 cm in diameter and 30 cm high. The tubes were sealed at the bottom with a plug of PVC in the centre of which was a 3/8 inch tap; prior to this procedure, nylon screens with sequential openings were placed into the PVC plug, so as to simulate a drainage gradient. Each column of soil was split into two layers, each 12.5 cm high. The soil was packed into the columns so as to approximate soil density under field conditions. The gypsum was added to the first layer before being packed into the columns. The soil columns were slowly moistened until saturated, remaining at that humidity for 24 hours in order to restore balance to the system. To avoid losses from evaporation, the columns were covered with plastic wrap. Three loads of leaching water were then applied to all the columns, corresponding to three pore volumes (3 PV), each one of 95.60 mm.

After leaching, the soils were air dried, broken up and passed through a 2 mm sieve, when the exchangeable sodium and the electrical conductivity (EC) of the soil saturation extract were determined using the methodology described above. From the data for exchangeable sodium and cation exchange capacity (CEC), the value of the exchangeable sodium percentage (ESP) was quantified.

The soils were homogenized in a greenhouse after correction, placed into plastic pots (2 kg) and 5 seeds of the cowpea cultivar *pele de moça* were planted, having been

Table 3 - Characterisation of soil fertility

Soil	Ca^{2+}	Mg^{2+}	K^{+}	Al^{3+}	P	Organic Material
	---------------------------- $cmol_c$ dm^{-3} -------------------------------				mg dm^{-3}	dag Kg^{-1}
S1	5.60	1.58	0.90	0.00	220	0.82
S2	3.95	1.45	0.54	0.00	210	1.58

inoculated with Rhizobium, strain BR 3267. After seven days the plants were thinned out, leaving two per pot.

Forty days after sowing, plant height was checked, taking the vertical distance between the ground surface and the insertion of the stem with the last leaf. The plants were then harvested to evaluate the number of nodules, dry matter weight of the nodules and shoots, and levels of absorbed nitrogen. The shoots were separated from the roots and the nodules removed, counted, and dried in a forced-ventilation oven at 65-75 °C until reaching a constant temperature, in order to obtain the nodular dry weight. The shoots were weighed, placed into paper bags and dried in a forced-ventilation oven at 65 °C until reaching a constant weight, in order to obtain the dry mass. Specific extracts were prepared for obtaining the Total Nitrogen (N) by the method of steam entrainment (Kjeldahl), as described by Bezerra Neto and Barreto (2011).

For the statistical analyses the SAS Institute (2009) software was used, and the data obtained then interpreted by variance and regression analysis, with various models being tested. The criterion for choosing the model was the highest value for the adjusted coefficient of determination, and the significance of the coefficients of the regression equation.

RESULTS AND DISCUSSION

The results for the averages obtained for exchangeable sodium and exchangeable sodium percentage (ESP) are found in Table 4. Analysing these figures it can be seen that regardless of the levels of gypsum requirement (GR) employed, there was a large decrease in the levels of exchangeable sodium in relation to the original values (Table 2). The data obtained for exchangeable sodium prove the efficiency of the gypsum applied to the soil in replacing the sodium adsorbed on the exchange complex by the calcium of the corrective agent. It can also be seen that application of the 50% level of gypsum requirement was not enough to fix the sodicity of the soils S1 and S2, as they continued to have a percentage of the exchangeable sodium (ESP) of > 15 %. Correction did not occur at this level probably due to the amount of correction agent applied not being sufficient for there to be a greater substitution of sodium by the calcium, consequently these soil samples still had a sodic character. It can be seen that for treatments with the application of levels of over100% of the gypsum requirement, there was no marked reduction in the exchangeable sodium in the soil, confirming that the 100% level of NG as determined by the Schoonover M-1 method was effective for the correction of sodicity in the soil samples (ESP < 15%), and that the use of higher levels than that determined by the Schoonover M-1 method is not recommended, as it results in more being spent on corrective agents, water and consequently on energy. This behaviour was also noted by Silveira *et al*. (2008) who, when working with saline-sodic soils in Custódia, Pernambuco, and adding gypsum to the irrigation water, found that the applied gypsum corrected the ESP of the soils for values of less than 15%.

After applying the corrective agent and amounts of water, the averages for the electrical conductivity (EC) of the saturation extract of the soil (Table 4) did not differ by Tukey test. Even though salinity was reduced from the initial values of the soil, they still remained at harmful levels to any plants which are sensitive to salts. The values obtained for the EC for the first three levels (50, 100 and 150%) of the soil GR (S1 and S2) were reduced to values of less than or equal to 4.00 dS m^{-1}. When levels of 200 and 250% of GR were used, it can be seen that the process of salinity correction did not occur. Ruiz *et al*. (2004), working with columns of saline-sodic soil, noted that the application of gypsum gave lower values for the EC

Table 4 - Average values for the variables: exchangeable sodium (Na+); exchangeable sodium percentage (ESP); electrical conductivity (EC); of the saturated paste extract

GR	Na^+		ESP		CE	
	S1	S2	S1	S2	S1	S2
	$cmol_c$ dm^3		%		dS m^{-1}	
50	2.93 aA	1.85 bA	21.67 aA	19.23 aA	3.37 aA	3.43 aA
100	1.54 aB	1.09 bB	11.39 aB	11.33 aB	3.82 aA	3.62 aA
150	1.33 aB	0.87 bBC	9.84 aBC	9.04 aBC	4.00 aA	3.85 aA
200	1.10 aBC	0.74 bBC	8.14 aBC	7.69 aBC	4.69 aA	4.10 aA
250	0.75 aC	0.53 aC	5.55 aC	5.51 aC	4.78 aA	4.21 aA

S1 - Loamy-clay-silt soil; S2 - Loamy soil. Averages followed by different lowercase letters in a column and uppercase on a line differ by Tukey test (value p= 0.05) of recently-corrected soils. These authors reported that when the EC has a value of over 4.00 dS m^{-1} it means that the volume of solution applied was not sufficient to dissolve the gypsum, or that too much corrective agent was applied.

The summary of the variance analyses relative to the effect of applying increasing levels of gypsum requirement (GR) on the number of nodules (NN), nodule dry weight (NDW), shoot dry weight (SDW), plant height (PH) and nitrogen content (N) absorbed by the shoots of the cowpea, for the soils S1 and S2 were significant, and are shown in Table 5.

In Figures 1 and 2 can be found the ratio between the variables, number of nodules (NN) and nodule dry weight (NDW), with the coefficients of determination for soil S1 (R^2 = 0.72 and 0.84) and soil S2 (R^2 = 0.96 and 0.98). These results show a good ratio between the variables and the applied levels of gypsum requirement.

It can be seen (Figures 1 and 2) that the application of increasing levels of gypsum requirement to soil S2 resulted in an increase in the number and dry weight of nodules, however for soil S1, an increase occurs in the number and dry weight of the nodules up to the level of 150% of the GR, and a decrease when the higher levels of the GR (200 % and 250 %) are applied. These results are possibly due to the electrical conductivity of the saturation extract of the soils at these levels presenting high values, approximately 4.70 dS m^{-1}, affecting the development of bacteria and resulting in fewer nodules and a consequent reduction in the nodule dry weight.

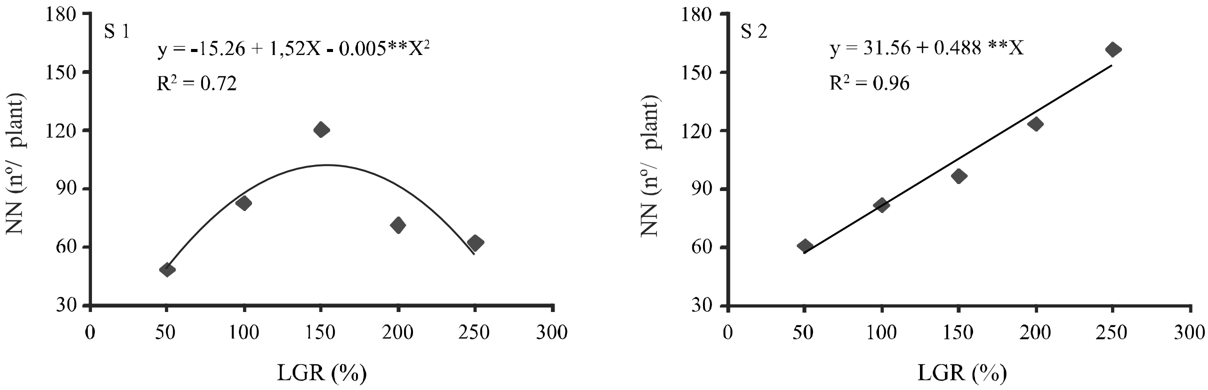
Ayers and Westcot (1999) reported that the cowpea is a plant which is moderately tolerant to salinity, showing no damage at salt concentrations measured by an electrical conductivity of around 4.9 dS m^{-1}. However, in this study, the EC was less than this value. Dantas *et al.* (2002), in research evaluating 50 cowpea genotypes under salinity,

Table 5 - Values of F resulting from variance analyses of the number of nodes (NN), nodule dry weight (NDW), shoot dry weight (SDW), height of shoots (PH) and nitrogen content (N) in the cowpea

Source of Variation	NN	NDW	SDW	PH	N
Soils (F1)	37.28**	6.14 *	401.80 **	140.97 **	279.54 **
GR (F2)	20.82**	12.96**	38.69 **	27.55 **	70.63 **
Int. F1 X F2	22.38**	14.33**	34.69 **	15.24 **	70.56 **
Coefficient of Variation (%)	13.9	22.92	3.90	3.63	2.55

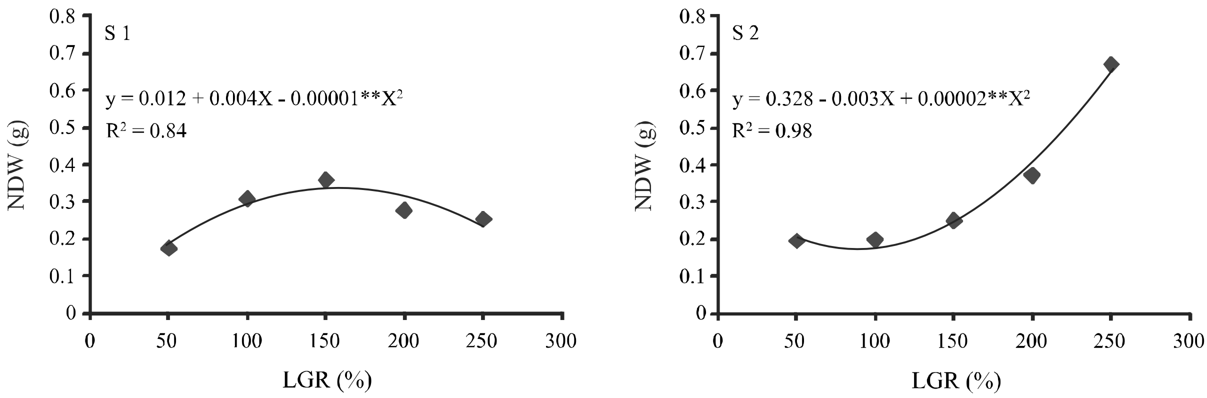
* e ** significant at 5% e 1% respectively, by F-test

Figure 1 - Ratio between the number of nodules (NN) in the root of the cowpea and levels of gypsum requirement (LGR) for soils S1 and S2



** Significant at 1%

Figure 2 - Ratio between nodule dry weight (NDW) in the cowpea and levels of gypsum requirement (LGR) for soils S1 and S2



** Significant at 1%

found that genotypes can be classified for tolerance to salinity based on a reduction in the percentage of shoot dry weight. These data are consistent with those found by Chagas Junior *et al*. (2010) in studies on the effectiveness of strains of Rhizobium when inoculated into cowpea. They found that BR 3267 has the lowest performance for the number of nodules in relation to the other strains studied. Differing data was found by Melo and Zilli (2009) in a greenhouse experiment, where a greater number of nodules and nodule dry weight were seen in plants inoculated with the BR 3267 strain, compared to those inoculated with BR 3262.

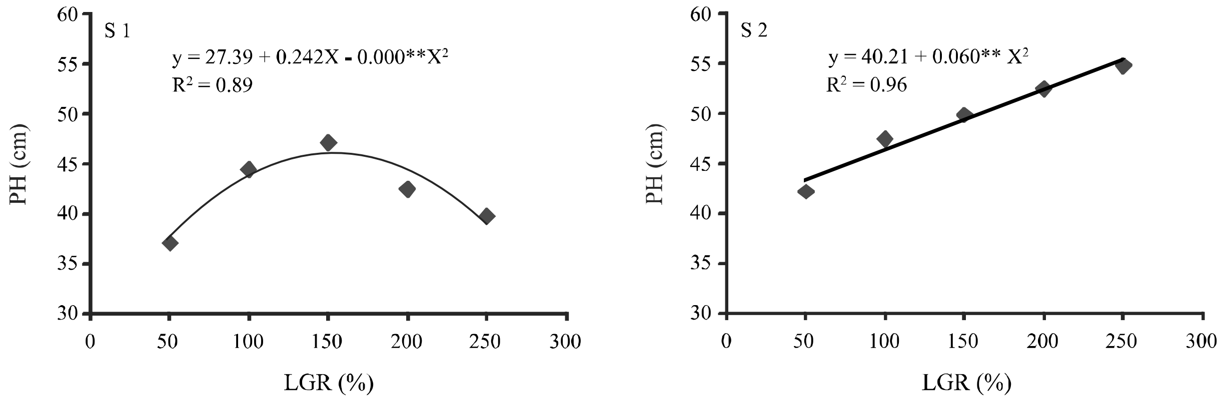
The ratio between the levels of gypsum requirement of soils S1 and S2 and plant height was tested, the results being shown in Figure 3. For soil S1, an increase can be seen in the height of the bean plants at levels of 100 and 150% of the GR, followed by a decrease at levels of 200 and 250%, when the EC of the saturation extract of the soil was around 4.7 dS m^{-1}. These results are similar to those reported by Coelho *et al*. (2013) who, working with cowpea and salinity levels (2.0, 4.0, 8.0 and 12.0 dS m^{-1}), found that from an EC level of the saturation extract of the soil equal to 4.00 dS m^{-1}, drastic reductions in plant height were seen, with values of 75 and 85% for the sandy-loam soil and clayey-loam soil respectively. The authors explain that this reduction in the growth of the vigna bean is due to a decrease in the osmotic potential of the soil solution, which caused a greater difficulty in the absorption of water and nutrients, as well as to the possibility of there being ion toxicity due to the possible excessive accumulation of the sodium cation and the chloride anion. In soil S2 different behaviour can be observed, indicating that up to a level of EC close to 4.0 dS m^{-1} the salinity did not affect the growth of the bean plant; a slight increase is also seen in plant height with the increased levels of EC. This fact is possibly explained by the higher calcium and magnesium content from the corrective agent being available for absorption by the plants, and as the EC seen in this soil sample was lower than the in soil S1, there was no adverse effect from this on plant growth.

Evaluating the ratio between levels of gypsum requirement of soils S1 and S2 and plant height for soil S1 (Figure 3), it is possible to affirm that a quadratic model is the best fit, with a coefficient of determination equal to 0.89. Similar behaviour was observed by Miranda *et al.* (2008) in studies where increasing concentrations of calcium chloride (2, 3, 5, 9, 15 e 25 $mmol_c$/L) were applied to saline-sodic soils in the state of Paraíba, and the behaviour of sorghum was evaluated. The authors found a coefficient of determination of 0.73, and assert that the salinity of the applied solutions becomes a limiting factor at higher concentrations of calcium chloride. Verifying the behaviour of this same ratio in soil S2, the linear, increasing model best represented plant response, with a coefficient of determination of 0.96. Differing results were reported by Silva *et al*. (2009) in studies with the cowpea. Irrigating at salinity levels of 0.5, 2.13, 2.94, 3.5, and 5.0 dS m^{-1}, a reduction in height of about 42.86% was found in plants irrigated with the highest saline level and a coefficient of determination equal to 0.89 was obtained.

The ratios between nitrogen levels, shoot dry weight and levels of gypsum requirement (LGR) were also tested and the results are shown in Figures 4 and 5.

It can be seen that the nitrogen level and shoot dry weight (Figures 4 and 5) displayed similar behaviour, with an increase up to the application of

Figure 3 - Ratio between shoot height (PH) in the cowpea and levels of gypsum requirement (LGR) for soils S1 and S2

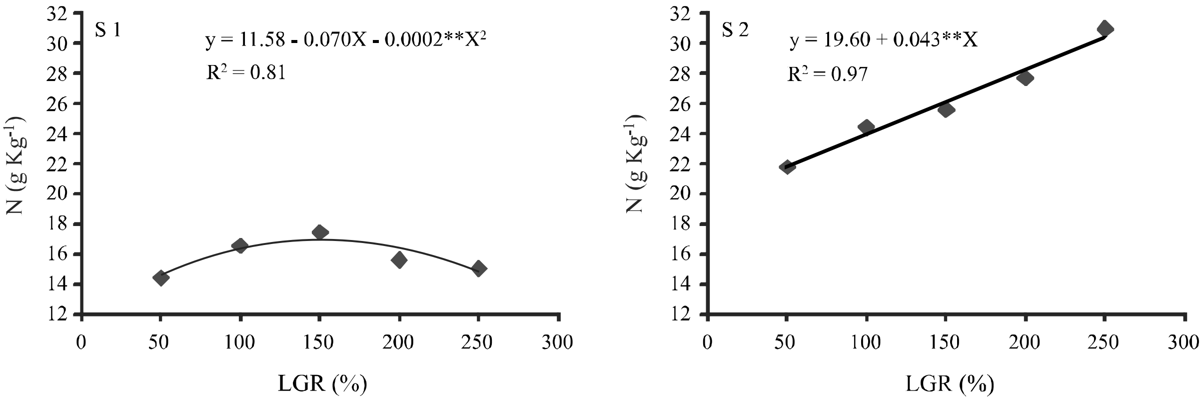


** Significant at 1%

150% of GR, followed by a decrease when the higher levels of GR were used. These results are due to less nodule formation, resulting in decreased symbiotic nitrogen fixation and thus less nitrogen available to the plants. As nitrogen is the nutrient responsible for vegetative growth, there is resultant decreased plant height and lower shoot dry weight. When a level of 50% of the GR was applied, the sodicity of the soil was not corrected (ESP = 21.64%), and the adverse effect of the levels of exchangeable sodium on such physical soil characteristics as water permeability and infiltration resulted in decreased values for all the variables studied. The results show that an EC of > 4.7 dS m^{-1} and ESP of > 21%, probably affect development in the strain of bacteria used.

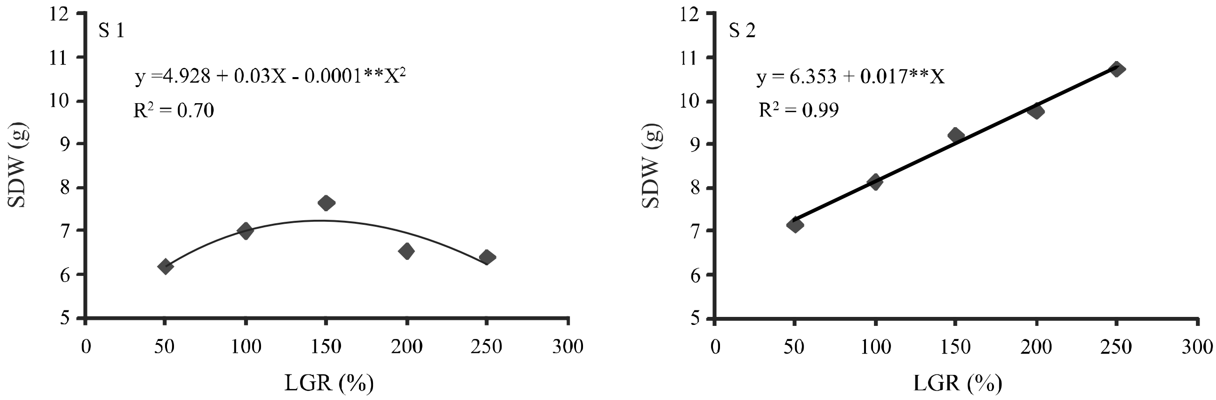
In the sample of loamy soil (S2), the results obtained for the number of nodules (Figure 1), the nodule dry weight (Figure 2) and the nitrogen content of the shoots (Figure 4) were proportional to the increase in gypsum levels applied. The smallest values for these variables were found with the application of the 50% level of gypsum requirement. This fact is explained by the higher exchangeable sodium content of the soil at this level; the physical characteristics of the sodic soil make the medium unfavourable to microbial life, i.e. less nodule formation and nodule dry weight with consequently lower levels of available nitrogen, resulting in less development of the plant. The interference of exchangeable sodium levels on development in the cowpea was studied by Smith, Chen and Chalk (2009), where the authors report that a reduction in the levels of exchangeable sodium in the soil helps to increase hydraulic conductivity, thereby facilitating the distribution of water and nutrients and consequently greater plant growth.

Figure 4 - Ratio between nitrogen levels (N), shoots in the cowpea and levels of gypsum requirement (LGR) for soils S1 and S2



** Significant at 1%

Figure 5 - Ratio between shoot dry weight (SDW) in the cowpea and levels of gypsum requirement (LGR) for soils S1 and S2



** Significant at 1%

CONCLUSION

1. The application of the 100% level of the gypsum requirement (GR) as obtained by the Schoonover M-1 method, followed by enough water for leaching, was effective in the correction of soil sodicity (ESP <15 %);
2. The application of increasing levels of the gypsum requirement of the soil resulted in an increase in the number and dry weight of the nodules, plant height and the nitrogen content absorbed by the plants, for soil S2;
3. The use of the 200 and 250% levels of the gypsum requirement in soil S1 caused a decrease in the number of nodules, in the nodule and shoot dry weight, in plant height and the nitrogen content absorbed by the plants.

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