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Zinc fertilization in cowpea cultivars[1]

Adubação com zinco em cultivares de feijão-caupi

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ABSTRACT - Zinc (Zn) is an essential micronutrient for plants, due to its role as a functional, structural, or regulatory cofactor in a large number of enzymes. The element is necessary for the synthesis of tryptophan, which is a precursor of indoleacetic acid. The aim of this study was to evaluate the behavior of two cowpea cultivars fertilized with four doses of Zn (0.0, 2.0, 4.0, and 6.0 kg of Zn ha^{-1}) regarding dry grain yield (PGS), in addition to quantifying leaf and grain Zn content and grain crude protein. The experiment was conducted in the town of Brejo, in the state of Maranhão, Brazil. A 4 x 2 factorial scheme was used with four replications. Response functions were adjusted by calculating the Zn doses which provided a maximum technical efficiency for each genotype. The tested cultivars of cowpea showed no great difference regarding Zn demand applied to the soil, ranging from 3.2 to 3.4 kg of Zn ha^{-1} for BRS Tumucumaque and BRS Guariba, respectively. Concerning Zn use efficiency, BRS Guariba was more responsive than was BRS Tumucumaque, i.e. the same dose of Zn produced 70 kg grain and 2.1 kg crude protein per hectare.

Key words: Mineral nutrition. *Vigna unguiculata* (L.) Walp. Grain yield.

RESUMO - O zinco (Zn) é um micronutriente essencial para as plantas em razão da sua participação como cofator funcional, estrutural ou regulador de grande número de enzimas. Esse elemento é necessário para a síntese do triptofano, que é um precursor do ácido indolacético. O objetivo desse trabalho foi o de avaliar o comportamento de duas cultivares de feijão-caupi em relação à aplicação de quatro doses de Zn (0,0; 2,0; 4,0 e 6,0 kg de Zn ha^{-1}), sobre a produtividade de grãos secos (PGS), bem como quantificar os teores de Zn na folha e no grão e proteína bruta no grão. O experimento foi conduzido no município de Brejo, MA. Foi utilizado um arranjo fatorial 4 x 2, com quatro repetições. Foram ajustadas funções de respostas, calculando-se as doses de Zn, que proporcionam as máximas eficiências técnicas para cada genótipo. As variedades de feijão-caupi testadas não apresentaram grandes diferenças na exigência da aplicação de Zn no solo, variando de 3,2 a 3,4 kg de Zn ha^{-1} para as variedades BRS Tumucumaque e BRS Guariba, respectivamente. Quanto a eficiência do uso do Zn, a variedade BRS Guariba foi mais responsiva, ou seja, com a mesma dose de Zn produziu 70 kg de grãos e 2,1 kg de proteína bruta a mais por hectare.

Palavras-chave: Nutrição mineral. *Vigna unguiculata* (L.) Walp. Produtividade de grãos.

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INTRODUCTION

The cowpea presents high contents of proteins, fibers, minerals such as iron, zinc, potassium, magnesium, and phosphorus, in its chemical composition, as well as low lipid content (FROTA *et al*., 2008). Currently, the culture has increased its expression in function of the biofortification process of its grains, aiming to provide the population with a diet rich in proteins and minerals such as iron and zinc, in order to combat anemia and strengthen this population's immune system (OLIVEIRA *et al*., 2011).

Considering the importance of this agricultural product as a primary nutrient source, larger nutrient amounts will be required with the increase in population. According to Godfray *et al*. (2010), the world population will continue to grow at an accelerated pace until 2050, when it will reach a plateau of approximately 9 billion people. The global food demand will also continue to grow and, as estimated, there will be a need to double global agricultural production for the period of 1990-2030, increasing by two and a half to three times in developing countries (GRAHAM; WELCH, BOUIS, 2001).

A recent study by Garvin, Welch, and Finley (2006) shows that the increase in productivity obtained through plant breeding has an inverse relation to grain micronutrient content over the years. This is a negative aspect since human wellbeing requires at least 22 mineral elements. Therefore, there is a need to associate the quality of agricultural and livestock production with health and nutrition, and the industry will be pressured to provide foods with greater nutritional quality.

Zinc (Zn) stands out among these mineral elements, being currently the most important concern in human health, especially in developing countries. One third of the world's population is estimated to be living in countries considered to be at high risk for Zn deficiency, suggesting that one-fifth of the world's population may not be ingesting enough of this nutrient (HOTZ; BROWN, 2004). Zinc deficiency is responsible for serious health complications, including damage to the immune system combined with increased risk of infections, physical growth impairment, delayed learning ability, damage to DNA development, and cancer (HOTZ; BROWN, 2004; ZEITUNE *et al*., 2001).

In Brazil, when used, micronutrient application is done empirically. The micronutrient is used by the producer without considering the specific crop need, or the amount of nutrient available in the soil to meet its demand (SILVEIRA; DYNIA; ZIMMERMANN, 1996). This factor promotes nutritional imbalance, considering the low Zn availability in the soil. Among the factors enhancing Zn deficiency in soils, high doses of phosphorus (FONSECA *et al*., 2011; MOUSAVI; GALAVI; REZAEI, 2012; ZUCARELI *et al*., 2011) and limestone for acidity correction stands out. The requirement of high-yield cultivars can be added to these factors, a fact that has been increasing the importance of micronutrients in agricultural production.

Several positive results with oilseeds such as canola, and grasses such as corn, millet, rice, and pastures were obtained and published in the literature (COUTINHO; SILVA; SILVA, 2007; HERNANDES *et al*., 2009; PEREIRA; ERNANI; SANGOI, 2007; SALIMPOUR *et al*., 2010; SILVA *et al*., 2010). However, there are very few studies of this nature involving cowpea (CARVALHO, 2014; FROTA *et al*., 2008).

The objective of this study was to evaluate the behavior of cowpea cultivars with the application of zinc doses in the production and grain yield components, as well as to determine the amount of zinc in the grain that is related to higher protein synthesis.

MATERIAL AND METHODS

The experiment was carried out between March 7 and May 30 of 2013, in the village of Palestina, Brejo city - MA (Brazil), in an area under cerrado (savannah-like vegetation). It is located at 03º40'45" S, 42º59'55" W, and 117-m altitude. The rainfall during the experimental period was 429 mm.

A Dystrophic Yellow Latosol was used with the soil samples collected in the surface layer (0 to 20 cm). Soil physical and chemical aspects were characterized according to the method described by Embrapa (1997).

The chemical and physical characteristics showed the following results: pH in H_2O = 4.9; 47 $mmol_c\ dm^{-3}$ H+Al; 0.7 $mmol_c\ dm^{-3}$ K^+; 6.0 $mmol_c\ dm^{-3}$ of Al^{-3}; 5 $mmol_c\ dm^{-3}$ of Ca^{2+}; 3 $mmol_c\ dm^{-3}$ of Mg^{2+}; 22 g dm^{-3} of organic matter; 2 g dm^{-3} of phosphorus (Mehlich); 16% base saturation; 0.5 mg dm^{-3} of Zn; 768 g kg^{-1} of the sand fraction; 66 g kg^{-1} of the silt fraction, and 166 g kg^{-1} of the clay fraction.

The experimental design was a randomized complete block design in a 4 x 2 factorial scheme with three replicates, i.e., four zinc levels (0.0, 2.0, 4.0, and 6.0 kg of Zn ha^{-1}), in the form of zinc sulfate (20% Zn) and two cowpea cultivars (BRS Tumucumaque and BRS Guariba).

The plots consisted of six rows of 5.0 m, spaced at 0.5 m, with a density of five plants m^{-1}.

Basal dressing with Zn doses (treatments), phosphorus (60 kg P_2O_5 ha^{-1}), and potassium (40 kg K_2O ha^{-1}) - triple superphosphate and potassium chloride respectively, were carried out manually in parallel furrows 0.15-m deep and 0.10-m apart from the planting rows. Yet nitrogen topdressing (40 kg of N ha^{-1}), in the form of urea, was performed at flowering.

Zinc contents of twenty plants per treatment located in the useful area of each experimental unit were determined in the third trifoliolate leaf from the apex down, which were sampled at R^2 phenological phase. After being harvested, identified, and properly washed, these samples were oven dried at 65 °C for 72 hours, and then milled. Zn extraction was performed by nitroperchloric digestion, whose contents were determined by atomic absorption spectrophotometry.

Grain Zn and crude protein contents were also evaluated. For zinc, the analyses were carried out following the same methodology used for the foliar analysis. For crude protein, the Kjeldahl method was used, following the methodology described in Silva and Queiroz (2002). The two analyses used grains of 10 pods sampled in the useful area of each treatment at the end of the culture cycle.

The variables evaluated were: dry grain yield (DGY), corrected for 13% moisture (kg ha^{-1}), leaf zinc content in mg kg^{-1} (LZC), grain zinc content, mg kg^{-1} (GZC), and grain crude protein in% (GCP).

Data were submitted to analysis of variance and polynomial regression study (SAS, 2016). First- and second-degree polynomial regressions were used for zinc doses. Based on the t-test, the best model was selected with the aid of the significance of each variable, accepting a significance level up to a 5% probability limit. Then, according to the response functions, Zn doses that provided the maximum technical efficiencies for each cultivar were calculated (ZIMMERMANN, 2004).

RESULTS AND DISCUSSION

The results of the data analysis of variance showed effects of the application of zinc doses on the yield of dry grains, leaf zinc content, grain zinc content, and grain crude protein contents of the cowpea cultivars BRS Guariba and BRS Tumucumaque (Table 1).

For Zn concentration in the cowpea plant tissue, a significant effect ($p<0.01$) was detected on the concentration of this micronutrient in the leaves and grains as a function of the Zn doses added to the soil, showing that the crop was responsive to fertilization with Zn.

This response was expected due to the low initial Zn content in the soil under study (0.5 mg dm^{-3}), which was below the critical level of the soil micronutrient (1.3 mg dm^{-3}) determined for the cowpea and obtained by Malavolta, Vitti, and Oliveira (1997).

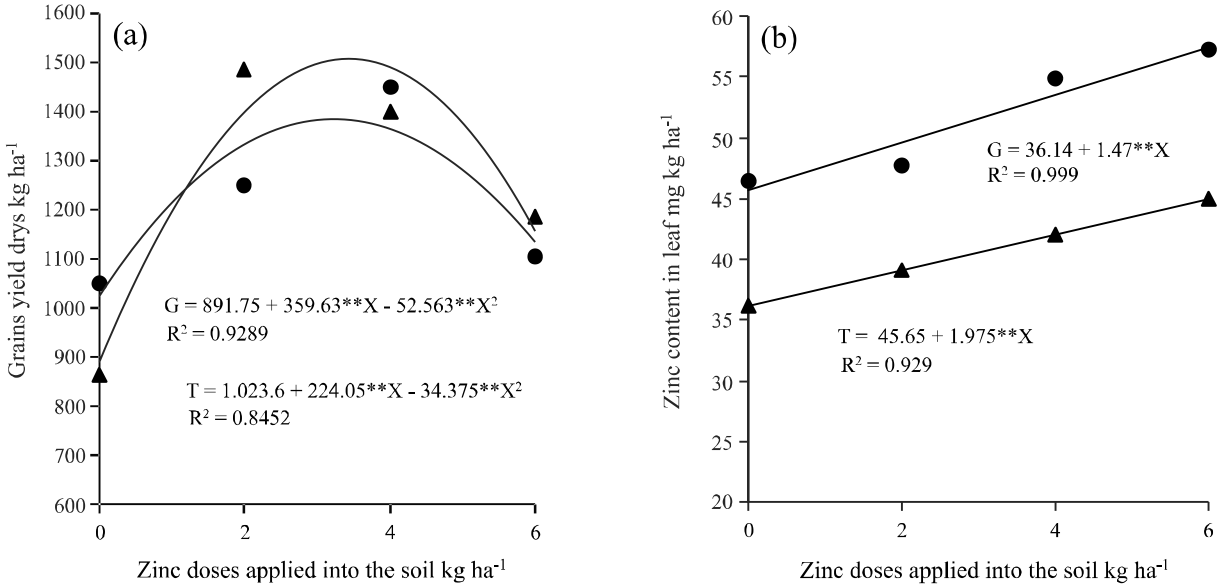
Leaf and grain zinc contents were adjusted to linear response functions in the two cultivars, with values ranging from 36 to 45, and 46 to 57 mg kg^{-1} (LZC), 46 to 54 and from 51 to 60 mg kg^{-1} (GZC) for cultivars BRS Guariba and BRS Tumucumaque, respectively, (Figure 1b and 2a).

Table 1 - Summary of variance analysis (mean squares) for dry grain yield in kg ha^{-1} (DGY), leaf zinc content in mg kg^{-1} (LZC), grain zinc content in mg kg^{-1} (GZC), and grain crude protein contents in% (GCP) in Brejo, MA, crop 2012/2013[1]

F. V.	GL	Mean squares			
		DGY	LZC	GZC	GCP
BL	2	224340.60**	15.47^{ns}	71.04**	0.22^{ns}
CULT	1	2644.87*	255.57 **	197.64**	6.83**
Zn’ in Cult1	1	23436.55^{ns}	174.49 **	133.22**	0.35^{ns}
Zn’’ in Cult1	1	149695.43**	8.09^{ns}	0.02^{ns}	0.96*
Zn’’’ in Cult1	1	45727.13*	6.12^{ns}	10.32^{ns}	0.01^{ns}
Zn’ in Cult2	1	289120.42**	1475.08**	199.22**	3.17**
Zn’’ in Cult2	1	416268.75**	4.27^{ns}	1.49^{ns}	2.08**
Zn’’’ in Cult2	1	8283.75^{ns}	40.35*	0.77^{ns}	0.01^{ns}
Error	14	5508.78	5.65	6.37	0.19
CV (%)	-	6.04	6.35	4.73	1.76

[1] ns: non-significant ($p>0.05$); *: significant ($0.05\geq p>0.01$); **: significant ($p\leq 0.01$) by the *t -test*

Figure 1 - Dry grain yield (**a**) and leaf zinc content (**b**) as a function of the application of Zn doses, Brejo, MA, 2012/2013 harvest, Cultivars BRS Tumucumaque (●) and BRS Guariba(▲)



Values for BRS Guariba are within the range considered adequate by Malavolta, Vitti, and Oliveira (1997), that is, from 30 to 40 mg kg^{-1}. In the case of cultivar BRS Tumucumaque, these higher values come from the fact that the breeding program of Embrapa Meio Norte had launched this cultivar as a biofortified material.

There was a response to zinc doses ($p<0.01$) by the F test for variables DGY, LZC, GZC, and GCP for cultivars BRS Guariba and BRS Tumucumaque , with maximum DGY of 1,460 kg ha^{-1} and 1,390 kg ha^{-1}, with application of doses of 3.4 and 3.2 kg of Zn ha^{-1}, respectively (Figure 1a).

Regarding the average grain yield, the two cultivars evaluated in the absence of zinc were verified to have yields above 900 kg ha^{-1}. When 3.0 kg of Zn ha^{-1} were applied, the same cultivars presented average maximum yields of 1,425 kg ha^{-1}, presenting a gain of 467 kg ha^{-1}, which is higher than the national average of 300 kg ha^{-1} (LEITE *et al*., 2009).

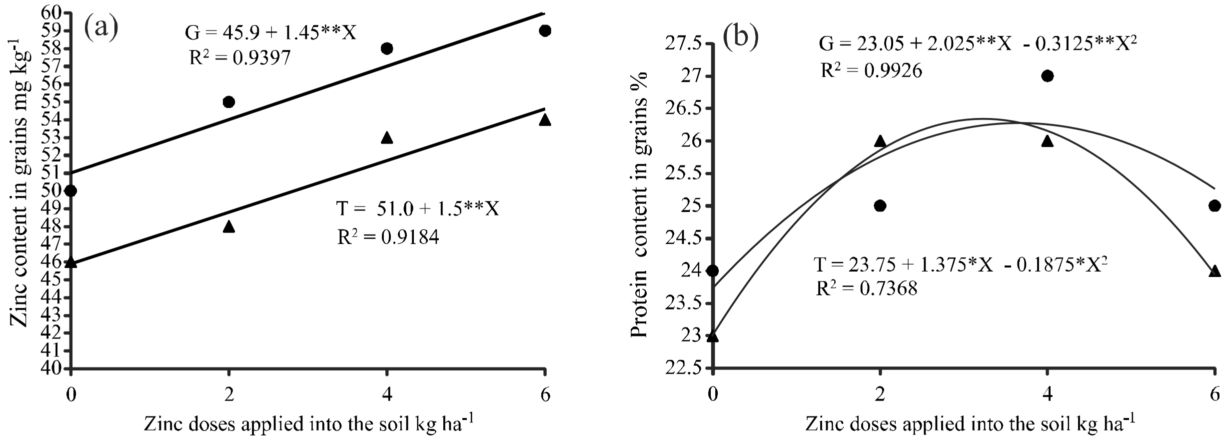
Another aspect to be considered is that the application of the chemical fertilizer input with zinc, in the balanced amount of each soil condition and genetic material, is of utmost importance both for productivity increase and for improvement in the grain bromatological quality, that is, the production nutritional quality.

The application of the 3.6 and 4.2 kg doses of Zn ha^{-1} gives values of 47 and 50 mg of Zn kg^{-1} of grains (Figure 2a). As for crude protein contents, the application of 3.6 and 4.2 kg of Zn ha^{-1} gives the value of 26% of crude protein, which corresponds to an increase of 3% in protein content in the two cultivars, when compared to the control (Figure 2b).

The results are in agreement with those obtained by Silveira, Dynia, and Zimmermann (1996), and the reports of Mousavi, Galavi and Rezaei (2012), when they affirm that zinc is an essential micronutrient for the plants due to its participation as a functional cofactor, structural or regulator of large number of enzymes. This element is necessary for the synthesis of tryptophan, which is a precursor of indoleacetic acid, an auxin, a hormone that stimulates the development of young plant parts, and is also involved in the metabolism of nitrogen. Marschner (1986) and Silva *et al*. (2010) claimed zinc as an important element for protein synthesis in plants, being also a ribosome component. Under zinc deficiency, there is a reduction of the accumulated amino acids in plant tissues, and of protein synthesis.

As for the results in grain composition, Frota, Soares, and Arêas (2008) obtained, on average, lower results than those obtained in this study, 24.5% of crude protein and 41 mg of Zn kg^{-1} of grains. The same authors verified that the cowpea amino acid profile was favorable to the reference standard, except for the sulfur deficiency, suggesting the need to combine this legume with other food sources.

Figure 2 - Dry grain Zn (**a**) and crude protein (**b**) contents of two cowpea cultivars submitted to the application of zinc doses, Brejo, MA, 2012/2013 harvest, Cultivars BRS Tumucumaque (●) and BRS Guariba (▲)



CONCLUSIONS

1. The tested cowpea cultivars showed no significant differences after applying Zn into the soil, which ranged from 3.2 to 3.4 kg Zn ha^{-1} for BRS Tumucumaque and BRS Guariba, respectively;
2. For Zn use efficiency, BRS Guariba was more responsive than was BRS Tumucumaque, i.e. lower Zn doses enabled a production of 70 kg grains and 2.1 kg crude protein per hectare.

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