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RESEARCH ARTICLE

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ZINC CONTENT AND PHYTOMASS ACCUMULATION IN GREEN CORN FERTILIZED WITH ZINC SULPHATE

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ABSTRACT

The low levels of zinc (Zn) in Brazilian semi-arid soil is one of the factors that most affect the yield and nutritional quality of the maize. The aim was to evaluate the influence of Zn doses via root and leaf on the production of phytomass and Zn contents in leaves and grains of green corn growing in alkaline soil. Two experiments were carried out under field conditions, in a randomized block design, with four blocks. In the first experiment five doses of Zn (0; 2.5; 5.0; 7.5 and 10.0 kg ha⁻¹) via soil, were tested. In the second experiment, treatments was five doses of Zn (0; 0.5; 1.0; 1.5 and 2.0 gL⁻¹) via leaf. The fertilization of green corn with zinc via soil increased the concentration of this nutrient in the soil and in the leaves, but it was not able to increase the production of phytomass, nor grains yield and the levels of Zn in grains. The supply of zinc via the leaves increases the leaf contents of Zn, dry mass of stem, leaves and ear, but does not affect the levels of Zn in green maize grains.

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INTRODUCTION

In Brazil, corn (*Zea mays* L.) is the second most important crop after soy in terms of planted area, production and export, being cultivated mainly in the Brazilian Midwest region for grains yield (Barbosa et al. 2015). The green corn cultivation, that is, corn with grains in the milky state is traditional in the Brazilian Northeast Region, but it has reach importance throughout Brazil, since the commercialization value is higher, when compared to corn in the form of dry grains (Paiva et al. 2012, Ribeiro et al. 2016). Zinc (Zn) is the micronutrient with the highest number of deficiency reports in the field, under natural conditions in Brazilian soils (Dalpisol et al. 2017), especially in demanding crops such as corn. This fact is related to the soil parent material, a high degree of weathering

of the soil in the rainiest regions and due to the chemical precipitation reactions in alkaline soil located in the Brazilian semi-arid regions (Biondi et al. 2011). The lack of Zn in the soil affects not only the productivity of the corn, but also the quality of the product harvested in nutritional terms, that is, low content of Zn in the grains. Zinc is a micronutrient of plants and humans. In plants, zinc is essential element for the activation of enzymes, protein synthesis and cell division, tryptophan synthesis (Marschner, 2012, Tavares et al. 2013). In animals and humans, Zn is involved in the activation of enzymes, participates in functions related to growth, development and neurological functions (Roohani et al. 2013). To improve the nutritional quality of the harvested product, the technique called biofortification has been used, which can be done through genetic improvement and gene and gene

manipulation (Garget *et al.* 2018, Kumar *et al.* 2019) or through fertilization, denominated agronomic biofortification (Maqbool & Beshir 2019, Gonçalves *et al.* 2019, Xia *et al.* 2019). Agronomic biofortification via soil, seed treatment and foliar application are characterized as less costly, more accessible and quick results, since it depends only on fertilization. For this reason, some research has been carried out for this purpose (Liu *et al.* 2016, Valcarengi *et al.* 2017, Fernandes & Dalchiavon 2019, Xia *et al.* 2019). The objective was to evaluate the influence of doses of Zn applied via root and leaves on the production of phytomass, the levels and accumulations of Zn in green grains and in the leaf tissues of green corn irrigated in alkaline soil.

MATERIAL E METHODS

Two experiments were carried out in a field condition, in a commercial production area, in the municipality of Vieirópolis - PB, mesoregion of the Sertão Paraibano, located at 06 ° 31'47.91" S and 38° 14' 29.42" W, with an altitude of 324 m. According to the Köppen (1956), the climate is semi-arid, hot and dry, with high temperatures during the day, softening the night, presenting annual variations within a range of 23 to 30° C, with possible higher peaks, mainly during the dry season period. The rainfall regime, in addition to being low, is irregular, with annual averages around 900 mm per year (CPRM, 2005). The soil in the experimental area was classified as carbonate Chromic Luvisol (Campos & Queiroz 2006, Santos *et al.* 2013). Before the installation of the experiment, the soil was sampled in the 0 - 0.20 m layer for its chemical and physical characterization, according to the methodology contained in Embrapa (1997). The soil analysis results were: $pH_{H_2O} = 7.23$, $Ca^{2+} = 7.6 \text{ cmol}_c \text{ dm}^{-3}$, $Mg^{2+} = 3.06 \text{ cmol}_c \text{ dm}^{-3}$, $K^+ = 0.60 \text{ cmol}_c \text{ dm}^{-3}$, $Na^+ = 0.70 \text{ cmol}_c \text{ dm}^{-3}$, $Al^{3+} = 0.00 \text{ cmol}_c \text{ dm}^{-3}$, $H + Al = 0.00 \text{ cmol}_c \text{ dm}^{-3}$, organic matter = 39.50 g kg^{-1} , sand = 567 g kg^{-1} , silt = 166 g kg^{-1} and clay = 267 g kg^{-1} . The first experiment was carried out in a randomized block design, with five treatments corresponding to five doses of Zn (0; 2.5; 5.0; 7.5 and 10.0 kg ha^{-1}), with four replications. The experimental plot consisted of three double rows of 5.0 m long planting, totaling approximately 100 plants in 30 m^2 of each plot. The useful portion was constituted by the central double row, with 0.5 m of each end neglected. The doses of Zn were applied in planting fertilization, in a single dose, in the form of commercial zinc sulfate (22% Fe and 11% S).

The second experiment was carried out in a randomized block design, with five treatments corresponding to five concentrations of Zn (0; 0.5; 1.0; 1.5 and 2.0 g/L) with four replications. The experimental plot consisted of three double rows of 5.0 m long planting, totaling approximately 100 plants in 30 m^2 of each plot. The useful portion was constituted by the central double row, with 0.5 m of each end neglected. For leaf fertilization, referring to each Zn concentration, commercial zinc sulfate (22% Fe and 11% S) was used. The applications were carried out using a manual costal applicator, with constant pressure and a spray volume of 200 L ha^{-1} in the phenological phase V6, that is, with six definitive leaves. The soil preparation of the experimental area was done through a plowing at 0.20 m depth. The hybrid corn, AG 1051®, was sown directly in the field, under a double row planting system, with 0.40 m spacing within the double row, 0.30 m between plants in the planting line and 2.0 m between the centers of each double row, obtaining an estimated population

density of $33,333 \text{ plants ha}^{-1}$. Sowing was carried out on March 28, 2018, at an average depth of 0.03 m, with two seeds per hole in the planting line, in which, subsequently, thinning was carried out in the vegetative phase "V3" (three leaves definitive), with only a single seedling remaining per hole. Low salinity water (0.1 dS m^{-1}) was used for irrigation of the cultivation area, using a localized irrigation system, pressurized by three-phase electrode, using a single drip tape, model Taldrip®, Naan Dan brand Jain®, nominal diameter of 17 mm, emitters spaced at 0.3 m and flow rate of 1.0 L h^{-1} at 100 KPa of nominal pressure, in the center of each double row. Planting fertilization consisted of the application of 20 kg of urea (45% N) and 70 kg/ha of P_2O_5 in the form of simple superphosphate (18% of P_2O_5). The cover fertilization with nitrogen and potassium was carried out following the recommendation of Freire *et al.* (1999), using urea (45% N) and potassium sulfate (48% K_2O and 17% S), respectively, dissolved in irrigation water (fertigation) and injected into the system using venturi-type equipment, totaling 120 kg/ha of K_2O and 180 kg/ha of N. The irrigation depth was determined based on the reference evapotranspiration estimates by the method of Hargreaves & Samani (Hargreaves 1974), with the help of the System for Evapotranspiration Estimation (SEVAP) (Silva *et al.* 2005); for this purpose, an analog dry bulb thermometer was installed, next to the cultivation area, to record the maximum and minimum daily temperatures during the conduct of the experiment, with readings obtained at 9:00 am. The control of weeds in the experimental area was done within the critical competition period, comprised between the vegetative phases "V3" and "V12" (third and twelfth final leaf) of the corn crop, following the recommendation of Vargas *et al.* (2006). For this purpose, spraying was carried out with 20 L spray equipment at constant pressure, based on the selective herbicide with systemic action, Atrazine Nortox 500 SC®, with 6-chloro-N²-ethyl-N⁴-isopropyl -1,3,5-triazine-2,4-diamine (Atrazine), 500 g L^{-1} , as an active ingredient, in the dosage of 5.0 L ha^{-1} of commercial product (2.5 L ha^{-1} of Atrazine). The control of the cartridge caterpillar (Spodoptera frugiperda JE Smith) was done whenever the presence of the first insects in the area was verified, with sprays from the vegetative phase "V3", based on the insecticide Lannate® BR, having S as the active principle. -methyl N-methylcarbamoyloxy) thioacetimidate (Metomil), 215 g L^{-1} , in the dosage of 0.6 L ha^{-1} of commercial product (129 g ha^{-1} of Metomil) (MAPA 2017). At 78 days after sowing, when harvesting the ears of green corn, five plants from the useful plot of each experimental unit were cut close to the soil, separated into stem, leaves, straw, cob and green grains. Subsequently, the materials of the stem, leaves, and straw of the ear of each repetition were crushed in a forage machine, model DPM 2 Nogueira, to reduce the size of the parts and consequent improvement in the drying process. Then, all parts of the plant were dried in an oven with forced air circulation at $\pm 65 \text{ }^\circ\text{C}$, until constant mass, to determine the dry mass of stem, leaves, straw, cob, grains and total. Samples of dry material from leaves and grains were crushed in a Willey mill to determine the levels of Zn in the extract resulting from the nitric-perchloric digestion of the tissues, through atomic absorption spectrometry, according to the methodology described in Malavolta *et al.* (1997). At the end of the first experiment, soil samples, in layer 0 - 0.20 m deep, were collected in the useful area of each experimental plot to determine the Zn levels according to the methodology contained in Embrapa (1997). The data were submitted to analysis of variance (ANOVA) using the F test (Tukey) and

polynomial regression analysis. All tests were carried out at the level of 5% probability using the SISVAR® software (Ferreira 2011).

RESULTS

In the first experiment, the levels of available Zn in the soil increased linearly with the doses of Zn sulfate applied, reaching an increase of 194% in relation to the zero dose, estimated by the regression function (Table 1). The Zn doses did not influence the dry mass of stem, leaves, straw, grains and cob, but there was a linear increase in the levels of Zn in the leaves as a function of the applied doses. Levels of zinc in the leaves, estimated by the regression function, increased by 83% in the highest when compared to the zero dose. The Zn doses did not influence the Zn levels in the grains. In the second experiment, the Zn concentrations promoted a linear effect on the dry mass of stem and quadratic for the dry mass of leaves and total (Table 2).

production of dry mass of leaves, stem, straw, and cob, nor for the levels of Zn in the grains. In other studies, there was also no effect of Zn doses via soil on grain and phytomass production (Ehsanullah *et al.* 2015, Abreu *et al.* 2016; Fernandes & Dalchiavon 2019). The minimum zinc content available in the soil, obtained at zero dose of zinc sulfate (0.62 mgdm⁻³) was considered low (Alvarez V. *et al.* 1999). In turn, the leaf contents of Zn, which in this same treatment was 14.09 mg kg⁻¹, corresponded to the lower limit of the sufficiency range for maize (Malavolta *et al.* 1997). Therefore, it is possible that other Zn fractions not extracted by the Mehlich-1 extractor were absorbed by corn (Leite *et al.* 2020), especially those linked to organic matter that was in content, considered suitable (Alvarez V. *et al.* 1999). However, the high pH of the soil probably decreased the efficiency of fertilization via suns, since, considering the higher dose of Zn (10 kg ha⁻¹) compared to the zero dose, the recovery of Zn by soil analysis was only 20, 2%. In addition to the genetic factor, several soil attributes such as texture, mineralogy, pH, organic matter content and

Table 1. Zinc contents in the soil, dry phytomass of the aerial part components and Zn contents in leaves and grains of green corn AG 1051 as a function of Zn doses applied via soil

Doses of Zn (kg ha ⁻¹)	Zn in soil (mg/dm ³)	stem	leaves	straw	grains	cob	total	Zn in leaves	Zn in grains
		-----g plant ⁻¹ -----						-----g kg ⁻¹ -----	
0.00	0.62	143.03	62.14	42.50	45.16	35.37	337.65	14.09	4.96
2.50	0.81	137.31	59.24	41.55	38.88	33.04	323.45	18.70	4.99
5.00	0.90	141.82	61.34	39.43	49.49	35.85	343.17	21.60	5.01
7.50	1.46	135.37	57.46	42.15	43.08	33.57	327.67	26.03	5.08
10.00	1.63	132.96	60.79	42.85	47.87	36.48	330.10	26.05	5.11
Average	1.08	138.10	60.19	41.70	44.90	34.86	332.41	21.29	5.03
Regression parameters significance									
β_1	-	ns	ns	ns	ns	ns	ns	*	ns
B_2	*	ns	ns	ns	ns	ns	ns	-	ns
VC (%)	13.50	12.14	8.52	7.83	17.06	6.1	8.81	9.03	13.69

β_1 and β_2 –linear and quadratic parameter respectively, of the adjusted functions: linear ($\hat{y} = a + \beta_1x$) and quadratic ($\hat{y} = a + \beta_1x + \beta_2x^2$). * P<0.05; ^{ns}P>0.05. VC: variation coefficient

Table 2. Dry phytomass of the aerial part components of green corn AG 1051 as a function of Zn concentrations applied via leaf

Zn concentrations(gL ⁻¹)	stem	leaves	straw	grains	cob	total	Zn in leaves	Zn in grains	
		-----g plant ⁻¹ -----						-----g kg ⁻¹ -----	
0,0	133.15	64.64	59.73	42.66	25.68	325.86	14.60	6.12	
0,5	160.52	77.83	79.85	39.48	29.23	386.91	13.37	5.94	
1,0	162.60	74.83	71.73	40.74	27.72	377.62	19.50	5.09	
1,5	160.62	75.44	74.20	41.76	25.56	377.58	20.81	5.21	
2,0	170.13	69.34	71.17	35.10	28.14	373.88	26.79	5.01	
Average	157.40	72.42	71.34	39.95	27.27	368.37	29.81	5.47	
Regression parameters significance									
β_1	*	*	ns	ns	ns	*	**	ns	
B_2	-	*	ns	ns	ns	*	-	ns	
VC (%)	24.89	12.32	20.54	32.79	15.98	14.38	11.63	18.25	

β_1 and β_2 –linear and quadratic parameter respectively, of the adjusted functions: linear ($\hat{y} = a + \beta_1x$) and quadratic ($\hat{y} = a + \beta_1x + \beta_2x^2$). ** P<0.01; * P<0.05; ^{ns}P>0.05. VC: variation coefficient

The Zn doses did not affect the dry mass of straw, grains and cob. The stem dry matter increased by 20.8% at the highest Zn concentration. The maximum values of dry mass of leaves and total were estimated by the respective regression functions at 1.07 g/L and 0.96 g/L, respectively. The Zn concentrations promoted a linear increase in the zinc leaf contents, whose maximum estimated value in the dose of 2g/L represented an increase of 45.7% in relation to the zero concentration (Table 2). As in the first experiment (Zn via soil), Zn concentrations did not promote significant changes in the levels of this nutrient in the grains.

DISCUSSION

In the first experiment (Zn via soil), although the doses of Zn applied via soil, increased the concentration of this nutrient in the soil and in the leaves, the same did not occurred for the

microbiological activity in the rhizosphere, influence the availability of Zn for the plant after its application via fertilization (Leite *et al.* 2020). This fact can explain the divergences between the responses of corn to fertilization with Zn in different types of soils. In the second experiment (Zn via leaf) the results partly differed from those obtained in the first experiment (Zn via soil). Foliar fertilization with Zn increased the production of dry stem, leaves and total phytomass. The linear adjustment for the stem dry matter, highlights the importance of Zn in cell division and in the elongation of stalks of grasses such as corn (Marschner2012). The positive effect of leaf application of Zn on corn phytomass may also be related to a possible increase in the photosynthetic rate and, consequently, of photoassimilates as observed by Liu *et al.* (2016). In this sense, the dry mass of stem is fundamental considering that it is the organ of support and translocation of

nutrients and redistribution in the plant. Other authors have also observed a positive effect of the application of zinc via the leaf on the production of dry mass of corn (Ehsanullah *et al.* 2015, Fernandes & Dalchiavon 2019). The application of Zn via leaf increased linearly the levels of Zn in the leaves, but did not interfere in the levels of this nutrient in the grains. This can be explained by the fact that the additional supply of Zn via leaf has promoted an increase in the production of vegetative parts of corn, especially of stem, to the detriment of grain production. In other studies, no direct relationship was observed between leaf content and Zn content in grains (Wang *et al.* 2012, Gonçalves 2017), in others (Saleem *et al.* 2016, Gonçalves *et al.* 2019, Xia *et al.* 2019) increases in Zn levels were observed in dry corn grains by applying Zn via leaf and, or root. The difficulty in increasing the levels of Zn in the grains may be related to the phase of harvesting the grains, suggesting that the translocation of the nutrient stored in the vegetative tissues up to the R3 phase is not decisive for raising its levels in the grains. According to Duarte *et al.* (2003) the extraction of Zn by corn occurs until the end of the cycle, with one third or more of the total absorption occurring from the beginning of the filling of the grains until maturation. In both experiments, despite the increase of Zn in the leaves, the levels of Zn in the grains were practically not altered by zinc fertilization. On average, the accumulated amount of Zn in fresh grains in the first and second experiments was 0.93 and 0.91 mg per ear. This means that when consuming two ears of green corn, 1.8 mg of Zn would be ingested, equivalent to about 16.7% of the daily requirement in this micronutrient (FAO, 2002), although in terms of human nutrition the bioavailability ie, the concentration of Zn linked to phytates is more important than the total levels (Xia *et al.* 2019).

Conclusions

The fertilization of green corn with zinc via soil increased the concentration of this nutrient in the soil and in the leaves, but it was not able to increase the production of phytomass, nor the production of grains and the levels of Zn in fresh grains. The supply of zinc via the leaf increased the leaf Zn content, the dry mass of stem, leaves and total, but did not affect the Zn content in the green corn grains. In alkaline soils, the fertilization of green corn with Zn via leaf is more advantageous than the fertilization via soil, due to the practicality, economy and gain in phytomass production.

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