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Carbonic anhydrase in maize plants submitted to different doses of zinc

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ABSTRACT
Carbonic anhydrase (EC 4.2.1.1) is an extremely important metalloenzyme in the metabolism of plants, animals, and microorganisms, taking part in photosynthesis, the metabolism of carbohydrates, proteins and lipids, and has zinc in the structure of its active site. Zinc is a micronutrient, but also a heavy metal, which can cause benefits or toxicity to plants, depending on the amount it is absorbed. The aim of this research was to evaluate the activity of carbonic anhydrase in maize plants (Zea mays L.) grown in a nutrient solution with different concentrations of zinc. The plants were grown in a greenhouse in washed sand contained in lysimeters with a capacity for 1,300 g of sand, which received a nutrient solution without Zn and individualized additions of increasing doses of Zn as ZnSO4.7H2O. The experiment was set up in a completely randomized experimental design with 6 treatments (0, 5, 10, 20, 40 and 80 mg Zn kg-1 of substrate), with 6 replicates. Carbonic anhydrase activity increased significantly with the dose of zinc, with the highest activity at a concentration of 10 mg kg-1 of substrate. The concentration of zinc in the plant increased significantly in the root and aerial part, reaching the maximum total value (root + shoot) at the highest dose of zinc applied to the substrate. The lowest dry mass production by the corn plant occurred at the highest dose of zinc (80 mg Zn kg-1 of substrate) as a response to possible phytotoxicity and there was no significant difference between the other treatments, indicating that the Zn present in the seed was sufficient for the initial phase of corn development. Carbonic anhydrase activity did not correlate significantly with Zn accumulated in the plant and with dry mass production.

Keywords: Trace elements, Phytotoxicity, Metalloenzymes, Heavy metals, Plant nutrition.

1 INTRODUCTION

Enzymes are important biomolecules that participate in the regulation of the metabolism of living beings, and among them is carbonic anhydrase (EC 4.2.1.1), an enzyme that contains zinc at the active site and participates in the metabolism of carbohydrates (such as photosynthesis), proteins, and lipids (KUDENKO et al., 2020). Under conditions of low activity, there is a decrease in plant development and even in its death.

Zinc is one of the micronutrients that most seem to limit corn yield in Brazil. Several studies have shown the response of zinc addition to yield (GOEDERT, 1987). In soils under cerrado vegetation, there is usually a low content of this element, usually less than 1 mg kg-1 of soil, which is considered a critical level for the good development of plants (LOPES, 1984).

Zn deficiency may also occur when high doses of lime are used (BARBOSA FILHO et al., 1990). The activity of carbonic anhydrase can also be altered in other unfavorable conditions that may cause stress in the plant.

In soils with deficiency or excess of zinc, there may be alteration of the activity of carbonic anhydrase, with damage to the development and production of the plant.

Early diagnosis of zinc deficiency, especially for annual crops, can help correct the deficiency in a timely manner, reducing yield losses.

Zn is an activator of carbonic anhydrase, an enzyme that catalyzes the CO2 hydration reaction, producing H+ and $HCO3^{-}$, acting on the electrolyte balance of the cell and allowing the conversion of the amino acid tryptophan into auxin, an important plant hormone (QUEIROZ, 1993).

The objective of this study was to evaluate the effect of zinc doses under nutrient solution conditions, in a greenhouse, on carbonic anhydrase activity and on the initial development of maize plants.

2 MATERIAL AND METHODS

The experiment was carried out in a greenhouse in a completely randomized design with 6 treatments (0, 5, 10, 20, 40 and 80 mg Zn $^{kg-1}$ sand) and 5 replications.

The maize plants (hybrid Braskalb XL 67) were sown in a substrate consisting of washed sand contained in lysimeters with a capacity of 1,300 g, which received complete nutrient solution, minus Zn (lysimeter capacity of 250 mL of nutrient solution) with different concentrations of Zn.

The nutrient solution used contained 0.43 g of KNO3, 0.12 g of K2HPO4, 0.20 g of CaCl2.2H2O, 0.11 g of MgSO4.7H2O, 0.96 g of H3BO3, 1.97 g of CuSO4.5H2O, 3.08 g of MnSO4.4H2O, 0.09 g of NaMo4.2H2O, 24.9 g of FeSO4.7H2O and 33.2 g of EDTA-Na in each liter of the nutrient solution.

On the day of sowing, 250 mL of nutrient solution and doses of zinc in the form of ZnSO4.7H2O were added to each lysimeter in order to reach the Zn concentration of each treatment, and then 4 corn seeds were sown per lysimeter.

Daily, until the fourteenth day after sowing, irrigation with distilled water was carried out in order to replace the water lost by evapotranspiration. After this period, 20 mL of the nutrient solution were added daily to each lysimeter for a period of 7 days. Water lost by evapotranspiration was replenished daily with distilled water until the end of the experiment.

At 30 days after sowing, the lysimeters were dismantled and the plants were separated from the sand and divided into shoots and roots. One of the plants of each lysimeter was immediately sent to the laboratory for determination of carbonic anhydrase activity, and the others were washed with running water, distilled water and deionized water, dried in an oven with forced air circulation maintained at a temperature of 50 ± 5 ºC for 72 hours and ground in a Willey mill with stainless steel knives and sieves (40 mesh mesh).

The samples were opened with a digestive solution composed of nitric and perchloric acids in a proportion of 3/1 to determine the Zn content by an air-acetylene atomic absorption spectrophotometer.

The determination of anhydrase activity was carried out according to the methodology proposed by Davis (1963) with modification in the determination of the CO2 reading in the hydration reaction medium.

Shoot samples (leaves) were cut into 1 cm diameter pieces to facilitate maceration, while root samples were cut into 1 cm long pieces.

In a test tube calibrated to a volume of 2 mL, at the bottom of the tube, 3 mL of veronal buffer – 0.02 mol $^{L-1\ NaOH,}$ pH 8 (diethyl barbiturate acid), 2.3 mL of 0.018% peptone solution, 0.12 mL of bromothymol blue in 0.2% ethanol were placed. To this solution, 2 mL of plant extract obtained by maceration of 2 g of fresh sample in a volume of 10 mL of deionized water were added, which was maintained at 0 $^{oC\ until\ the\ moment}$ of use.

After the addition of the plant extract to the test tube, 1 mL of carbonated water (saturated in CO2) was immediately added, when the change from blue to yellow color began, which went up from the bottom of the test tube. When the color reached the 2 mL mark in the test tube, the time spent in seconds was recorded, which was used to express the carbonic anhydrase activity.

The results obtained were submitted to analysis of variance, and when the F value was signified at 5% probability, Tukey's test ($p<0.05$) was used to compare treatment means. Pearson's linear correlation analyses were also performed.

3 RESULTS AND DISCUSSION

The proof that Zn is essential for higher plants was made by Maize in 1919 in an experiment in nutrient solution and its deficiency in corn plants was first observed by Barnette and collaborators in 1936 (THORNE, 1957).

Zn participates in the formation of biomolecules such as some carbohydrates and proteins, chlorophyll and hormones such as auxins, important regulators of plant growth, such as stem elongation. In its deficiency, the plant can stop growing. Zinc is a trace metal element (Zn2+), which is part of the constitution of carbonic anhydrase, an enzyme that catalyzes the hydration of carbon dioxide with the production of H+ proton and bicarbonate ($HCO3^-$).

The accumulated Zn increased significantly in maize plants with the dose of the nutrient applied to the substrate used for cultivation (Table 1, Figure 1), consisting of washed sand that received doses from 0 to 80 mg Zn kg-1. Levels much higher than the level considered critical for Brazilian soils (evaluated by Mehlich 1 extractors, DTPA at pH 7.3 and HCl 0.1 mol L-1), which is 1 mg Zn kg-1 soil (RIBEIRO and SANTOS, 1996).

Table 1. Zinc in root, shoot and total (mg $^{plant-1}$) in maize plants grown in washed sand substrate irrigated with nutrient solution and receiving increasing dose of zinc.

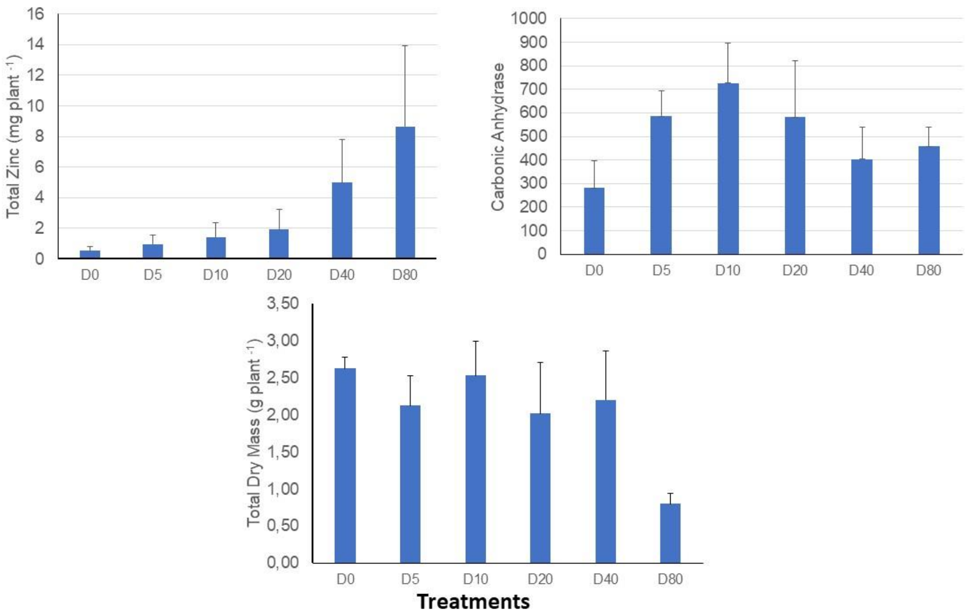
Treatment	Root	Aerial part	Total
D0	0.26 ± 0.13 B	0.28 ± 0.12 C	0.54 ± 0.24 B
D5	0.61 ± 0.57 B	0.35 ± 0.05 AB	0.96 ± 0.58 B
D10	0.92 ± 0.84 B	0.50 ± 0.13 AB	1.42 ± 0.98 B
D20	1.33 ± 1.20 B	0.62 ± 0.22 AB	1.96 ± 1.25 B
D40	3.53 ± 3.04 AB	1.45 ± 0.33 B	4.98 ± 2.85 B
D80	5.57 ± 5.46 A	3.09 ± 0.59 A	8.66 ± 5.25 A

D0= control (no zinc added), D5, D10, D20, D40 and D80 = treatments that received doses of 5, 10, 20, 40 and 80 mg Zn $^{kg-1}$ substrate. Means followed by the same capital letter in the same column did not differ from each other according to the Tukey test ($p<0.05$). Means are followed by the standard deviation.

The Zn content in the seed varies with the species, the variety, and the soil and climatic conditions of where the plant was grown. In maize plants, evaluations in 49 lines revealed concentrations ranging from 15.5 to 70 mg kg^{-1} Massa seca (MASSEY and LOEFFEL, 1966, HINESLY et al., 1978).

This high concentration of Zn in the seed is one of the possible explanations for the fact that the production of total dry matter up to the dose of 40 mg Zn kg-1 substrate, at this stage of development, did not differ significantly from the control treatment, which did not receive Zn, and from the treatments that received up to 40 mg Zn kg-1 of substrate (Figure 1, Table 2).

Figure 1. Zinc content (mg plant-1), carbonic anhydrase activity (second 2 mL-1 solution) and total dry matter production (g plant-1) in maize plants grown in nutrient solution containing increasing doses of Zn. D0, D5, D10, D20, D40 and D80 represent doses of 0, 5, 10, 20, 40 and 80 mg kg-1 Zn in the substrate. Lines over the bars indicate the standard deviation of the mean.



The production of total dry matter, roots and shoots by corn plants was significantly affected by the treatments. In all cases, the production was lower in the treatment that received the dose 80 mg Zn kg^{-1} substrate (Table 2 and Figure 1).

Therefore, the higher dose of Zn applied to the substrate had a negative effect on plant development. This negative effect may have been caused by the reaction of the element with some biomolecule important for metabolism. Another possible cause is competition in the absorption of other nutrients with phosphorus, iron, copper, and manganese.

Table 2. Dry mass of maize plants, g plant-^{1}, cultivated in washed sand substrate irrigated with nutrient solution and receiving increasing doses of zinc.

Treatment	Root	Aerial part	Total
D0	0.93 ± 9.09 A	1.70 ± 0.16 A	2.63 ± 0.15 A
D5	0.75 ± 0.24 AB	1.38 ± 0.18 AB	2.13 ± 0.40 A
D10	1.09 ± 0.39 A	1.45 ± 0.14 AB	2.53 ± 0.46 A
D20	0.71 ± 0.33 AB	1.31 ± 0.38 AB	2.02 ± 0.69 A
D40	0.93 ± 0.25 A	1.27 ± 0.20 B	2.19 ± 0.67 A
D80	0.28 ± 0.05 B	0.52 ± 0.10 C	0.80 ± 0.14 B

D0= control (no zinc added), D5, D10, D20, D40 and D80 = treatments that received doses of 5, 10, 20, 40 and 80 mg Zn $^{kg-1}$ substrate. Means followed by the same capital letter in the same column did not differ from each other according to the Tukey test ($p<0.05$). Means are followed by the standard deviation.

Despite the negative effect of high doses of Zn, the plants did not show symptoms of phytotoxicity, characterized by smaller leaf size, chlorosis of the vein, necrosis at the tips, reduced root development.

Carbonic anhydrase is an enzyme that catalyzes the reversible hydration of carbon dioxide, giving rise to proton (H+) and bicarbonate ($HCO3^-$), products that participate in numerous metabolic pathways such as gluconeogenesis, lipogenesis, ethylene synthesis and many others in higher plants and algae and help maintain electrolyte balance and pH (RUDENKO et al., 2020).

The carbonic anhydrase activity increased with the dose of Zn applied, reaching a maximum value at the dose of 10 mg Zn $^{kg-1}$ substrate, to decrease soon after (Figure 1, Table 3). At doses 5, 10 and 20 mg Zn $^{kg-1}$ substrate, carbonic anhydrase activity was significantly higher than the control treatment.

Rahimi and Schropp (1985) found low carbonic anhydrase activity in plants with Zn deficiency.

Wood and Sibly (1952) reported that oat plants treated or not with Zn (0.2 mg $^{L-1}$) showed similar carbonic anhydrase activity up to 39 days after sowing (DAS) and then decreased until the end of the experiment, always being superior in the treatment that received Zn.

Table 3. Carbonic anhydrase activity, expressed in seconds 2 mL-1 of solution, and zinc absorbed by maize plants, plant -1, cultivated in washed sand substrate that received nutrient solution with increasing doses of zinc.

Treatment	Carbonic Anhydrase	Absorbed Zn
D0	281.78 ± 114.66 C	0.54 ± 0.24 B
D5	584.72 ± 107.93 AB	0.96 ± 0.58 B
D10	726.64 ± 168.30 A	1.42 ± 0.98 B
D20	583.60 ± 236.84 AB	1.96 ± 1.25 B
D40	403.70 ± 134.69 BC	4.98 ± 2.85 B
D80	457.62 ± 83.49 ABC	8.66 ± 5.25 A

D0= control (no zinc added), D5, D10, D20, D40 and D80 = treatments that received doses of 5, 10, 20, 40 and 80 mg Zn $^{kg-1}$ substrate. Means followed by the same capital letter in the same column did not differ from each other according to the Tukey test ($p<0.05$). Means are followed by the standard deviation.

In oats grown in a solution containing Zn (0.2 mg $^{L-1}$) or not, the nutrient content in the leaves was similar until 21 days after sowing (DAS), increased until 49 days and decreased until the end of the experiment, being higher in the treatment with Zn (WOOD and SIBLY, 1952).

4 CONCLUSIONS

Carbonic anhydrase activity increased significantly with zinc dose up to a concentration of 10 mg Zn kg^{-1} substrate, when it has reached its maximum activity.

The zinc concentration in the plant increased significantly, reaching the maximum value at the highest dose.

The lowest dry matter production by the maize plant occurred at the highest dose of zinc (80 mg Zn kg^{-1} and there was no difference between the other treatments, indicating that the zinc present in the seed is apparently sufficient for the initial phase of corn development.

The carbonic anhydrase activity was not significantly correlated with the zinc content and dry matter production by the corn plant.

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