

FOLIAR FEEDING WITH ZINC AS A BIOFORTIFICATION STRATEGY IN MAIZE

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ABSTRACT Human zinc malnutrition is a concern in the contemporary scenario. Ranked among the serious micronutrient deficiencies and considered a major cause of child mortality, especially in developing countries. Cereals, such as maize (*Zea mays* L.), food source in these countries, show low zinc concentration. In view of this, biofortification has been shown promise to increase the nutrient content in staple foods and to improve their nutritional quality. The aim of this study was to evaluate the potential of maize genotypes in relation to yield and zinc concentration in grains and leaves as a function of zinc sulfate doses in two growing seasons. A total of 17 commercial hybrids were evaluated in three doses (0, 5, and 10g L⁻¹ ha⁻¹). The zinc concentration was quantified by acid digestion, followed by atomic absorption spectrophotometer analysis. Any change was observed in grain yield as a function of the doses. Higher doses applied near flowering resulted in higher concentrations of the micronutrient in the grains. However, the concentrations in the grains were, on average, below the value established by the Harvest Plus biofortification program. The results provide information for the selection of genotypes with zinc uptake potential aiming at food safety through agronomic biofortification.

Keywords: *Zea mays* L, zinc deficiency, micronutrients, food safety, plant nutrition.

ADUBAÇÃO FOLIAR COM ZINCO COMO ESTRATÉGIA DE BIOFORTIFICAÇÃO EM MILHO

RESUMO A desnutrição humana por zinco é uma preocupação no cenário contemporâneo. Classificada entre as graves deficiências de micronutrientes, sobretudo em países em desenvolvimento, ela é considerada uma das principais causas da mortalidade infantil. Cereais como o milho (*Zea mays* L.), fonte de alimento nesses países, apresenta baixa concentração de zinco. Frente a isso, a biofortificação tem se mostrado promissora para incrementar o teor de nutrientes em alimentos básicos e melhorar a sua qualidade nutricional. O objetivo desse estudo foi avaliar o potencial de genótipos de milho quanto à produtividade e a concentração de zinco nos grãos e nas folhas, em função de doses de sulfato de zinco, em duas épocas de cultivo. Foram avaliados 17 híbridos comerciais, em três doses (0, 5 e 10g L⁻¹ ha⁻¹). A concentração de zinco foi quantificada por meio da digestão ácida, seguida de análise via espectrofotômetro de absorção atômica. Observou-se que não houve alteração na produtividade de grãos em função das doses. Maiores doses aplicadas próximo ao florescimento resultaram em maiores concentrações do micronutriente nos grãos. No entanto, as concentrações nos grãos ficaram, em média, abaixo do valor estabelecido pelo programa de biofortificação Harvest Plus. Os resultados fornecem informações para seleção de genótipos com potencial de absorção de zinco, visando à segurança alimentar por meio da biofortificação agrônômica.

Palavras-chave: *Zea mays* L, deficiência de zinco, micronutrientes, segurança alimentar, nutrição vegetal.

The world population is estimated to reach 9.1 billion people in 2050. In order to meet this food demand, world food production will have to grow by approximately 70% (FAO, 2009). Despite the gradual increase in grain yield recorded in recent years due to plant breeding and technologies associated with phytotechnical management, their relationship is inversely proportional to the micronutrient content found in them (Garvin et al., 2006). The lack of micronutrients at the minimum desirable doses in cereals, such as rice, wheat, beans and maize, the main sources of nutrients in developing countries, can contribute to malnutrition problems, hence leading to infant mortality.

Malnutrition by zinc (Zn) in humans is considered a major cause of infant mortality and is classified as one of the most serious micronutrient deficiencies, affecting approximately one third of the world population. The Zn, in turn, is related to the activity of more than 300 enzymes and acts as a cofactor in thousands of proteins, including transcription factors. The major consequences of its deficiency on the human body are damage to brain functions, immune system and physical growth (Cakmak et al., 2010).

To ensure food safety, the food production must be essentially sufficient not only in quantity but also in nutritional quality. It is estimated that at least 50% of the world's grain areas are under Zn-deficient soils, which reduces the yield and nutritional quality of cereals grown in these areas. In Brazil, similar conditions are found mainly in the savanna. In order to overcome this scenario, agronomic biofortification has shown to be a promising technique, aiming at increasing the selected micronutrient content in staple foods.

Biofortification of cereals can be performed by applying micronutrients in the soil, in the leaves

(foliar feeding), in the seeds, or by fertigation. When applied via leaf, the probability of micronutrients being transported to fruits and other growing regions is greater than when the application is performed via soil or seeds (Welch, 1995).

Therefore, the aim of this study was to evaluate the potential of maize genotypes for grain yield and Zn accumulation in grains and leaves as a function of Zn sulfate doses applied via leaf in different growing seasons.

Materials and Methods

Experimental area

The experiments were conducted in two growing seasons (December/2015 and January/2016), at the Center for scientific and technological development of the Federal University of Lavras, in the city of Lavras - Minas Gerais, Brazil. The soil of the experimental area was classified as red-yellow latosol and showed high concentrations of Zn^{2+} (4.5 mg dm^{-3}) in the 0-20 cm layer.

Seeding was performed under direct seeding system. At the date in question, 350 kg ha^{-1} of the formulation 08-28-16 (N, P_2O_5 , K_2O) + 0.5% Zn were used. The topdressing fertilization (phenological stage V5) was performed with urea ($140 \text{ kg of N ha}^{-1}$). Pest and weed control were performed according to the technical recommendations for the crop.

Treatments and experimental design

A total of 17 commercial hybrids of maize and three doses of Zn sulfate (0, 5, and $10 \text{ g L}^{-1} \text{ ha}^{-1}$) were evaluated in two growing seasons, in the factorial design with three replicates. The experimental design was the complete randomized blocks. The plots

consisted of four rows of 5m each, spaced 0.60 m apart, with a density of four plants per linear meter and a population density of approximately 65,000 plants ha⁻¹. In order to collect data and observations, the two central lines were considered as a useful plot.

Foliar application of zinc sulfate (ZnSO₄·7H₂O) was performed with CO₂-pressurized backpack sprayer in the morning to avoid product evaporation. The treatments consisted of: D₀ - plots that did not receive any foliar application of Zn sulfate (Control - 0 g L⁻¹ ha⁻¹); D₅ - foliar application of Zn sulfate at the vegetative stage V5 (5g L⁻¹ ha⁻¹); and D₁₀ - foliar application of Zn sulfate in the vegetative stages V5 (5g L⁻¹ ha⁻¹) and reproductive R1 (5g L⁻¹ ha⁻¹), totalizing a dose of 10g L⁻¹ ha⁻¹.

Assessments

At 73 days after emergence, in order to avoid the leaf's residual effect application made in R1, and when 50% of the plants of the plot already had emission of the tassel and style-stigma, five plants were selected for the collection of the opposite leaf and below the upper ear, considering the middle third and excluding its midrib, according to the recommendations of Martinez et al. (1999) for Zn quantification on the leaves. When the plants reached physiological maturity, the ears from the useful area of each plot were harvested and the grain samples were corrected to 13% moisture. The grain yield and their respective Zn concentrations were evaluated.

In order to quantify Zn contents in leaves and grains, nitro-perchloric extraction was performed in a digester block, as described by Malavolta et al. (1997). After the digestion, Zn concentrations were determined by atomic absorption spectrophotometer by flame atomic absorption spectroscopy (FAAS).

Samples were analyzed in triplicate, using a standard sample NIST SRM 1573a - Tomato leaves as reference for concentrations of elements in plants and a blank sample for analytical quality control purposes.

Statistical analysis

The Zn concentration on the leaves and grains and the grain yield data, at each growing season, were submitted to individual and joint analysis of variance (ANOVA). When there was significance among treatments, the Scott-Knott grouping test with 5% significance level or regression analysis was performed.

For the leaf Zn characteristic, the data were transformed aiming at reducing the existing variability and normalizing the residues, according to the expression proposed by Box and Cox (1964): , where: $\lambda = -0.5$.

However, the averages of these analyses were expressed without transformation, using the inverse operation of the previous expression (Banzatto & Kronka, 1995). All the statistical analyses were performed in the SISVAR 5.1 software (Ferreira, 2011).

Results and Discussion

Grain yield

The effect of growing seasons, hybrids and Zn sulfate doses significantly influenced grain yield (p-value < 0.05). There was a 39.3% reduction in grain yield between December (with an average yield of 10768 kg ha⁻¹) and January (6532 kg ha⁻¹) crops. This fact was expected due to the more favorable climatic conditions in the December crop for maize, with a regular distribution of rains (average rainfall

of 177.85 and 84.20 mm for December and January, respectively), especially in the bolting period until begins the filling of grains (Bergamaschi et al., 2004). The water deficit, during critical development periods of the maize plant in the second growing season, certainly contributed to a reduction in grain yield.

The effect of hybrids was significant for yield (p -value < 0.05). According to the Scott-Knott means grouping test, the different maize genotypes were divided into two groups. This fact occurred due to the genetic superiority of the higher average hybrids, which showed greater adaptability and phenotypic stability, as well as greater resistance to biotic and abiotic stresses.

Although the effect of doses was significant for grain yield (p -value < 0.05), Zn sulfate had little influence on it, since the control treatment ($0 \text{ g L}^{-1} \text{ ha}^{-1}$) was not statistically different of the highest dose of applied Zn sulfate ($10 \text{ g L}^{-1} \text{ ha}^{-1}$) by the Scott-Knott test (Table 1). However, due to Zn's performance in plant development, as in the tryptophan production, precursor of indole-3-acetic acid, plant hormone growth promoter, some studies have shown positive responses to the application of Zn in maize culture (Galvão, 1994).

In the literature, foliar application of Zn sulfate provided a positive response in grain yield with the

application of 1% Zn sulfate solution (23% Zn) and 0.75% of the same solution in soils with Zn contents varying from low to medium, respectively (Galvão, 1996; Rastija et al., 2002).

In the present study, the lack of response in grain yield due to leaf Zn application can be justified because the Zn present in the soil is within the levels considered as relatively high for the crop supply, being sufficient to meet the demand of the plant during the whole cycle (Sousa & Lobato, 2004). Jamami et al. (2006) also found no positive response to Zn application in soils with high micronutrient contents, corroborating the results found in this study.

Due to the low Zn mobility in the phloem, the application of Zn sulfate via foliar does not always result in gains in yield, being often the application via soil more efficient, especially broadcasting (Galvão, 1996). According to Marschner (1995), the translocation of Zn in the plant depends on its availability in the vegetative part. On the other hand, the lack of response to foliar spraying may occur due to the preference for post-flowering applications, since late applications have a greater impact on Zn concentration in the grain, with lower impact on yield (Cakmak et al., 2010).

According to Joy et al. (2015), high yield responses due to the Zn application ($> 150\%$ higher than the control) occur in soils with severe micronutrient deficiency. In these cases, there is also a high Zn concentration in the grain, making it clear that the yield response is highly dependent on the soil Zn content.

Leaf Zn concentration

The effect of growing seasons, Zn doses and the interaction of seasons \times sulfate doses were significant

Table 1 - Average yield of maize grains as a function of Zn sulfate doses ($0, 5, \text{ and } 10 \text{ g L}^{-1} \text{ ha}^{-1}$) considering the two growing seasons and hybrids.

Dose (g L^{-1})	Yield ¹ (Mg ha^{-1})
0	8.87a
5	8.25b
10	8.83a

¹Averages followed by the same letter does not differ among themselves by the Knott means grouping test at 5% probability.

for Zn foliar concentration (p-value <0.05). However, regardless of the dose of applied sulfate, leaf Zn concentrations were lower in the first growing season (Table 2). This fact can be justified due to the higher rainfall at December crop, consequently promoting a greater translocation of nutrients to the grains, called “dilution effect”, resulting in yield gains, as verified.

In general, the leaf Zn concentration increased with the applied doses of Zn sulfate. At the dose D_0 (0 g L⁻¹ ha⁻¹), the evaluated hybrids showed, on average, leaf Zn concentrations within the range established for maize, which according to Malavolta et al. (1997) and Martinez et al. (1999), should be between 20 and 70 mg kg⁻¹ Zn. As reported, the high concentration may be resulting from the high micronutrient content present in the soil. On the other hand, at dose D_{10} (10 g L⁻¹ ha⁻¹), values up to 165.47 mg kg⁻¹ Zn were obtained (Figure 1). Decaro et al. (1983) studied the effect of doses (0, 5, 10, and 15 kg ha⁻¹) and Zn sources (sulfate and oxide) applied via soil and verified increases in leaf Zn concentrations and that the dose of 5 kg ha⁻¹ was sufficient to promote an adequate concentration of the micronutrient in the crop.

When the highest dose of Zn sulfate (10 g L⁻¹) was applied, no visible anomaly in the plants or damage in grain production was found. Amaral et al. (1996) observed similar results, where concentrations up to 322.0 mg kg⁻¹ of leaf Zn did not cause phytotoxicity in maize. However, high levels of Zn in leaves (165.47 mg kg⁻¹) are considered as sufficient to promote the appearance of visual toxicity symptoms (Jones et al., 1991).

Leaf applications of Zn sulfate at the dose of 10g L⁻¹ plotted in stages V5 and R1, conditioned the highest concentrations of the micronutrient in the leaves for both seasons. On the other hand, doses of 0 and 5g L⁻¹ leaf Zn sulfate provided similar

concentrations and maintained within the sufficiency range (20-70 mg kg⁻¹) (Malavolta et al., 1997; Martinez et al., 1999).

No significant correlations were found between leaf Zn concentrations at 73 days after emergence with grain production ($r = -0.16$; $\alpha = 0.05$), and therefore this variable should not be used as a predictive measure of harvest level. Ferreira et al. (2001) found similar results. According to the authors, increases in leaf Zn concentrations were observed as the reproductive stages approached. However, this fact did not result in yield gains probably due to the high Zn content in the soil (0.713 mmol dm⁻³ of Zn²⁺).

Concentration of Zn in the grain

The effects of hybrids, Zn doses and the hybrid \times doses \times seasons interaction influenced the Zn concentrations in maize grains (p-value <0.05). On the other hand, the hybrids submitted to the control (0 g L⁻¹) treatment did not present significant differences among the analyzed periods. In general, the Zn concentrations in the grains were higher when the dose of 10 g L⁻¹ sulfate was applied.

When the 0 g L⁻¹ dose was compared with the mean effects of leaf Zn application on Zn concentration in the grains, an increase of 17% was observed above the control. Joy et al. (2015), however, conducted a systematic review of the literature for studies on the impact of Zn fertilization and its concentrations on maize grains, and concluded that the average effects micronutrients applied via leaf on grain concentration were 30% above the control.

A low coefficient of correlation among Zn concentrations in leaf tissues at 73 days after emergence and their respective grain concentrations ($r = 0.28$; $\alpha = 0.05$) was observed, suggesting that the

Table 2 - Average leaf Zn concentrations (mg kg⁻¹) as a function of growing seasons and Zn doses.

Doses (g L ⁻¹)	Seasons ¹	
	December 2015	January 2016
0	18.14 b	67.66 a
5	18.25 b	47.59 a
10	88.50 b	165.47 a

¹Averages followed by the same letter on the line does not differ among themselves by the Knott means grouping test at 5% probability.

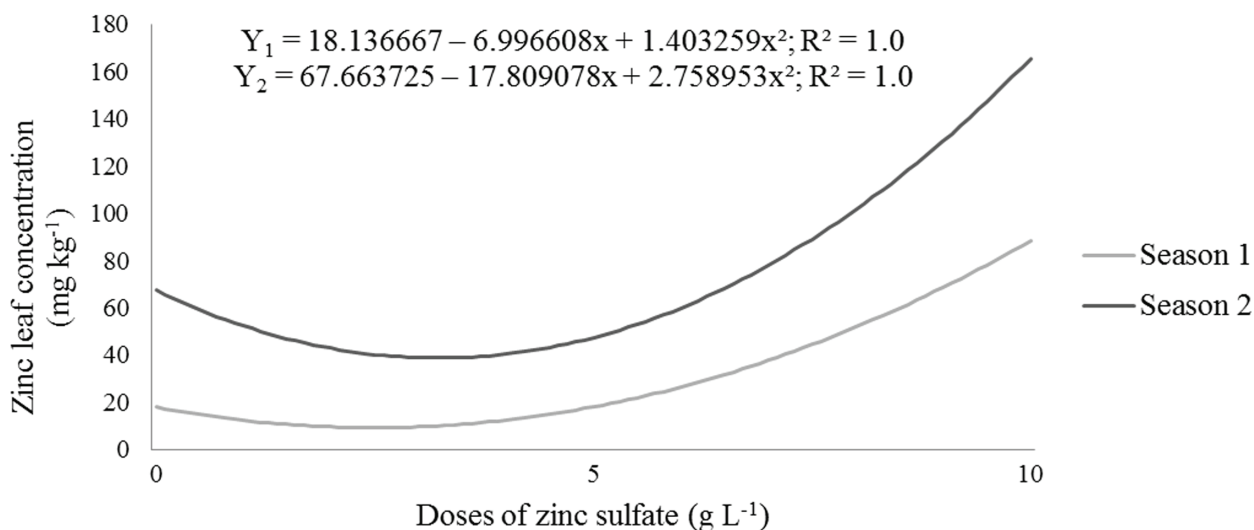


Figure 1 - Regression for leaf Zn concentration (mg kg⁻¹) as a function of the doses for each growing season (E₁ - December/2015, E₂ - January/2016).

translocation of the nutrient stored in tissues up to this period was not determinant for the elevation of their grain contents. This fact can be justified since the Zn extraction by maize occurs until the end of the cycle, with one third or more of the total absorption occurring from the beginning of grain filling until maturation (Duarte et al., 2003). These results comply with the reports of Cakmak et al. (2010), where leaf sprays, performed post-flowering, caused a greater impact on the Zn concentration in the grains.

On average, the Zn concentrations in the grains showed by the hybrids were below the target value (40 mg kg⁻¹) established by the biofortification program,

Harvest Plus. However, it is believed that the Zn application in later phenological stages can promote a higher Zn concentration in the grains due to the high translocation of nutrients during their filling phase.

The Zn mobility in the phloem depends on its availability in the vegetative part, since when in higher concentrations, this micronutrient can be bound to organic compounds of low molecular weight (Marschner, 1995).

Foliar applications of Zn have become an effective strategy to increase Zn concentrations in the grains, since the micronutrient absorbed by the leaf epidermis, remobilized and transferred to the grain

through the phloem, avoiding its fixation in the soil (Fernández & Eichert, 2009). Fahad et al. (2015) obtained significant results on yield and concentration of Zn in grains (51.5 mg kg^{-1}), using leaf application with Zn sulfate ($0.5 \text{ kg Zn ha}^{-1}$) in the bolting phase. On the other hand, Borges (2006) highlights that the accumulation of micronutrients such as Zn in the shoot of maize cultivars was almost nil until the 29 days after emergence, reaching the maximum accumulation after 100 days, thus coinciding with the filling of grains.

The application of fertilizers with Zn via soil and/or leaf are able to increase the micronutrient concentration in the grain between two to three times. Superior results are obtained with the application of Zn sulfate due to its high solubility when compared to Zn oxide (ZnO) and ZnEDTA. As observed in the present study and reported by Cakmak et al. (2010), two distinct leaf applications with 0.5% (p/v) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ before or after bolting are effective in increasing grain concentration, being the applications realized in the later stages of growth, responsible for the greatest concentrations.

Finally, corroborating to the results found in the present study, data related to yield are important to exclude the “concentration effect”, since low grain yields can lead to higher Zn concentrations in the grains, inasmuch as Zn absorbed by plant is distributed to smaller grains or to smaller quantities of grains.

Conclusions

The grain yield did not change as a function of the doses of Zn sulfate. The cultivation performed in the second season and higher doses of Zn sulfate provided higher leaf Zn concentrations in maize

hybrids. The Zn concentration in the grains varied according to the hybrids, the doses of Zn sulfate and the growing season. Higher doses of Zn applied near flowering resulted in higher micronutrient concentrations in the grains. The Zn concentration in the grains from the evaluated hybrids was, on average, below the target value established by the Harvest Plus biofortification program. The results provide information for the selection of genotypes with the highest Zn uptake potential aiming at food safety through agronomic biofortification.

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