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Enrichment of bean seeds (*Phaseolus vulgaris L.*) using zinc (Zn) through foliar application

Enriquecimento de sementes de feijão (Phaseolus vulgaris L.) através da aplicação foliar de zinco (Zn)

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Abstract: Increasing the concentration of micronutrients in harvested products, especially zinc, is a growing challenge for agriculture, with the possibility of bringing benefits to human health and crop productivity. Therefore, this paper aimed to: (i) elucidate the effect of foliar application of zinc, starting at flower anthesis as a method to increase the content of the micronutrient in bean (Phaseolus vulgaris L.) grains in a substrate with appropriate content of the micronutrient, (ii) the use of different sources of zinc applied in a foliar via, to establish the best basis for increasing the micronutrient content in common beans, and (iii) the determination of the most suitable doses of zinc, applied by foliar spraying, to increase the nutrient content of common bean plants. Based on the results obtained, we concluded that the foliar application of zinc carried out periodically from the floral anthesis can increase the content of the micronutrient in bean grains. The most viable source for the enrichment of bean grains is zinc sulfate and all doses tested (125, 250, 375 and 500 g Zn ha^{-1}) applied via foliar were efficient for the enrichment of bean grains concerning the control (0 g Zn ha^{-1}).
Keywords: Biofortification. Grain. Beans.

Resumo: O aumento da concentração de micronutrientes nos produtos colhidos, especialmente de zinco é um desafio crescente para a agricultura, com possibilidade de trazer benefícios à saúde humana e à produtividade da cultura. Assim sendo, objetivou-se: (i) elucidar o efeito da aplicação foliar de zinco, a partir da antese floral como método para incrementar o teor do micronutriente nos grãos de feijão (*Phaseolus vulgaris* L.) em substrato com teor adequado do micronutriente (ii) utilização de diferentes fontes de zinco aplicadas via foliar visando estabelecer melhor fonte para aumento do teor do micronutriente no grão de feijoeiro e (iii) determinação das doses de zinco mais adequadas a serem aplicadas através da pulverização foliar visando o aumento do teor do nutriente no grão de plantas de feijão. Com base nos resultados obtidos, concluiu-se que a aplicação foliar de zinco realizada periodicamente, a partir da antese floral é capaz de aumentar o teor do micronutriente em grãos de feijão. A fonte mais viável para o enriquecimento de grãos de feijão é o sulfato de zinco e todas as doses testadas (125, 250, 375 and 500 g Zn ha^{-1}) aplicadas via foliar na forma de sulfato de zinco foram efetivas para o enriquecimento de grãos de feijão, em relação ao controle (0 g Zn ha^{-1}).
Palavras-chave: Biofortificação. Grão. Feijão.

1 INTRODUCTION

The current model of agricultural production increasingly requires the use of technological tools to improve productivity and the quality of harvested products. This use benefits society, and generates an agribusiness aware of ecological issues, which will boost income for rural producers and guarantee food security for the population (LEACH *et al.*, 2012). The increased concentration of micronutrients, especially zinc, in products harvested is a growing challenge for agriculture with the potential to bring benefits to human health and crop productivity.

Zinc deficiency causes challenges for crop yields (CAKMAK, 2008), as soils poor in Zn are very common all over the world, and in Brazil. An increase of Zn content in seeds and grains can generate agronomical improvements (vegetative growth and crop productivity) in plants from these seeds, also bringing benefits to human health (PALMGREN *et al.*, 2008). Zinc deficiency affects the development of roots (FAGERIA, 2004) causing reduction of crop growth and yield (EPSTEIN AND BLOOM, 2005). Improvement of Zn-enriched seeds generates better commercial input for the seed producing areas as well as for farmers using these seeds. In this context, the periodic application of Zn is an agricultural practice that presents the potential to increase micronutrient content in grains and seeds.

In addition to agronomic benefits, the biofortification of grains, which is the process of enriching the nutritional content of the crop while developing in the field for human consumption, promotes a sustainable solution for increasing nutrition in the world (JEONG AND GUERINOT, 2008). Micronutrient deficiencies in humans, especially in the case of Zn, have caused serious health problems for over more than 2 billion people worldwide (VELU *et al.*, 2014). A study conducted by Hotz and Brown (2004) reported that Zn deficiency affects a third of the world population, with the occurrence of cases of Zn deficiency ranging from 4 to 73% of the population, in different countries. This deficiency leads to severe complications in human health, including problems in physical growth, and problems with the immune system and learning ability, combined with an increased risk of infection, DNA damage, and development of cancer (HOTZ AND BROWN, 2004). Insufficient Zn intake during pregnancy can also cause slower brain development of the foetus (HAFEEZ, 2013).

For a measurable biological impact on human health, Zn content in wheat must be increased by at least 10 mg kg^{-1}, to a value of 400 g per day for adult women in countries such as India, where whole-wheat flour is used to make food (PFEIFFER AND MCCLAFFERTY, 2007).

The increase in zinc concentration in seeds leads to higher crop yields from these seeds and improvement of human health (CAKMAK, 2008). Enriching seeds with zinc, especially in crops that are primary sources of food for human consumption, such as beans, is a technique that can reduce health problems arising from Zn deficiency in humans. The high consumption of cereal-based foods with low concentrations and bioavailability of Zn is one of the main reasons why zinc deficiency is so common in humans (PRASAD, 1984).

In addition, the method replaces the application of nutrients in seeds and soil cover, as it does not involve osmotic and fixation problems. This practice can also bypass the genetic improvement strategy for biofortification, as crop breeding to develop plants with better nutritional content is an effort at long-term improvement that requires extensive germplasm screening, crossbreeding and development, as well as performance tests in target areas under different environmental conditions (CAKMAK, 2008). Finally, it avoids problems of contamination of the soil by excessive fertilization containing Zn.

Therefore, the purpose of this study was to shed light on the effect of foliar application of Zn, from floral anthesis, as a method to increase the micronutrient content of bean grains (*Phaseolus vulgaris* L.). We also sought to investigate the use of different sources of Zn in order to establish the best source for increasing the micronutrient content in the common bean, and to determine the most suitable doses of Zn to be applied by foliar spraying in order to enrich the nutrient content in the grain.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL AND GROWING CONDITIONS

The experiments were carried out at the "Professor Walter Radamés Accorsi" Experimental Garden at the Department of Biological Sciences of the "Luiz de Queiroz" School of Agriculture, University of São Paulo, conducted in a greenhouse. In all experiments, plastic pots with a volume capacity of 20 dm^3 containing substrate composed of a mixture of clay, sand, and organic matter (2:1:1) were used under N-P-K fertilization, according to the conventional fertilization recommended for each crop. Irrigation was performed aiming to leave the substrate close to the field capacity. Chemical analysis of the substrate was made in order to better observe the characteristics of the soil to be planted.

In both experiments, the sowing of the bean cultivar BRS Estilo (*Phaseolus vulgaris* L.), with set growth and with uniformity of colour and grain size, was carried out. The cultivar presented resistance to the common mosaic virus and to the anthracnose, standing erect with greater resistance to lodging, a cycle of 85 to 90 days and high productivity (EMBRAPA, 2009). In each vase, 15 seeds were sown at a depth of 2 cm, with thinning 7 days after emergence, leaving 4 plants per vase, characterizing the experimental plot.

The first experiment aimed to elucidate the effects of foliar application of zinc, from floral anthesis, as a method to increase the micronutrient content in beans, in a substrate with adequate micronutrient content. In this same experiment, different sources of zinc with the potential to enrich the bean grains were compared in order to establish the best source of zinc.

The second experiment was carried out using different doses of zinc, applied starting with the anthesis, corresponding to the source of zinc that gave the best response (regarding to productive aspects and biofortification of the grain), as previously established in the first experiment. Thus, the best dose to be applied by foliar spraying to biofortify the bean grain, cultivated on a micronutrient-corrected substrate, was confronted.

2.2 ZINC TREATMENTS

The first experiment consisted in these treatments: control (without application of Zn), zinc sulfate zinc chloride, zinc nitrate, phosphorus zinc and finally NHT zinc, in which the equivalent of 250 g of zinc per hectare was used in each application, regardless of the source, from the anthesis of the bean plants, at 47, 54, 61 and 69 days after sowing.

The phosphorus zinc product consists of a foliar fertilizer containing mixture of P_2O_5 (40%) and Zn (8%). NHT zinc is a fluid fertilizer (concentrated suspension), applied to the substrate, with gradual release of the nutrient, manufactured by an industrial process that produces small-sized particles.

The second experiment, as predicted, was carried out using different doses of zinc ($ZnSO_4.7H_2O$), corresponding to the source of zinc that presented the best response (in relation to productive aspects and seed enrichment) in experiment 1.

The treatments were: control (without application of Zn), zinc sulfate 125 g h^{-1}, zinc sulfate 250 g ha^{-1}, zinc sulfate 375 g ha^{-1}, and finally zinc sulfate 500 g ha^{-1}. The application of treatments started 43, 55, 61 and 70 days after sowing.

2.3 PLANT BIOMETRICS

The variables in all the experiments were analyzed to compare productive variables, especially the zinc content in the grains, with the purpose of biofortification of the vegetal material. The number of pods, the number of spikelets and the number of beans were obtained by collecting and counting these variables. The dry weight of pods, dry weight of spikelets, dry weight of grains and aerial dry weight were established by collecting the plant material, which were packed in properly identified paper bags, then dried in an oven at 75°C for 72 h and measured with precision scales to obtain the dry weight.

2.4 SEED PROTEIN CONTENT

The crude protein content was determined by the decomposition of the proteins and other nitrogenous compounds in the presence of hot concentrated H_2SO_4, using the Kjeldahl method, multiplying the total N value by a factor of 6.25 (AOAC, 1995).

2.5 CHEMICAL ANALYSIS OF NUTRIENTS

Finally, the analysis of zinc in the grains was carried out by chemical analysis of 0.25 g of the dry plant material. The material was mechanically grounded to be subjected to nitric-perchloric digestion and quantification by atomic emission spectrometry with argon plasma (ICP-AES) (MALAVOLTA *et al.*, 1997). Nitric-perchloric digestion was performed by adding 2.5 mL of a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a digestion tube containing the sample. Afterwards, the tubes remained in the digester block gradually increasing the temperature every 30 min, until reaching a temperature of 210°C (MALAVOLTA *et al.*, 1997). After a few hours, the samples were colourless and ready for analysis; however, the analyses were performed only 24 h later by atomic emission spectrometry with argon plasma (ICP-AES).

2.6 EXPERIMENTAL DESIGN

The first experiment was composed of 6 treatments, with 6 replicates, making 36 plots, in which different zinc sources were compared using a randomized complete block design. The second experiment was a randomized block design, consisting of 5 treatments, with 8 replicates, making 40 plots of different zinc doses, using the zinc sulfate source, applied from the anthesis of bean plants.

The analyses of variance were obtained through the SAS statistical program (SAS, 1999) and the differences between the means of the evaluated parameters were obtained by Tukey tests at the 5% probability level.

3 RESULTS

3.1 PLANT PRODUCTION - NUMBER OF PODS, POD DRY WEIGHT, NUMBER OF GRAINS AND GRAIN DRY WEIGHT

In the analysis of plant production, it was observed that the number of pods harvested was higher for the common bean that received control treatments and with foliar application of $ZnSO_4.7H_2O$ (250 g Zn ha^{-1}) sprayed from the anthesis, compared to the treatment with $ZnCl_2$ (250 g Zn ha^{-1}) sprayed from the anthesis (Table 1).

The dry weight of the pods was significantly higher in plants treated with leaf sprays from $ZnSO_4.7H_2O$ (250 g Zn ha^{-1}) compared to treatments receiving leaf applications from the zinc phosphorus (250 g Zn ha^{-1}), $Zn(NO_3)_2$ $.6H_2O$ (250 g Zn ha^{-1}) and $ZnCl_2$ (250 g Zn ha^{-1}) (Table 1).

The number of grains harvested was higher in the treatments with foliar sprays from the anthesis of 250 g Zn ha^{-1} with $ZnSO_4.7H_2O$ and from the control, in relation to treatments with foliar sprays from the anthesis of 250 g Zn ha^{-1} of the $Zn(NO_3)_2 .6H_2O$ and $ZnCl_2$ sources (Table 1).

The dry weight of grains obtained was higher in the common bean treated with leaf sprays from $ZnSO_4.7H_2O$ (250 g Zn ha^{-1}), compared to treatment with phosphorus zinc, zinc nitrate and zinc chloride, also applied in the anthesis at the dose of 250 g Zn ha^{-1} (Table 1).

3.2 ZINC CONTENT IN THE SEEDS THROUGH THE FOLIAR APPLICATION OF ZINC

In the analysis of the zinc content in the grain, we observed that according to the Tukey test, $ZnCl_2$ (250 g Zn ha^{-1}), $ZnSO_4.7H_2O$ (250 g Zn ha^{-1}) and $Zn(NO_3)_2 .6H_2O$ (250 g Zn ha^{-1}) presented increase in Zn content of the grain in relation to treatments with NHT zinc (Fig 1).

The treatments with sprays of different doses of $ZnSO_4.7H_2O$ from the anthesis did not affect shoot dry weight, number of pods, dry weight of pods (g), number of grains, and dry weight of bean plant grains (g) established in the anthesis (Table 2).

The Zn content in the grains was significantly higher in all treatments where leaf sprays were observed from the anthesis with $ZnSO_4.7H_2O$ (125, 250, 375 and 500 g Zn ha^{-1}) in relation to the Tukey test (Fig 2). Leaf application of $ZnSO_4.7H_2O$ (0, 125, 250, 375 and 500 g Zn ha^{-1}) sprayed on anthesis in common bean plants showed no significant difference in the variable protein content in the grains.

Treatment with sprays of different doses of $ZnSO_4.7H_2O$ (0, 125, 250, 375 and 500 g Zn ha^{-1}) from the anthesis did not affect the number of spikelets, dry spikelets, number of grains and dried weight bean plants (Table 1).

3.3 DISCUSSION

The plant productive variables among the treatments that received foliar application of different sources of zinc (first experiment) did not differ from the control treatment because the micronutrient had higher concentration in the soil. These data are in agreement with those obtained by Teixeira *et al.* (2008), in which beans cultivated in soils with a Zn content of 2.1 mg dm^{-3} did not increase productivity with either zinc chloride or sulfate applied through the leaf.

In addition, according to Ram *et al.* (2016), the foliar application of $ZnSO_4.7H_2O$ carried out alone or in conjunction with agricultural pesticides did not influence the production of beans in five different experiments carried out in different regions of Brazil. Regarding the zinc content in the grains using different zinc sources, applied foliarly, from the anthesis, it is noticed that the results corroborate those of Teixeira *et al.* (2005), in which the foliar fertilization with zinc promoted increases in the Zn content in the beans. Similar results were also obtained by Ram *et al.* (2016), in which the foliar application of zinc sulfate in five experiments from different regions of Brazil significantly increased the concentration of Zn in the beans.

The data for the productive variables between the treatments that received foliar application of different doses of zinc sulfate were not influenced by the treatments that received foliar application of zinc and the control treatment because the content of the micronutrient in the soil was high. Also according to Ram *et al.* (2016), the foliar application of $ZnSO_4.7H_2O$, either alone or combined with agricultural pesticides, did not influence the production of beans in five different experiments carried out in different regions of Brazil.

The zinc content in the beans, after foliar application of different doses of zinc sulfate, is in agreement with the findings obtained by Teixeira *et al.* (2005), in which zinc leaf fertilization led to additions to the Zn content in the beans. Similar results were also obtained by Ram *et al.* (2016) in which the foliar application of zinc sulfate in five experiments from different regions of Brazil significantly increased the concentration of Zn in the beans.

A study by Yilmaz *et al.* (1997) proposed the effect of six different methods of zinc application on grain yield, in addition to the concentration of this micronutrient on shoots and grain of wheat cultivars, observing that Zn concentrations in shoots and grain were significantly increased by different treatments containing zinc, compared to wheat plants without zinc application. In this case, the treatments were: control (without zinc application), application via soil, seed application, foliar application, application via soil + foliar application, and application via seed + foliar application. The application treatment via soil + foliar application with Zn was the one that brought the greatest increase of zinc concentration in the grains.

The effect of foliar zinc application on the concentration of this micronutrient in wheat seeds was also increased in the work of Ozturk *et al.* (2006). The authors had the objective of investigating the foliar application of zinc as a method for enriching wheat seeds, concluding that treatments with Zn applications were effective in increasing the concentration and total amount of Zn in the seeds, and increments became more pronounced with the application of ten foliar sprays of zinc. Foliar zinc applications were performed through three or ten sprays of 0.68 kg ha^{-1} of $ZnSO_4$ $.7H_2O$ during panicle development.

Considering the productive data and nutrient content in the grain, we see that any dose used in the study is capable of significantly increasing the zinc content in the grain compared with the control, without affecting the bean crop production.

4 CONCLUSION

The zinc foliar application performed periodically from the floral anthesis is able to increase the content of the micronutrient in beans. The most viable source for the enrichment of bean grains is $ZnSO_4.7H_2O$. All the doses tested (125, 250, 375 and 500 g Zn ha^{-1}) applied via foliar in the form of zinc sulfate, were effective for the enrichment of the bean grains. In addition, all the doses tested (125, 250, 375 and 500 g Zn ha^{-1}) applied via leaf in the form of zinc sulfate were efficient for the enrichment of the bean grains, in relation to the control (0 g Zn ha^{-1}).

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Table 1: Plant dry weight (g), number of bean pods, dry weight of bean pods (g), grain number and dry weight (g) of bean 'BRS Estilo' under different zinc sources

Dose (g Zn.ha^{-1})	Number of bean pods (g)	Pods dry weight (g)	Dry weight of bean pods (g)	Grain number
Control	22.8±5 a	14.6±6.5 abc	11.3±5.6 abc	64.2±21 a
Z. sulfate	22±5.8 a	17.8±6.4 a	13.3±4.6 a	70.2±20.6 a
Z. chloride	12.8±5.8 b	5.5±2.9 d	3.9±2.0 d	24.2± 11.4 c
Z. nitrate	14.8±3.4 ab	8.3±3.8 cd	5.7±2.5 cd	33.3±13.3 bc
Z. Phosphorus	15.5±2.2 ab	8.4± 3.1 cd	6.2±2.3 bcd	40.8±15.2 abc
NTH zinc	19.3±2.2 ab	15.6±3.3 ab	11.7±2.7 ab	62.7±13.2ab

*Averages followed by the same letter were not different by Tukey test (5% probability).

Table 2: Plant dry weight (g), number of bean pods, dry weight of bean pods (g), grain number and dry weight (g) of bean 'BRS Estilo' under different zinc doses

Dose (g Zn.ha^{-1})	Plant dry weight (g)	Number of bean pods	Dry weight of bean pods (g)	Grain number	Dry weight of bean (g)
0	20.27±2.1a	33.25±4.0a	53.618±5.5a	163.37±16.0a	43.19±6.9a
125	19.12±3.2a	35.62±5.7a	57.181±7.0a	170.75±25.5a	45.75±5.1a
250	20.58±3.9a	38.37±6.9a	56.169±5.5a	176.00±22.5a	45.13±4.0a
375	18.24±2.3a	35.75±4.0a	54.453±4.9a	170.37±17.6a	43.68±4.2a
500	20.93±3.4a	36.50±4.4a	53.506±4.7a	171.00±16.8a	43.06±3.6a

*Averages followed by the same letter were not different by Tukey test (5% probability).

Figure 1: Grain zinc concentration of bean 'BRS Estilo' under different zinc sources. The values of the bars are averages (n = 6), columns with different letters indicate a significant difference by the Tukey test (p <.01)

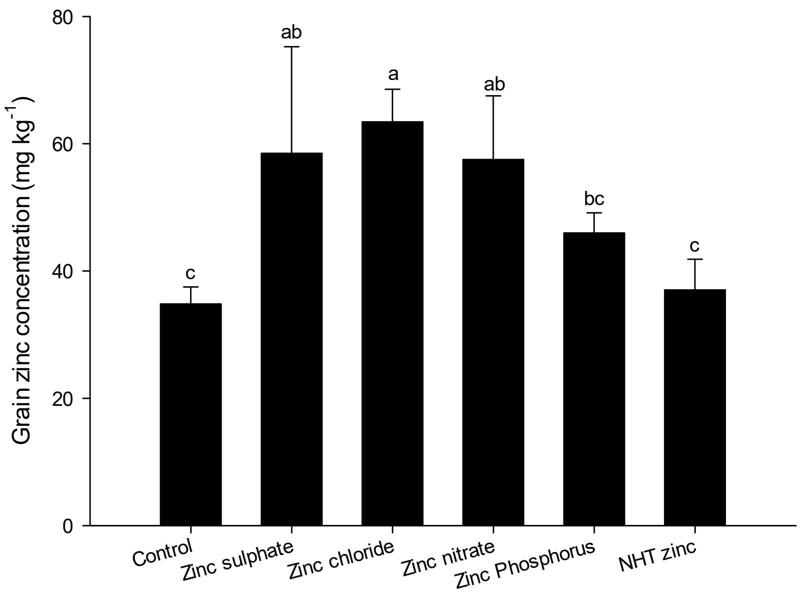

Figure 2: Grain zinc concentration (mg kg⁻¹) of bean 'BRS Estilo' under different zinc doses