Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.15, n.4, e8456, 2020
Recife, PE, UFRPE. www.agraria.pro.br
DOI:10.5039/agraria.v15i4a8456
Protocol 8456 - 11/04/2020 • Approved in 22/06/2020

Agronomic biofortification with zinc in curly lettuce cultivars

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ABSTRACT: In underdeveloped countries, zinc deficiency is a public health problem. The main foods consumed in these countries have low levels of the nutrient, making their consumption insufficient to meet the minimum daily requirements. Therefore, the aim of this study was to evaluate agronomic biofortification with zinc in curly lettuce cultivars. The experiment was carried out at the Universidade Federal de Uberlândia - Monte Carmelo Campus, using a complete randomized block design in a 4 × 5 factorial scheme with four replications. The factors consisted of four curly lettuce cultivars (Brida, Isabela, Thaís, and Vanda) and five doses of leaf zinc (0, 400, 800, 1200 and 1600 g ha^{-1} of Zn). The following traits were evaluated: zinc leaf content, plant height, SPAD index, total fresh mass, stem diameter, head diameter, number of leaves per plant, and estimated average yield. Data were subjected to analysis of variance by the F test ($\alpha = 0.05$) and means were compared by Tukey test at 5% probability and regression analyzes. According to the results obtained, the cultivar Thaís can be considered the lettuce with highest leaf zinc content and the most biofortified. It is advisable to use 700 g ha^{-1} of leaf zinc to obtain biofortified lettuce with increased yield.

Key words: foliar application; *Lactuca sativa*; plant nutrition; zinc doses

Biofortificação agronômica com zinco em cultivares de alface crespa

RESUMO: Em países subdesenvolvidos, a deficiência de zinco é um problema de saúde pública. Os principais alimentos consumidos nesses países apresentam baixos teores do nutriente, tornando seu consumo insuficiente para atender aos requerimentos mínimos diários. Diante disso, objetivou-se avaliar a biofortificação agronômica com zinco, em cultivares de alface crespa. O experimento foi realizado na Universidade Federal de Uberlândia - Campus Monte Carmelo, utilizando delineamento de blocos casualizados, em esquema fatorial 4 × 5 com quatro repetições. Os fatores consistiram em quatro cultivares de alface crespa (Brida, Isabela, Thaís e Vanda) e cinco doses de zinco foliar (0, 400, 800, 1200 e 1600 g ha^{-1}). Avaliaram-se os seguintes caracteres: teor foliar de zinco, altura de planta, índice SPAD, massa fresca total, diâmetro do caule, diâmetro da cabeça, número de folhas por planta e produtividade média estimada. Os dados foram submetidos à análise de variância, pelo teste F ($\alpha = 0{,}05$) e as médias foram comparadas pelo teste Tukey a 5% de probabilidade e análises de regressão. De acordo com os resultados obtidos, a cultivar Thaís destacou-se por acumular o maior teor de zinco na folha. É recomendável o uso de 700 g ha^{-1} de zinco via foliar para obter alface biofortificada com maior produtividade.

Palavras-chave: aplicação via foliar; *Lactuca sativa*; nutrição de planta; doses de zinco

Introduction

Zinc deficiency affects approximately one third of the world's population, accounting for about 16, 18 and 10% of respiratory infections, malaria rates and diarrhea, respectively (Rios et al., 2009). Zinc is essential for humans because it is related to immune system functioning, sensory function, neurobehavioral development, reproductive health, growth, and physical development (Hotz & Brown, 2004). In underdeveloped countries, zinc, iron, and vitamin A deficiencies are considered public health problems.

Despite their importance in human health, the main foods consumed in developing countries have low micronutrient levels, making their consumption insufficient to meet the minimum daily requirements (Carvalho & Vasconcelos, 2013). Therefore, some methods are being used to attend the need of zinc in humans is supplied (Cakmak, 2008), highlighting among them the agronomic biofortification, which aims to enrich the food through fertilization (Cakmak et al., 2010; Hussain et al., 2012) via soil and / or leaf. Both forms of zinc application have shown satisfactory results regarding their elevation in cereal grains (Cakmak, 2009; Hussain et al., 2012), beans (Cambraia, 2015) and onion (Almendros et al., 2015). However, there is still little research related to agronomic biofortification in leafy vegetables.

Lettuce (*Lactuca sativa* L.) is the most consumed leafy vegetable in the world, being considered of great nutritional importance, providing considerable contents of phosphorus (20 mg $100g^{-1}$), potassium (141 mg $100g^{-1}$), vitamin C (2.8 mg $100g^{-1}$), and vitamin A (25µg $100g^{-1}$) as well as being a source of fiber and low in calories (USDA, 2019). However, humans need about 22 nutrients to maintain their proper and healthy metabolism (Graham et al., 2007), and some of these are not present or present in low levels in lettuce leaves. Among these nutrients present in low amounts is zinc (Zn), a micronutrient with daily requirements of about 11 mg for adults (NIH, 2020).

Because lettuce is a vegetable that is increasingly present in several dishes, and is easy to prepare and low cost, any strategy aimed at enhancing agronomic biofortification can result in several health benefits. Biofortification of lettuce cultivars with zinc may contribute to supply nutritional deficiencies of low-income populations, as it presents itself as an affordable and easily produced food. Therefore, the aim of this study was to evaluate the agronomic biofortification with zinc in curly lettuce cultivars.

Materials and Methods

The experiment was carried out from March 05 to June 09, 2018, in Monte Carmelo-MG, belonging to the Universidade Federal de Uberlândia (altitude 873 m, 18º43'37" S and 47 ° 31'27" W). The climate, according to the Köppen climate classification, is humid temperate, with hot summers and dry winters.

A randomized complete block design in a 4 × 5 factorial scheme with four replications was used. The treatments resulted from the combination of four curly lettuce cultivars (Brida, Isabela, Thaís, and Vanda) and five doses of zinc applied via foliar (0, 400, 800, 1200 and 1600 g ha^{-1}), using as source zinc sulfate PA with 20% zinc. Each experimental plot consisted of four planting lines, containing 20 plants per plot, arranged in a spacing of 0.25 m between rows and 0.25 m between plants (1.25 m^2 $plot^{-1}$) of each plot.

Sowing was carried out on March 5, 2018, in 200-cell polyethylene trays filled with commercial coconut shell fiber-based substrate. Prior setting up the experiment, soil was sampled at a depth of 0 to 20 cm for chemical and physical analysis, presenting the following results: very clayey texture, containing 73.5% of clay; pH in $CaCl_2$ = 5.3; Pmehlich = 23.3 mg dm^{-3}; K = 0.50 cmole dm^{-3}; Ca = 3.5 cmole dm^{-3}; Mg = 1.03 cmole dm^{-3}; Zn = 4.7 mg dm^{-3}; B = 0.30 mg dm^{-3}; Fe = 16 mg dm^{-3}; Cu = 4.0 mg dm^{-3}; Mn = 4.2 mg dm^{-3}; H + Al = 3.10 cmole dm^{-3}; SB = 5.05 cmole dm^{-3}; T = 8.15 cmole dm^{-3}; and V% = 52%.

Two months before transplant, the soil acidity of the area was corrected with liming, aiming to increase the base saturation to 70%. After limestone application, the soil was plowed and barred; and two days before transplanting, the beds were raised and fertilization was performed, according to the results of soil analysis and recommendation for the crop (Ribeiro et al., 1999). When the seedlings had three definite leaves they were transplanted to the definitive site.

The fertilization consisted of 150 kg ha^{-1} of N and 50 kg ha^{-1} of P_2O_5, using as source urea and simple superphosphate, respectively. In planting fertilization, 20% of N was used and the total P_2O_5 recommended. In cover fertilizers, 20%, 30% and 30% of the total recommended N were used, respectively, at 15, 30, and 40 days after transplantation (Ribeiro et al., 1999).

Zinc foliar applications were carried out in a single dose with the aid of a Guarani® manual costal pump, with a capacity of 20 liters of syrup, and a volume of 4 liters of syrup for each 16 plots, which corresponded to the point of dripping. The application was performed on May 13, 2018, fifteen days after transplantation (DAT), in the late afternoon. After application, irrigation was stopped for a period of 24 hours. To avoid drift, a 1 m high plastic curtain was installed around the plot at the time of application.

The irrigation system used was by spraying, with two daily watering shifts, to keep the soil always moist and suitable for the best crop development. Phytosanitary treatments were carried out throughout the crop cycle, according to the incidence of pests and diseases, with registered products. Weeding was done manually to keep the crop always clean.

Harvest was performed at 43 DAT, when the plants showed their maximum vegetative development. Before harvesting, the chlorophyll content of the plants in the useful plot was measured using the Minolta SPAD-502 CFL1030 chlorophyll meter on the median leaf of the plant in the morning. After harvesting, the heads were taken to the Phytotechnic Laboratory (LAFIT) to determine the following traits: plant height (cm), total fresh mass (kg $plant^{-1}$), stem diameter (mm), head diameter (cm), number of leaves per plant, and estimated average yield (kg m^{-2}).

The leaf zinc content (mg kg^{-1}) was determined at the Soil Laboratory of the Universidade Federal de Uberlândia - Campus Uberlândia (LABAS). The samples were washed in running water and subsequently in distilled water. They were placed in an oven with forced air circulation at 65 °C until they reach a constant mass and then ground and subjected to chemical analysis. Zinc leaf contents were determined by nitric-perchloric extract by atomic absorption spectrophotometry (Silva, 2009).

For statistical analysis, Shapiro-Wilk, Levene and Tukey tests were used for ANOVA residual normality, homogeneity between variances and block additivity, respectively ($p < 0.01$), using the IBM SPSS Statistics version software 20.0 (Marôco, 2018). Given these assumptions, the data were subjected to analysis of variance by the F test ($p < 0.05$) and, when there were significant effects, the averages of the cultivar factor were compared by the Tukey test ($p < 0.05$) using the SISVAR program® (Ferreira, 2008). For the dose factor, regression analysis was performed using the statistical software Sigma Plot® version 14 (Systat Software Inc, 2008).

Results and Discussion

Biofortification with zinc of curly lettuce cultivars

The leaf zinc content of curly lettuce was influenced by cultivar and doses; however, no significant interaction was verified among these factors (Figure 1). Higher rates of zinc application promote linear increases in the leaf zinc content, with an increment rate of 0.049 mg kg^{-1} of zinc leaf for each zinc gram (g ha^{-1}) applied (Figure 1A). In addition, the cv. Thaís responded with greater Zn content in the leaf tissue, presenting an average content of 213 mg kg^{-1} (Figure 1B). However, the cv. Vanda presented lower Zn content (141 mg kg^{-1}). Thaís had 51% more leaf zinc content than cv. Vanda (Figure 1 B).

According to the USDA (2019), the average leaf Zn content in lettuce is 180 mg kg^{-1}. Based on this value, the cv. Thais has higher leaf zinc content in all doses of zinc used, and this cultivar also had the highest responses to increasing doses (Figure 1C). The cultivars Brida and Isabela responded with increments above the reference value when the doses of 1200 and 1600 g ha^{-1} were used.

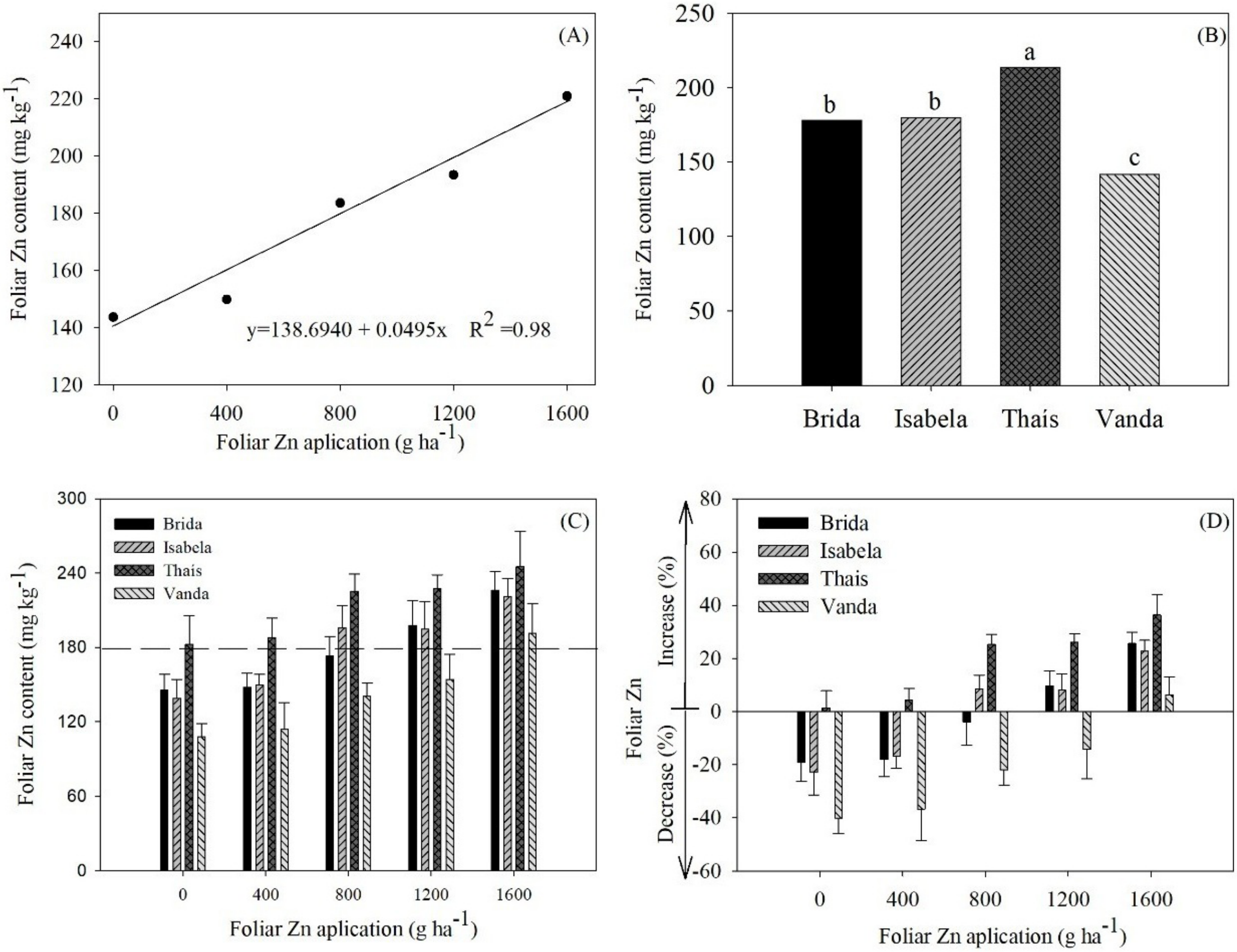

Figure 1. (A) Leaf zinc content in curly lettuce as a function of increasing doses of zinc via leaf; (B) Average leaf zinc content in curly lettuce cultivars (different letters on the bars indicate difference between the means by Tukey test ($p < 0.05$); (C) Mean leaf zinc content as a function of cultivars and doses, with their respective standard deviations. Dashed line indicates zinc reference value in lettuce leaves (180 mg kg^{-1} - USDA, 2019); (D) Change of leaf zinc content from the reference value (180 mg kg^{-1} - USDA, 2019).

The relative increments of leaf zinc for each cultivar at all evaluated doses are shown in Figure 1D, based on the reference value of 180 mg kg^{-1}. It is verified that at the zinc dose of 1600 g ha^{-1} all cultivars presented leaf zinc content higher than the reference, especially cv. Thais showed an average increase of 36%. In the highest dose, the cultivars Brida, Isabela, and Vanda presented average increments of 25, 22 and 6%, respectively. It is noteworthy that at the dose of 800 g ha^{-1} from zinc to cv. Thaís presented a significant increase of 25%, that is, with half of the maximum dose used, this cultivar showed satisfactory response to biofortification. The cv. Vanda had lower zinc content in leaf tissue compared to the other cultivars and reference value.

These results demonstrate the high agronomic biofortification potential of zinc curly lettuce, since significant increases of leaf zinc were obtained in the cultivars and doses used. Considering the content of zinc in the leaf tissue, it can be said that all cultivars reached levels that made them biofortified, besides, the zinc content is within the limit that can be consumed by people without harm to health (25-250 mg kg^{-1}) (Martinez et al., 1999). However, one must pay attention to the results of agronomic traits, so that there is no productive damage.

Agronomic traits of curly lettuce submitted to zinc biofortification

There was significant interaction between cultivars and doses of zinc for plant height, SPAD index, total fresh mass and average yield (Table 1). Plant height ranged from 15.76 cm (cv. Brida at a dose of 0 g ha^{-1}) to 19.58 cm (cv. Vanda at a dose of 800 g ha^{-1}). In general, the highest values were obtained for the cv. Thaís, except at the dose of 800 g ha^{-1}, in which it did not differ from the others.

Plant height of lettuce cultivars presented quadratic polynomial adjustment with increasing Zn doses (Figure 2A), with the application of 812, 632, 703, and 737 g ha^{-1} (maximum curve point) resulted in the highest plant height for the cultivars Brida, Isabela, Thaís, and Vanda, respectively. These doses promoted increases of 20.3, 7.9, 5.9, and 16.2% in plant height for cultivars Brida, Isabela, Thaís, and Vanda, respectively. This result indicates that zinc may aid both production and biofortification, as it is a component of growth hormones, such as auxins, which are directly linked to plant development (Brennan, 2005).

These data corroborate with data obtained by Reyes et al. (2019), who applied zinc in a single dose via arugula leaf for biofortification, despite observing phytotoxic effects on plants between 1 to 1.5 kg ha^{-1} did not show a decrease in productivity. The values found in the current experiment are close to the values found by other authors (Silveira et al., 2015; Vargas et al., 2017). Thus, the biofortified plants obtained in this study have standard height and are acceptable to the consumer market, that is, plants whose harvest point was not exceeded and, consequently, that were not clogged. In this sense, agronomic biofortification can increase the nutritional value of lettuce without compromising the characteristics required by consumers.

Table 1. Plant height, SPAD index, total fresh mass, and yield of four curly lettuce cultivars as a function of foliar zinc doses.

Cultivar	Doses of Zn (g ha^{-1})				
	0	400	800	1200	1600
	Plant height (cm)				
Brida	15.76 b	18.14 b	19.46 a	17.73 ab	16.19 b
Isabela	17.58 a	18.90 ab	19.13 a	17.56 ab	15.88 b
Thaís	18.20 a	19.32 a	19.48 a	18.50 a	17.72 a
Vanda	16.34 b	19.27 a	19.58 a	17.38 b	16.02 b
MSD	0.98				
CV (%)	2.93				
	Index SPAD				
Brida	20.17 a	20.31 a	20.32 ab	21.15 a	22.00 a
Isabela	17.54 b	20.76 a	21.15 a	21.54 a	22.50 a
Thaís	15.21 c	15.77 c	15.98 c	16.32 c	18.42 b
Vanda	18.25 a	18.77 b	19.17 b	19.52 b	19.67 b
MSD	1.49				
CV (%)	4.14				
	Total fresh mass (kg plant^{-1})				
Brida	0.25 ab	0.26 a	0.23 b	0.21 ab	0.20 a
Isabela	0.22 b	0.24 a	0.24 ab	0.22 ab	0.21 a
Thaís	0.25 ab	0.27 a	0.28 a	0.19 b	0.17 a
Vanda	0.27 a	0.25 a	0.27 ab	0.24 a	0.21 a
MSD	0.04				
CV (%)	9.28				
	Yield (kg m^{-2})				
Brida	4.04 ab	4.13 a	3.81 b	3.35 ab	3.20 a
Isabela	3.54 b	3.88 a	3.90 ab	3.59 ab	3.41 a
Thaís	4.08 ab	4.38 a	4.48 a	3.01 b	2.80 a
Vanda	4.27 a	4.03 a	4.29 ab	3.89 a	3.43 a
MSD	0.66				
CV (%)	9.28				

(a) MSD = Minimum significant difference; CV (%) = Coefficient of variation.
(b) Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% significance level.

Analysis of chlorophyll content in leaves is a method used to monitor plant development, providing information on physiological state, nitrogen levels in leaves, and photosynthetic potential of plants (Riccardi et al., 2014; Yang et al., 2014). The SPAD index is an indirect measure of chlorophyll content in leaves because it correlates with chlorophyll content and nutritional status of plants. The SPAD index values ranged from 15.21 (cv. Thaís when no zinc was applied via leaf) to 22.50 (cv. Isabela when 1600 g ha^{-1} of Zn was applied via leaf) (Table 1). Linear increase in SPAD index values was observed at each 1 g ha^{-1} increase in zinc doses, regardless of cultivar (Figure 2B). This can be reflected in excellent quality leaves, as found by Cassetari et al. (2015), since chlorophyll content is directly related to photosynthetic activity and plant nutritional status.

Some authors (Kaya & Higgs, 2001; Anwaar et al., 2015; Roosta et al., 2018) reported that the decrease in chlorophyll content may be caused by both zinc deficiency and toxicity. Because, zinc is directly involved with the process of photosynthesis, nitrogen metabolism, respiration, and protein synthesis in plants (Malavolta, 2006; Cardoso et al., 2012; Mascarenhas et al., 2014).

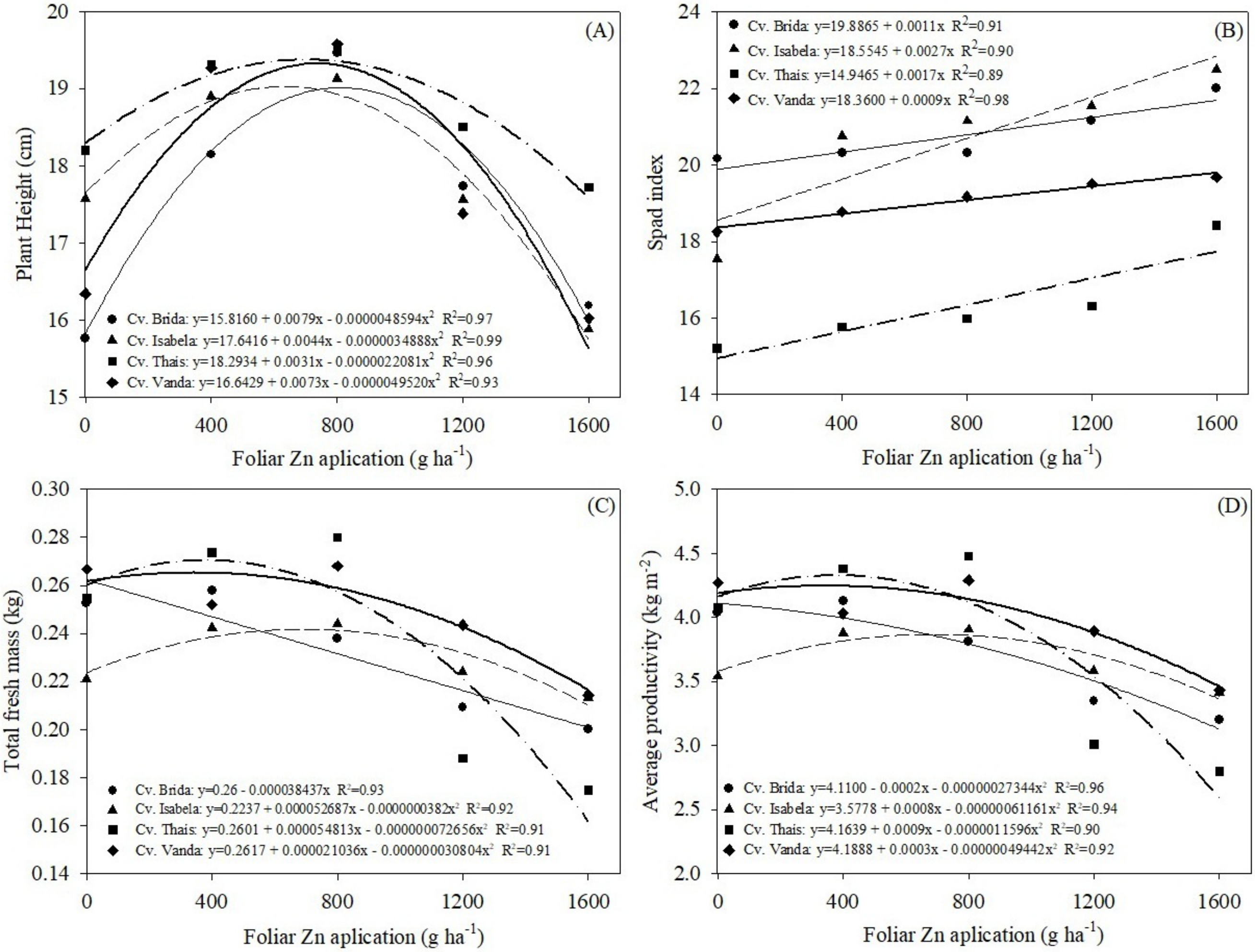

Figure 2. (A) Plant height, (B) SPAD index, (C) total fresh mass, and (D) average yield of the four curly lettuce cultivars as a function of zinc doses.

The cultivar Thaís had more Zn absorption, hence more Zn content in the leaves (Figure 1B). In the other hand, this cultivar showed reduction in the fresh weight on higher doses of Zn (Figure 2C). Fresh masses of cv. Thaís ranged from 0.16 kg when 1600 g ha^{-1} zinc was applied to 0.25 kg when 800 g ha^{-1} was applied (Figure 2C). The application of 1600 g ha^{-1} zinc reduced 64% the fresh weight of cv. Thais in relation to 800 g ha^{-1} dose of Zn. This reduction could be related to phytotoxicity due application of higher rates of Zn.

Although, Zn is an essential micronutrient it could affect the growth and plant metabolism when this element is present in the environment in toxic levels (Marschner, 1995). Only the cultivar Thais were able to accumulate more than 200 mg kg^{-1} of Zn (Figure 1B). According to Martinez et al. (1999) the ideal content of Zn for lettuce range from 25 to 250 mg kg^{-1}, whereas for Trani & Raij (1997) it would be between 30 and 100 mg kg^{-1}. Therefore, high doses of Zn could affect plant growth due phytotoxicity. In this study, for all tested cultivars, the least fresh weight was observed when the highest Zn dose was applied (Figure 2C).

According to Yuri et al. (2006), the largest fresh mass of iceberg lettuce, was found when 2.01 kg ha^{-1} of zinc sulfate was applied via leaf, differing from the current experiment, where the largest fresh mass was found at doses of 663.98, 425.90, and 277.78 g ha^{-1} for cultivars Isabela, Thaís and Vanda, respectively (Figure 2C). For cv. Brida, there was a decrease of 0.0375 g in fresh lettuce mass every 1 g ha^{-1} of zinc applied (Figure 2C). This difference may have been due to the fact that the soil of the area in which the current experiment was implemented was high in zinc compared to the soil of the place where the experiment by Yuri et al. (2006), which had a low zinc content (1.2 mg dm^{-3} zinc). Although the soil of the current experiment was high in zinc, there was a positive response to the total fresh mass of the plants against micronutrient application. Sago et al. (2018) observed drastic reductions in lettuce fresh mass values with increasing zinc content from 0.15 to 0.45 mM in nutrient solution.

The different results found for fresh mass can be justified by the genetic difference among curly lettuce cultivars, which can lead to distinctions in morphological and productive characteristics, even under similar climatic conditions; different sources of mineral fertilizers used, which may or may not facilitate absorption by the leaf cuticle. In addition, due to the split application of the nutrient application, as a single application of zinc via leaf 14 days after transplant may cause better utilization of the nutrient by the plant, as observed by Yuri et al. (2006).

For the estimated average yield trait, there was a difference among cultivars at doses of 0, 800 and 1200 g ha^{-1} of zinc (Table 1). For all cultivars, according to the doses used, there was an adjustment of the quadratic polynomial model. The cultivars Brida, Isabela, Thaís, and Vanda had higher yields at doses of 397.11, 694.44, 389.27, and 305.50 g ha^{-1}, respectively (Figure 2D). After reaching the maximum doses, yield decreases were observed with increasing leaf zinc doses, regardless of cultivar.

For cv. Vanda, the average yield in this experiment ranged from 42.9 to 34.3 t ha^{-1}; already in the experiment by Resende et al. (2018), the maximum yield obtained for the same cultivar was 49.3 t ha^{-1}. Despite the difference in productivity in both experiments, this is not significant (14.9% between the highest yield of the current experiment and the yield found by the author in question), since the main objective, biofortification and, consequently, the availability of the product. nutrient through a low-cost food and easily available to the whole population, has been reached.

For the traits head diameter, stem diameter, number of leaves per plant and leaf zinc content, there was no significant interaction between the evaluated factors. Thus, for these trits, cultivars and zinc doses were evaluated separately (Table 2). The values for head diameter were from 27.90 to 28.43 cm, not statistically different among cultivars (Table 2). Because head diameter is a very relevant feature on the part of consumers at the time of lettuce acquisition, leaf application of zinc may be a promising alternative for growers, as it does not interfere with production characteristics and has beneficial to population because it is important for the synthesis and repair of DNA, RNA and proteins, as well as influencing biochemical and physiological processes related to growth, division, cell differentiation, development, and aging (Fukada et al., 2011).

The values for this trait, due to the application of zinc doses via leaf, adjusted to the second-degree polynomial equation, and the largest head diameter (29.56 cm) was found when the 706 doses was applied 76 g ha^{-1} zinc (Figure 3).

Because the packaging of leafy vegetables for later transport is done in plastic or wooden boxes, traits such as head diameter and plant height are of great importance (Sala & Costa, 2012). However, despite the relentless pursuit by producers of larger plants, this is not always beneficial as larger plants are the most damaged in packaging and transport processes, thus reducing the commercial quality of the final product (Suinaga et al., 2013).

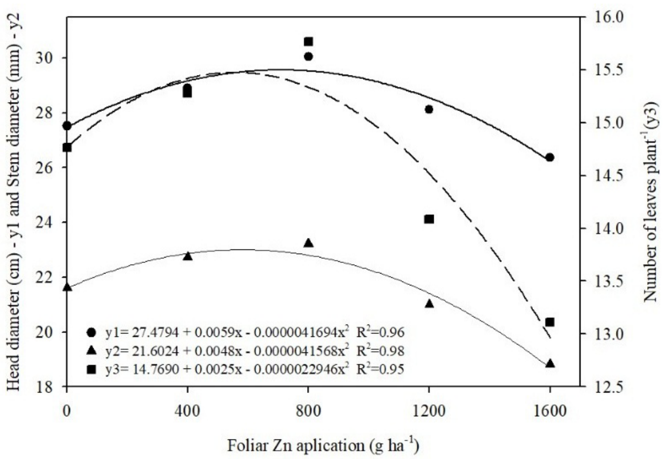

Figure 3. (y1) Head diameter (cm), (y2) stem diameter (mm), and (y3) number of leaves plant^{-1} as a function of zinc doses.

Regarding the stem diameter, it was possible to verify that the cv. Vanda differed from the others, with an average of 24.15 mm (Table 2). The values for these traits, as a function of the different doses of leaf zinc, regardless of the cultivar evaluated, were adjusted to the second-degree polynomial equation, and the 577.41 g ha^{-1} dose provided the largest stem diameter. with 22.98 mm (Figure 3).

The number of leaves in the current experiment ranged from 12.31 to 16.32 leaves plant^{-1}, and cv. Vanda differed statistically from the others, with higher number of leaves (Table 2). The dose of 545.85 g ha^{-1} zinc reflected in the largest number of leaves (15.45 leaves plant^{-1}), regardless of cultivar. From this dose, there was a decrease in the number of leaves with the increase of the applied dose (Figure 3).

The number of leaves is an extremely important traits, due to the fact that the leaves constitute the commercial part of lettuce (Filgueira, 2008) and the consumer's attention at the moment of purchase is focused on the appearance, volume and indirectly to the number of leafs. In addition, the number of leaves contained in each plant may indicate the adaptation of genetic material to the environment (Diamante et al., 2013), especially to temperature, photoperiod (Oliveira et al., 2004) and the management employed in the crop.

Zinc Doses higher than 545.85 g ha^{-1} caused a reduction in the number of leaves (Figure 3), probably due to phytotoxicity

Table 2. Head diameter (cm), stem diameter (mm), number of leaves per plant, and leaf zinc content of four curly lettuce cultivars as a function of different doses of zinc.

Cultivar	Head diameter (cm)	Stem diameter (mm)	Number of leafs	Zinc leaf content (mg kg^{-1})
Brida	27.90 a	21.28 b	15.54 b	177.95 b
Isabela	28.43 a	20.01 b	14.24 c	179.95 b
Thaís	28.43 a	20.46 b	12.31 d	213.66 a
Vanda	28.01 a	24.15 a	16.32 a	141.65 c
MSD	0.61	1.70	0.51	14.62
CV (%)	2.58	9.46	4.18	9.80

(a) MSD = Minimum significant difference; CV (%) = Coefficient of variation.
(b) Means followed by the same letter in the column do not differ from each other by Tukey's test ($p \geq 0.05$).

caused by high leaf Zn content obtained in the highest doses evaluated (Figure 1A). Other studies (Queiroz et al. 2014; Nespoli et al. 2017) reported a higher number of leaves than the present study. Although the plant size influences the choice of the consumer market, the search for a healthier lifestyle is increasingly part of the routine of Brazilians. According to Euromonitor International (2017), the healthy food and beverage market in Brazil has grown over the last five years by an average of 12.3% per year.

Given this, some factors may have contributed to the results on the productive traits of the crop, in this work, among them, the high zinc content in the soil (4.7 mg dm^{-3}). According to Ribeiro et al. (1999), soils with high zinc content are those with values above 2.2 mg zinc per dm^3 of soil. Therefore, the soil in which the experiment was implemented had about 105% more zinc than the critical level, which may have influenced the development of some lettuce cultivars, since zinc is a micronutrient related to nitrogen metabolism, in addition to growth hormone components, such as auxins, which are directly linked to plant development.

For the leaf zinc content, the cv. Thaís presented the highest micronutrient content in the leaf (231.66 mg kg^{-1}), differing from the other cultivars, with 61.14% more zinc than the cv. Vanda (Table 2). It can be observed, according to the regression analysis graph, that the zinc rates for leaf zinc adjusted to the growing linear model for all cultivars, and for each 1 gram of zinc applied via leaf, There was an increase of 0.0495 mg kg^{-1} in the zinc leaf content of lettuce cultivars (Figure 3). Similarly, Sago et al. (2018) observed that the zinc leaf content of lettuce gradually increases as the zinc concentrations in the nutrient solution increase. Seema et al. (2017), in a study with spinach, also observed that leaf zinc content increased as there was an increase in zinc content applied to the soil. According to Reyes et al. (2019), foliar fertilization with up to 1.5 kg ha^{-1} of zinc, even when applied in soils with high micronutrient content, does not affect the physiological parameters and biomass of arugula.

According to WHO (2006), the tolerable daily intake limit of zinc for an adult is 21-70 mg and cannot exceed this limit due to micronutrient toxicity. The daily intake indicated in the US according to the ICZ (2019) is 12 and 15 mg day^{-1}, respectively, for women and men, and pregnant and lactating women need 19 mg day^{-1}. Thus, considering the amounts of zinc found in the cultivars evaluated in the current experiment and how crisp lettuce already participates in the composition of Brazilian daily meals, in the form of salads, such vegetables would contribute part of the nutritional needs required by humans daily.

In order to supply the daily nutrient requirement by the human body (average of 15 mg day^{-1} in adult men), the necessary consumption of lettuce of cultivars Brida, Isabela, Thaís and Vanda would be 84.29, respectively; 83.36, 70.20, and 105.90 g day^{-1}. Adequate zinc fertilization is an important factor for lettuce cultivation, since in addition to increasing zinc concentration, reducing NO_3 levels and increasing the concentration of essential amino acids, all with beneficial properties for human nutrition (Barrameda-Medina et al., 2016). Zinc is found in many foods, including beef, oysters, almonds, Brazil nuts, liver, mollusks, eggs, soy flour (TACO, 2011). However, these sources of zinc may not be accessible to low-income people. Thus, biofortification of leafy vegetables such as curly lettuce is a good alternative to supply nutritional deficiency.

Conclusions

The curly lettuce cv. Thaís had the highest leaf zinc content and can be considered the most biofortified, while cv. Vanda did not show satisfactory responses with the different doses of leaf zinc applied.

The use of 700 g ha^{-1} of leaf zinc is recommended to obtain biofortified lettuce with yield increase.

Acknowledgements

We are grateful for research support from the Lettuce breeding program at the Universidade Federal de Uberlândia, and for financial support from the Brazilian CAPES (Finance Code 001), CNPq and FAPEMIG institutions.

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