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Agronomic biofortification with zinc in hydroponically cultivated lettuce

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Abstract

Lettuce is the most cultivated leafy vegetable in hydroponic systems. The biofortification of lettuce with zinc (Zn) can contribute to relieving nutritional deficiencies in socially vulnerable populations. Biofortification is affordable, easy and fast, even in urban areas. Our objective was to evaluate the effect of Zn concentration in nutrient solutions on the production and agronomic biofortification of lettuce cultivars. The experimental design was completely randomized, in a split-plot design, with four replicates. We tested Zn concentrations of 0.3, 1.0, 1.7 and 2.4 mg L^{-1} in the hydroponic solution and the cultivars 'Vanda' and 'Saladela'. We evaluated plant diameter, number of leaves, fresh and dry mass of the aerial part and root, a chlorophyll index, Zn content and the accumulation of Zn in leaves and roots. 'Vanda' lettuce had the largest diameter, and 'Saladela' lettuce had the most leaves. The fresh mass of the aerial part was higher in 'Vanda' lettuce. For a Zn concentration of 2.4 mg L^{-1}, the Zn contents of the leaves and roots were 733.3 and 2441 mg kg^{-1} and the accumulations of Zn in the leaves and roots were 931.66 and 4890 mg $plant^{-1}$, respectively. Zn content and accumulation were higher in 'Saladela' lettuce. FM, DM did not decrease with increasing Zn concentration in the nutrient solution. Agronomically biofortifying the lettuce produced hydroponically using the nutrient film technique was thus possible.

Keywords: *Lactuca sativa* L.; leafy vegetable; nutrient content; supplying nutritional; zinc sulfate.
Abbreviations: NFT_nutrient film technique; Zn_zinc; FMAP_fresh mass of the aerial part; DMAP_dry mass of the aerial part; RFM_root fresh mass; RDM_root dry mass; RCI_relative chlorophyll index; FM_fresh mass; DM _dry mass; PD_plant diameter; NL_number of leaves; LZC_leaf zinc content; LZA_leaf zinc accumulation; RZC_root zinc content; RZA_root zinc accumulation; SMD_significant minimum difference; SPAD_soil plant analysis development.

Introduction

Lettuce is the most produced and consumed leafy vegetable in Brazil and the world and is one of the most cultivated species in hydroponic systems (Sala and Costa, 2012), where the nutrient film technique (NFT) is most commonly used. One of the benefits of this cultivation system for plants is the passage of the nutrient solution only through the root zone, without wetting the leaves.

Hydroponic systems allow greater productivity because they are closed nutritional systems and can more easily supply micronutrients than can conventional systems. This cultivation technique is increasing in popularity, with the potential for agronomic biofortification, especially for lettuce (Rouphael et al., 2018).

Lettuce is nutritionally important, providing high levels of phosphorus (20 mg 100 g^{-1}), potassium (141 mg 100 g^{-1}), vitamin C (2.8 mg 100 g^{-1}) and vitamin A (25 mg 100 g^{-1}), and is a source of fiber and low-calorie content (USDA, 2022). Humans, however, need about 22 nutrients to maintain an adequate and healthy metabolism (Graham et al., 2007), and some of these nutrients are not present or are at low levels in lettuce leaves, especially zinc (Zn) (National Institutes of Health, 2020).

Zn nutritional deficiency affects more than 2 billion people worldwide, affecting even children, and prevails in low-income countries, causing various health problems (Beal et al., 2017). The daily recommendations of Zn intake are 11 mg d^{-1} for men and 8 mg d^{-1} for women. The requirement of this mineral, however, is higher in some phases of life, such as during pregnancy, childhood, puberty and senility (Hambidge et al., 2008). Various methods for meeting the daily requirement of Zn are therefore being studied (Cakmak, 2008), particularly agronomic biofortification (Moraes et al., 2022).

Agronomic biofortification with Zn has recently been studied in some crops, mainly in cereals such as wheat, rice and corn, because these crops represent the majority of calories consumed, especially in developing countries (Hussain et al., 2012; Cakmak and Kutman, 2018). Clemens (2017), however, suggested that the biofortification of leafy vegetables would be a complementary strategy, because lettuces contain a lower percentage of phytic acid compared to cereals, so more Zn would be bioavailable for consumption. The intake of biofortified foods can thus help us to meet our nutritional requirements (Almeida et al., 2016).

The lack of research on agronomic biofortification in leafy vegetables in hydroponic crops reinforces the importance of such studies, which have hypothesized that an increase in Zn concentration in nutrient solutions would increase Zn contents in lettuce without affecting the productive characteristics of plants.

Results and discussion

An analysis of variance indicated that Zn concentrations and the lettuce cultivars did not interact for all traits evaluated. Zn concentration, however, had isolated effects on root fresh mass (RFM), root dry mass (RDM), leaf Zn content (LZC), leaf Zn accumulation (LZA), root Zn content (RZC) and root Zn accumulation (RZA). For the cultivars, there was an isolated effect for plant diameter (PD), number of leaves (NL), relative chlorophyll index (RCI), fresh mass aerial part (FMAP), leaf Zn content (LZC) and leaf Zn accumulation (LZA).

Plant diameter and number of leaves

The cultivar 'Vanda' had the largest PD, with an average of 41.1 cm (Table 1). The availability of Zn is strongly correlated with plant growth, because Zn is necessary for the synthesis of nucleic acids, proteins and carbohydrates (Lukaski, 1995). Leafy vegetables are packaged for transport in plastic or wooden boxes, so parameters such as PD are important (Sala and Costa, 2012).

PD is very relevant for consumers when they buy lettuce, so the application of Zn in the nutrient solution can be a promising alternative for producers, because it does not interfere in production. PD in our study was larger with Zn in the nutrient solution. Zn also benefits the population, because it is important for the synthesis and repair of DNA, RNA and proteins and influences biochemical and physiological processes involved in growth, cell differentiation, development and aging (Fukada et al., 2011).

The lettuce cultivar 'Saladela' had a higher NL, with an average of 29 leaves (Table 1). NL is a relevant parameter because leaves constitute the commercial part of the lettuce, and the attention of consumers at the time of purchase is focused on appearance, volume and NL (Filgueira, 2008).

NL in each plant may also indicate the adaptation of genetic material to the environment (Diamante et al., 2013), mainly temperature, photoperiod (Oliveira et al., 2004) and management used in crop production.

Ceccherini et al. (2020) tested 'Vanda' lettuce in various volumes of hydroponic trays and in the field. A volume of 50 cm^3 in the field had a circumference larger than 10 cm^3 in hydroponic production, but plants averaged fewer than 1.25 leaves, indicating the influence of better nutrition on production. A volume of 40 cm^3 in hydroponic production had the highest NL (37 leaves $plant^{-1}$). Hydroponically produced 'Vanda' i had 29 leaves $plant^{-1}$, even at the highest ZN concentration applied.

Fresh and dry masses of the aerial part and roots

Zn concentration did not have an isolated effect on FMAP or APDM, indicating that these characteristics did not decrease as the Zn concentration increased, with averages of 241.45 g and 13.6 g $plant^{-1}$ for 'Vanda' and 185.16 g and 13.6 g $plant^{-1}$ for 'Saladela', respectively (Table 1), but increased Zn content, as discussed below. RFM and RDM increased up to 20.27 and 2.40 g $plant^{-1}$ at Zn concentrations of 1.9 and 1.2 mg L^{-1}, respectively (Fig. 1A, B).

Sago et al. (2018) reported large reductions in fresh mass in baby leaf lettuce as Zn concentrations in the nutrient solution increased from 0.15 to 0.45 mg L^{-1}. The leaves became necrotic at Zn concentrations ≥0.15 mg L^{-1}, but higher concentrations in our study had no deleterious effects on the plants. This discrepancy between our study and the study by Sago et al. (2018) may have been due to differences in genotypes and cultivation conditions, because Sago et al. (2018) investigated Zn accumulation under various conditions, including wind speeds and temperatures in the root zone (30 °C), i.e. controlling environmental factors that can affect transpiration and decrease baby leaf fresh mass.

The different results for fresh mass among the cultivars can be accounted for by the genetic differences between the cultivars. FMAP differed between 'Vanda' and 'Saladela', which can lead to differences in morphological and productive parameters, even under similar climatic conditions. Ceccherini et al. (2020) reported an increase in fresh mass in 'Vanda' lettuce as the volume of seedlings produced in trays increased, which was tested in hydroponic production and the field.

Ozdener and Aydin (2010) evaluated concentrations of 0, 250, 500, 1000 and 2000 mg Zn L^{-1} in 'Istanbul'

Table 1. Plant diameter (PD), number of leaves (NL), fresh mass of the aerial part (FMAP), relative chlorophyll index (RCI), leaf zinc content (LZC) and leaf zinc accumulation (LZA) for the lettuce cultivars.

Cultivar	PD (cm)	NL	FMAP (g)	RCI (SPAD)	LZC (mg kg^{-1})	LZA (mg $plant^{-1}$)
'Saladela'	26.7b	29.0a	185.1b	25.1b	61.5a	750.9a
'Vanda'	41.1a	20.0b	241.4a	31.6a	50.4b	637.7b
SMD (5%)	1.7	1.2	16	5.7	6	112.4

Different letters within a column indicate significant differences identified by a Tukey's test ($p < 0.05$).

arugula cultivated in soil and found that leaf Zn contents were 8, 24, 147, 157 and 251 mg kg^{-1}, respectively, but did not observe phytotoxicity or reductions in the fresh and dry masses of the aerial part and roots. Much of the Zn absorbed by the plant remained in the roots. Zn content in our study was between seven- and eight-fold lower in the leaves than the roots. A large amount of Zn accumulated in the roots but did not inhibit growth. White and Broadley (2005) found that most plant species with leaf Zn contents >100 mg kg^{-1} had reduced growth. Barrameda-Medina et al. (2014) reported that leafy vegetables such as lettuce with leaf Zn contents >218 mg kg^{-1} could have lower dry masses of the roots and aerial parts, but the leaf Zn content was higher and lettuce dry mass was lower than in our study.

Moraes et al. (2022) found that RDM in 'Vanda' and 'Saladela' plants cultivated in soil was 2.1 and 2.4 g $plant^{-1}$ at Zn doses of 11 and 2 mg dm^{-3}, respectively. APDM and RDM decreased as Zn dose increased, contrary to our results for both cultivars in hydroponic production, i.e. leaf and root masses did not decrease as Zn dose increased, and the plant cycle was faster (28 days after transplanting) than with soil cultivation.

Relative chlorophyll index

'Vanda' averaged 31.7 SPAD units (Table 1). RCI is used to monitor plant development, providing information on the physiological state, nitrogen content in leaves and the photosynthetic potential of plants (Riccardi et al., 2014; Yang et al., 2014).

The application of high Zn concentrations to plants did not have a phytotoxic effect, with a slight increase (6%) in RCI of 'Saladela' for 'Vanda'. This finding may indicate leaves of excellent color quality, because chlorophyll content is directly correlated with photosynthetic activity and nutritional status. Roosta et al. (2018) reported a decrease in chlorophyll content caused by both Zn deficiency and toxicity (Anwaar et al., 2015). Ebbs and Uchil (2008) found that iron (Fe) deficiency due to high doses of Zn was one of the causes of decreased RCI in *Brassica juncea* L. Fe content in our study decreased from 0.9 to 0.2 mg L^{-1} as the lettuce Zn concentration increased, but RCI did not decrease.

Some studies have demonstrated the efficiency of cultivation in hydroponic systems, such as Souza et al. (2019), who evaluated the development of 'Crocantela' lettuce plants compared to soil cultivation. Their hydroponic system provided better nutritional and environmental conditions, so the plants differed significantly in chlorophyll content: 0.448 mg g^{-1} for chlorophyll a, 0.123 mg g^{-1} for chlorophyll b and 0.571 mg g^{-1} for total chlorophyll. The plants also had a higher leaf mass (305 g) compared to plants grown in soil (35 g).

Zn content and accumulation in plants

Zn content increased linearly for leaves and quadratically for roots as the Zn concentration in the nutrient solution increased (Fig. 2). Zn contents in the leaves and roots were 733.3 and 2441 mg kg^{-1}, respectively, at the highest concentration of 2.4 mg L^{-1}. When Zn is supplied to plants, contents in plant tissues decrease in the order roots > leaves > fruits > seeds, because the reserve organs obtain most of their minerals through the phloem, where Zn has little mobility (Broadley et al., 2012). Lettuce can thus have a higher amount of the nutrient than do grains and cereals when biofortified, because the leaves are the edible part, so consuming smaller portions (smaller quantities) but with the same Zn concentration in the nutrient solution is possible.

Zn contents are higher in root than leaf tissues when Zn is applied in larger amounts due to the genetic regulation of the absorption and transport of this micronutrient, among other factors (Gupta et al., 2016), accounting for the higher content and accumulation in the roots (seven-to eight-fold less Zn accumulated in the leaves than the roots). We do not consume lettuce roots, but Zn content was high in our study. These roots thus have the potential to concentrate the micronutrient, so consuming an organic product rich in root Zn is possible, i.e. powdered roots could be useful for the supplementation of Zn for humans. Hydroponics could thus facilitate this supplementation, because hydroponic products maintain the roots, facilitating their harvest/extraction, unlike cultivation in the field.

LZC was higher in 'Saladela' (Table 1). White and Broadley (2011) reported that species of the

Table 2. Amount of Zn in 50 g of fresh leaf mass for the lettuce cultivars 'Vanda' and 'Saladela' and its percentage of the recommended daily intake.

Zn concentration (mg L^{-1})	Zn (mg)	Zn daily recommendation[1] (%)
	'Vanda'	
0.3	0.08	8
1.0	0.13	13
1.7	0.12	12
2.4	0.17	17
	'Saladela'	
0.3	0.10	10
1.0	0.18	18
1.7	0.21	21
2.4	0.26	26

[1]Recommended intake of 10 mg Zn d^{-1} (Department of Health – UK, 1991; Russell et al., 2001).

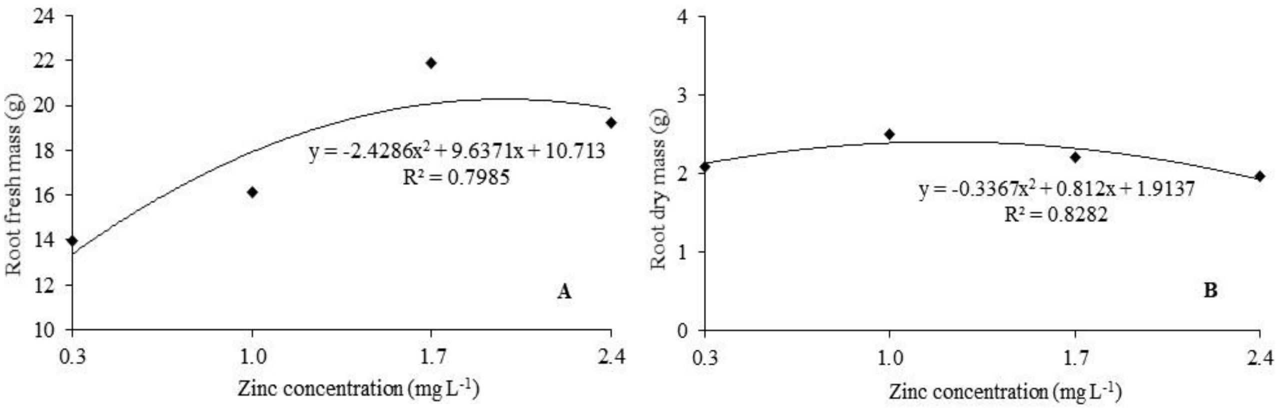

Fig 1. Root fresh mass (A) and root dry mass (B) for the lettuces 28 days after transplanting as a function of the zinc concentration in the nutrient solution in hydroponic cultivation.

Asteraceae family tended to have high Zn contents in the leaves and roots, consistent with our results for the Zn contents in both tissues. Zn contents in roots and leaves can be increased by applying Zn fertilizers. Root Zn contents of up to 500-5000 mg kg^{-1} of dry mass and leaf Zn contents of up to 100-700 mg kg^{-1} of dry mass can be achieved without the loss of yield. Zn content in our study was even higher in the roots than the leaves.

Zn accumulated linearly in the leaves and roots (Fig. 3). Zn contents were 931.66 and 4890 mg $plant^{-1}$ in the leaves and roots, respectively, at the highest Zn concentration of 2.4 mg L^{-1}. Based on the accumulation of Zn, considering the estimate of the contribution of biofortified lettuce with Zn in the recommendation of daily intake of 10 mg of Zn, it was observed that the amounts of Zn in the 50 g part obtained at the concentrations of Zn applied, especially for 'Saladela' at the concentration of 2.4 mg L^{-1}, indicated contribution of up to 26% more in daily intake, while for 'Vanda' can contribute up to 17%, in the highest concentration of Zn applied (Table 2).

A daily intake of 50 g of biofortified lettuce can thus contribute more Zn than can larger intakes of grains and cereals, because they contain large amounts of phytic acid. Chattha et al. (2017) found that three wheat cultivars biofortified with Zn sulfate contained between 4.0 and 5.6 mg of Zn in 100 g of grains, but these grains contained between 911 and 1090 mg of phytic acid. Saha et al. (2017) observed that the Zn contents in cooked rice of six cultivars biofortified with Zn ranged from 11.2 to 15.6 mg Zn kg^{-1}, and phytic acid ranged from 1400 to 2600 mg kg^{-1}, so 300 g of cooked rice had between 0.15 and 0.19 mg of bioavailable Zn, corresponding to 15 and 19% contributions to the recommended daily Zn intake, respectively.

The 'Saladela' lettuce accumulated more Zn in the leaves compared to 'Vanda' (Table 1). The micronutrient in lettuce grown in nutrient solution may be effectively absorbed, which is a characteristic of plants grown in media with a high availability of mineral nutrients. Zn at the concentrations applied in our study did not burn the lettuce leaves (Fig. 4).

Symptoms of Zn phytotoxicity include low productivity, slow growth and chlorosis induced by Fe deficiency due reductions in chlorophyll synthesis and chloroplast degradation and interference in the

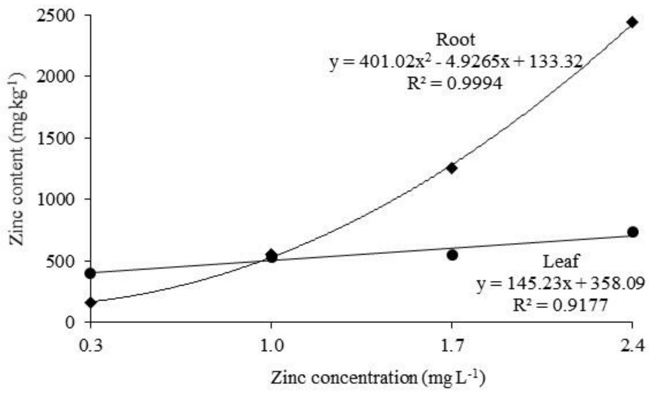

Fig. 2. Zinc content in the leaves and roots for the lettuces 28 days after transplanting as a function of the zinc concentration in the nutrient solution in hydroponic cultivation.

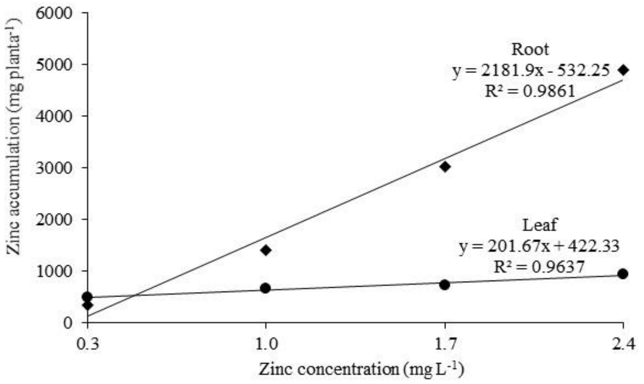

Fig. 3. Zinc accumulation in the leaves and roots for the lettuces 28 days after transplanting as a function of the zinc concentration in the nutrient solution in hydroponic cultivation.

absorption of phosphorus, magnesium and manganese (Chaney, 1993). Studies of biofortification, however, indicate that vegetables can tolerate high leaf Zn contents, with values of 259 (Padash et al., 2016) and 1000 mg Zn kg^{-1} of dry mass in lettuce and 465 mg Zn kg^{-1} (Barrameda-Medina et al., 2017) in cabbage. Zn in our study did not reduce the growth or the chlorophyll content of the leaves, indicating an increase in the accumulation of Zn in the leaves.

White and Broadley (2011) reported dry masses ranging from 0.074 to 1.201 mg Zn g^{-1} for cabbage genotypes and from 0.117 to 1.666 mg Zn g^{-1} for broccoli genotypes. The highest contents exceeded the values estimated by White and Broadley (2011) for the potential of leafy vegetables to absorb Zn, supporting the hypothesis that they could reach higher Zn contents compared to grains, roots or tubers.

More studies of the bioavailability of nutrients from vegetables for human absorption, however, need to be conducted, because current research for this purpose and research focusing on the biofortification of leafy vegetables in hydroponic cultivation is limited.

Materials and methods

Experimental site

The experiment was conducted in a protected environment in a hydroponic system in the experimental area of the Department of Biotechnology and Plant and Animal Production, sector Horticulture, at the Center of Agrarian Sciences, University Federal of São Carlos, in Araras, SP (22°21'S, 47°23'W; 640 m a.s.l.).

Treatments and experimental design

The experimental design was completely randomized, in a split plot scheme were plots were zinc concentrations and subplots lettuce cultivars, with four replicates. The following zinc (Zn) concentrations in the nutrient solution were based on the standard Zn concentration of 0.3 mg L^{-1} recommended by de Furlani et al. (1999): 0.3 1.0 1.7 and 2.4 mg L^{-1}. 'Vanda' and 'Saladela' lettuces were cultivated using the nutrient film technique, with spacings of 0.25 × 0.25 m (plant and profile).

Experimental procedure

Lettuce seedlings were produced in a commercial nursery (IBS Mudas in Piracicaba, SP) in polypropylene trays with 128 cells. The seedlings were subsequently transplanted for hydroponic cultivation in a protected environment.

The nutrient solution we used followed the recommendation by Furlani et al. (1999), with 500 g L^{-1} calcium nitrate, 500 g L^{-1} potassium nitrate, 100 g L^{-1} monoammonium phosphate and 350 g L^{-1} magnesium sulfate. ConMicros fertilizer was used, with the following composition: iron (7.26%), copper (1.82%), manganese (1.82%), boron (1.82%), molybdenum (0.36%), nickel (0.36%) and Zn (0.73%). The Zn concentrations for each treatment were obtained based on the concentration of Zn in ConMicros and the amount of Zn (20%) in zinc sulfate ($ZnSO_4 \cdot 7H_2O$).

The composition of the zinc treatments were: treatment 1 (41 g L^{-1} of ConMicros); treatment 2 (41 g L^{-1} of ConMicros + 3.5 g L^{-1} of zinc sulfate); treatment 3 (41 g L^{-1} of ConMicros + 7.0 g L^{-1} of zinc sulfate); treatment 4 (41 g L^{-1} of ConMicros + 10.5 g L^{-1} of zinc sulfate). Obtaining the final concentrations of 0.3 1.0 1.7 and 2.4 mg Zn L^{-1}.

The plants were harvested 28 d after transplantation. Three plants of each replicate were evaluated for agronomic characteristics. Two plants were evaluated for the relative chlorophyll index.

Characteristics assessed

Plant diameter (PD), number of leaves (NL), fresh and dry masses of the aerial part (leaves and stem) and roots were evaluated. The plants were dried in an oven with forced air circulation at 65 °C to a constant mass to obtain the dry masses. The relative chlorophyll index was obtained using the SPAD-502

Fig 4. Photographs of the cultivars 'Saladela' (left) and 'Vanda' (right) 28 days after transplanting hydroponically cultivated at the four zinc concentrations (mg L^{-1}) in the nutrient solution.

Plus device model in two readings per leaf and two leaves/plant.

The dried leaves and roots were sent to the Soil and Plant Analysis Laboratory of the Agronomic Institute in Campinas, SP, to determine their Zn contents using metod perchloric nitric digestion and spectrometric optical emission with inductively coupled plasma (Johnson and Ulrich, 1959).

Zn accumulation was calculated as:

$$A = DM \ x \ NC$$

where A is accumulation, DM is dry mass of the aerial part (mg) and NC is nutrient concentration (mg kg^{-1}).

The contribution of biofortified lettuce based on Zn accumulation and fresh leaf mass was estimated using the recommendation of a daily intake of 10 mg of Zn (Department of Health - UK 1991). We used a consumption of 50 g of fresh leaf mass of the cultivars to determine the amount of Zn in the leaves.

Statistical analysis

The data were submitted to analyses of variation. Regression analysis was performed for the Zn concentrations, using the highest coefficient of determination (R^2) and the lowest standard error to determine the best fit (Epad). The Tukey test was used for the cultivars, for both analyses Sisvar software was used and Excel 2016 software was used for the preparation of tables and graphs.

The Tukey test at p<0.05 was used for the characteristics that differed significantly between the cultivars, and regression analysis was used for the characteristics that differed significantly between the Zn concentrations.

Conclusion

Lettuce produced hydroponically using NFT could be agronomically biofortified. FM, DM did not decrease with increasing Zn concentration in the nutrient solution and the Zn content increased up to 733.3 mg kg^{-1} in leaves and up to 2441 mg kg^{-1} (2.4 mg Zn L^{-1}) in roots.

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