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Soil Type and Zinc Doses in Agronomic Biofortification of Lettuce Genotypes

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Received: 29 November 2019; Accepted: 8 January 2020; Published: 15 January 2020

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Abstract: The incidence of many malnutrition-related diseases among the populations of developing countries is closely related to low dietary zinc (Zn) intakes. This study evaluated the potential of agronomic biofortification of lettuce genotypes with Zn in different soils. We evaluated the ability to biofortify three lettuce genotypes ('Grand Rapids', 'Regina de Verão', and 'Delícia') in two soils (Red-Yellow Latosol and Dystroferric Red Latosol) using five doses of Zn (0, 5, 10, 20, and 30 mg kg^{-1}). At 55 days after sowing, the plants were harvested. There was an interaction among the soils, genotypes, and Zn doses. Regardless of the soil and genotype, the increase in Zn supply promoted a linear increase in shoot Zn concentration. However, shoot and root dry matter yields were differentially affected by Zn supply according to the genotype and soil type. Overall, the Red-Yellow Latosol provided a higher shoot Zn concentration but also caused greater growth damage, especially in 'Regina de Verão' and 'Delícia'. 'Grand Rapids' was biofortified the most in Red-Yellow Latosol.

Keywords: *Lactuca sativa*; nutritional value; cultivars; micronutrient; food safety

1. Introduction

The population growth rate on the planet is 1.08% per year. The current population count is around 7.6 billion [1]. It is estimated to exceed nine billion by 2050 [2]. Considering that the primary sources of nutrients come directly or indirectly from foods produced by agriculture, this population increase strongly pressures the environment to meet such a demand [3,4]. Increasing crop yields has been important for reducing global undernourishment from about 19% to 13% between 1990 and 2010 [5]. However, micronutrient deficiency currently affects almost half of the world's population, and Zn deficiency is one of the major public health concerns in developing countries [6].

In humans, Zn deficiency is associated with poor dietary diversification [7] and is exacerbated by its poor availability in soils [8]. Tropical soils present naturally low fertility and low Zn concentration (0.6 to 2.0 mg kg^{-1}), affecting approximately 90% to 95% of the native Brazilian Cerrado soils [9]. Moreover, the availability of this micronutrient is dependent on several soil properties, such as pH or capacity for phosphorus adsorption [10]. Thus, fertilization with Zn is a good strategy to enrich agricultural products and increase the daily Zn intake of the population [11–13], especially for those who consume it in small amounts daily. According to the National Research Council, the recommended dietary allowance (RDA) of Zn that an adult needs is about 11 mg; however, one-fifth of the world's population consumes deficient quantities of this micronutrient [6].

In this sense, agronomic biofortification seeks to increase the nutrient concentration and bioavailability in the edible parts of plants. It is considered the most economical solution for micronutrient deficiency in humans and animals [14]. Several studies have shown that Zn fertilization is efficient at increasing the Zn concentration in foods [13,15,16]. Yet, leafy vegetables are more suitable for achieving higher Zn concentrations than fruits, tubers, or seeds, since Zn is mainly transported through the xylem of plants [8,17]. White et al. [16] found a linear increase in shoot Zn concentrations in different cabbage and broccoli genotypes grown under Zn applications with the Zn nitrate as the substrate. Those authors found a wide range of critical shoot Zn concentrations for cabbage (74–1201 mg kg^{-1}) and broccoli (117–1666 mg kg^{-1}), which exceeded the estimated potential for Zn biofortification of leafy vegetables (up to 700 mg kg^{-1}) [17]. For lettuce, the range of optimal leaf Zn concentrations is 20 to 60 mg kg^{-1} [18]. However, Padash et al. [19] found increases up to 185 mg kg^{-1} without toxicity effects on lettuce growing with 10 mg L^{-1} of Zn. This could be a significant contribution as a biofortified crop, since lettuce is the main leafy horticultural produce worldwide, with a production increase of about 62% in the least two decades [20].

Although studies have been carried out to prove the agronomic efficiency of Zn biofortification in agricultural products, it is interesting to address genotype–soil interactions and their effects on agronomic biofortification efficiency [8,17].

Thus, a better understanding of how Zn biofortification of lettuce is influenced by production factors may provide greater efficiency for this technology. This study evaluated the response of lettuce genotypes to Zn application for biofortification in two soils.

2. Materials and Methods

2.1. Location and Experimental Design

The experiment was carried out in greenhouse conditions at the Federal University of Lavras, Brazil (21°13′35″ S, 44°58′43″ W, altitude of 918 m). According to Koppen's classification (1936), the climate is humid subtropical (Cwa), mesothermal, with dry winters and the temperature of the coldest month is between −3 and 18 °C, with a rainy subtropical summer. The temperature of the hottest month is higher than 22 °C.

The experiment was completely randomized and distributed in a 5 × 3 × 2 factorial design, as follows: Five doses of Zn (0, 5, 10, 20, and 30 mg kg^{-1}) as Zn sulphate ($ZnSO_4$), three lettuce genotypes ('Grand Rapids', 'Regina de Verão', and 'Delícia'), and two soil types (Red-Yellow Latosol, RYL; and Dystroferric Red Latosol, dRL) in three replications.

2.2. Installation and Conduction of the Experiment

Samples from two soils were collected at a depth of 0–20 cm. The dRL and RYL samples were collected from a native forest and grazing soil, respectively, of the Lavras municipality. They were air-dried, sieved to <2 mm, and subjected to physicochemical soil analysis according to Corguinha et al. [10] and mineralogical analysis according to Souza et al. [21] (Table 1).

Table 1. Physicochemical and mineralogical features of Red-Yellow Latosol (RYL) and Dystroferric Red Latosol (dRL) at the 0–20 cm layer.

Soil Attribute	Soil		Soil Attribute	Soil	
	RYL	dRL		RYL	dRL
pH (H_2O)	5.0	4.9	CEC-T ($cmol_c\ kg^{-1}$)	4.3	9.1
Al^{3+} ($cmol_c\ kg^{-1}$)	0.7	1.3	V (%)	16.3	3.3
Ca^{2+} ($cmol_c\ kg^{-1}$)	0.4	0.1	Sand ($g\ kg^{-1}$)	730.0	240.0
Mg^{2+} ($cmol_c\ kg^{-1}$)	0.2	0.1	Silt ($g\ kg^{-1}$)	50.0	110.0
K^+ ($mg\ kg^{-1}$)	41.0	37.0	Clay ($g\ kg^{-1}$)	220.0	650.0
H^++Al^{3+} ($cmol_c\ kg^{-1}$)	3.6	8.8	Ds ($kg\ dm^{-3}$)	1.2	1.0
P ($mg\ kg^{-1}$)	0.8	0.8	SiO_2 ($g\ kg^{-1}$)	95.1	130
P rem ($mg\ kg^{-1}$)	26.5	8.5	Al_2O_3 ($g\ kg^{-1}$)	97.4	319
S ($mg\ kg^{-1}$)	6.2	7.1	Fe_2O_3 ($g\ kg^{-1}$)	36.2	172
B ($mg\ kg^{-1}$)	0.7	0.4	TiO_2 ($g\ kg^{-1}$)	6.2	22.0
Cu^{2+} ($mg\ kg^{-1}$)	0.9	1.7	P_2O_5 ($g\ kg^{-1}$)	0.0	0.9
Fe^{2+} ($mg\ kg^{-1}$)	64.7	93.4	Fe_d ($g\ kg^{-1}$)	10.8	102
Mn^{2+} ($mg\ kg^{-1}$)	5.9	3.3	Fe_o ($g\ kg^{-1}$)	0.1	9.2
Zn^{2+} ($mg\ kg^{-1}$)	0.6	0.8	Ct ($g\ kg^{-1}$)	752	293
OM ($g\ kg^{-1}$)	24.0	48.0	Gibbsite ($g\ kg^{-1}$)	63.0	359
BS ($cmol_c\ kg^{-1}$)	0.7	0.3	Ki	1.0	0.4
CEC-t ($cmol_c\ kg^{-1}$)	1.4	1.6	Kr	0.7	0.3

P rem = remaining phosphorus; CEC-T = cation exchange capacity at pH 7; OM = organic matter; CEC-t = effective cation exchange capacity; V = base saturation; Ds = apparent density; Ki = (% SiO_2 × 1.70)/% Al_2O_3; Kr = (% SiO_2 × 1.70)/[%Al_2O_3+ (% Fe_2O_3 × 0.64)]. These two indices indicate the degree of change or weathering of tropical soils.

Liming was performed with lime (CaO: 38.8%, MgO: 13.4%, PRNT: 94.4, Ca: 27.72%, and Mg 8.0%). The soil remained incubated for 30 days, with 70% of the total pore volume occupied by deionized water. After the incubation period, soil pH was 6.0. Then, soil samples received the following basic fertilization: 100 mg kg^{-1} of N, 200 mg kg^{-1} of P (MAP—60% of P_2O_5 and 11% of N), 100 mg kg^{-1} of K (KCl), 37.5 mg kg^{-1} of Mg, 50 mg kg^{-1} of S ($MgSO_4$ $7H_2O$), 0.5 mg kg^{-1} of B (H_3BO_3), 1.5 mg kg^{-1} of Cu ($CuSO_4$ $5H_2O$), 5 mg kg^{-1} of Mn ($MnSO_4$ H_2O), and 0.1 mg kg^{-1} of Mo (Na_2MoO_4 $2H_2O$). Along with the basic fertilization, the treatments (Zn doses) were applied.

Samples of each soil were again incubated for 10 days with 70% of the total pore volume occupied by deionized water. After this second incubation, the soil samples were individually harrowed. Then, the lettuce was sown, and after seven days, the most vigorous plant was left in each pot containing 5 dm^{-3} of soil. The water was replaced daily. At 25 and 40 days after sowing, 100 mg kg^{-1} of N (NH_4NO_3) and 100 mg kg^{-1} of K (KCl) were applied as a cover fertilization. Harvesting was performed 55 days after sowing by cutting lettuce plants close to the soil.

2.3. Growth and Biofortification with Zn

After harvesting the plants, the shoots and roots were washed with running deionized water, and the samples were placed to dry in a forced air oven at 65 to 70 °C. The shoots (SDM) and roots (RDM) dry matter were determined. Only the shoots were ground and subjected to Zn analysis. Subsequently, the dried tissues (approximately 200 mg) were weighed and acid-digested in 2.0 mL of HNO_3 with 2.0 mL of $HClO_4$ at 120 °C for 60 min, and then at 220 °C until $HClO_4$ fumes were observed. The Zn concentration in SDM was determined by an atomic absorption spectrophotometer (PerkinElmer Inc., AAnalyst 800®, San Jose, CA, USA). A standard reference material from the National Institute of Standards and Technology (NIST 1573a containing 30.94 mg kg^{-1} of Zn), as well as a blank sample were used in each batch of digestion for quality assurance and quality control in Zn determinations, which were considered satisfactory (>90% recovery).

2.4. Statistical Analysis

Data were subjected to analysis of variance and, when significant at 5% by the F test, a polynomial regression analysis was performed for Zn doses. The significant equations with the highest coefficient of determination were chosen (R^2). Linear correlation analyses were also performed between the Zn contents of the lettuce genotypes and the Zn doses applied to the soil. Analyses were run on the AgroEstat software [22].

3. Results

3.1. Growth of Lettuce Genotypes

The growth of the three lettuce genotypes varied, and it was affected by soil types and Zn doses. The shoots of lettuce genotypes were always larger when they were grown in RYL. However, Zn application caused a significant reduction in the shoot dry matter (SDM) of 'Regina de Verão' and 'Delícia' genotypes grown in RYL, which was not observed when grown in dRL (Figure 1).

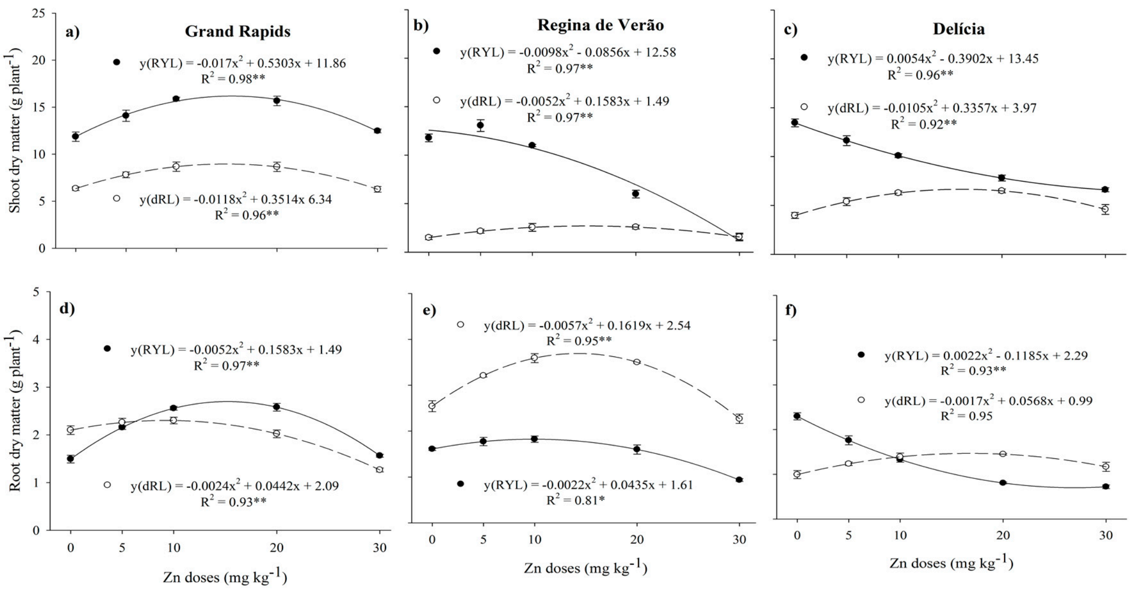

Figure 1. Shoots and roots dry matter of three lettuce genotypes grown in Red-Yellow Latosol (RYL) and Dystroferric Red Latosol (dRL) with different Zn doses. (**a**,**d**) presents the dry matter production in 'Grand Rapids'; (**b**,**e**) presents the dry matter production in 'Regina de Verão'; and (**c**,**f**) presents the dry matter production in 'Delícia'. *, ** and ns means significance at 1%, 5% probability and not significant by F test, respectively.

'Grand Rapids' had the best growth in response to Zn application in soils, yielding 16.0 and 9.0 g SDM per plant in RYL and dRL, respectively, with Zn doses of up to 15.6 mg kg^{-1} (Figure 1a). The 'Regina de Verão' and 'Delicia' genotypes had different responses to the tested soils. When both were grown in RYL, growth was constantly reduced by increasing Zn applications, achieving about 91% ('Regina de Verão') and 42% ('Delicia') lower yields of SDM at the maximum dose compared to the control. By contrast, 'Regina de Verão' and 'Delícia' yielded maximum values of 2.7 and 6.7 g SDM per plant, at the estimated Zn doses of 15.2 and 16.0 mg kg^{-1} in the dRL (Figure 1b,c).

Root system growth was affected by treatments in all three lettuce genotypes. The 'Grand Rapids' lettuce reached 2.7 g of RDM per plant in RYL with Zn doses of 15.2 mg kg^{-1}. On the other hand, the RDM yield in dRL remained stable (2.0 g per plant) at up to 10 mg kg^{-1} of Zn and thereafter decreased (Figure 1d). 'Regina de Verão' genotypes showed maximum RDM (3.7 g per plant) in dRL at 14.2 mg kg^{-1} of Zn, which is equivalent to a 48% increase over not applying Zn to the soil (Figure 1e). This lettuce genotype ('Regina de Verão') had a similar response to Zn fertilization when grown in RYL as that observed for 'Grand Rapids' grown in dRL, whose RDM yield remained around 1.8 g per plant up to a dose of 10 mg kg^{-1}, decreasing thereafter up to the Zn dose of 30 mg kg^{-1} (Figure 1e).

The 'Delícia' lettuce root growth decreased with the Zn application when grown in RYL, reaching the lowest RDM yield (0.8 g per plant) at a Zn dose of 20 mg kg^{-1}. This is a 65% lower RDM than in plants grown without Zn fertilization (Figure 1f). However, the 'Delicia' genotype had increased growth in dRL up to the Zn dose of 16.7 mg kg^{-1} but with maximum yields lower than those reached for the other genotypes.

3.2. Leaf Zn Concentration and Accumulation-Biofortification

The Zn concentration and accumulation in lettuce genotypes were affected by both soil and micronutrient doses. The highest Zn concentration and accumulation in the leaves of 'Grand Rapids', 'Regina de Verão', and 'Delícia' were found in the RYL (Figure 2).

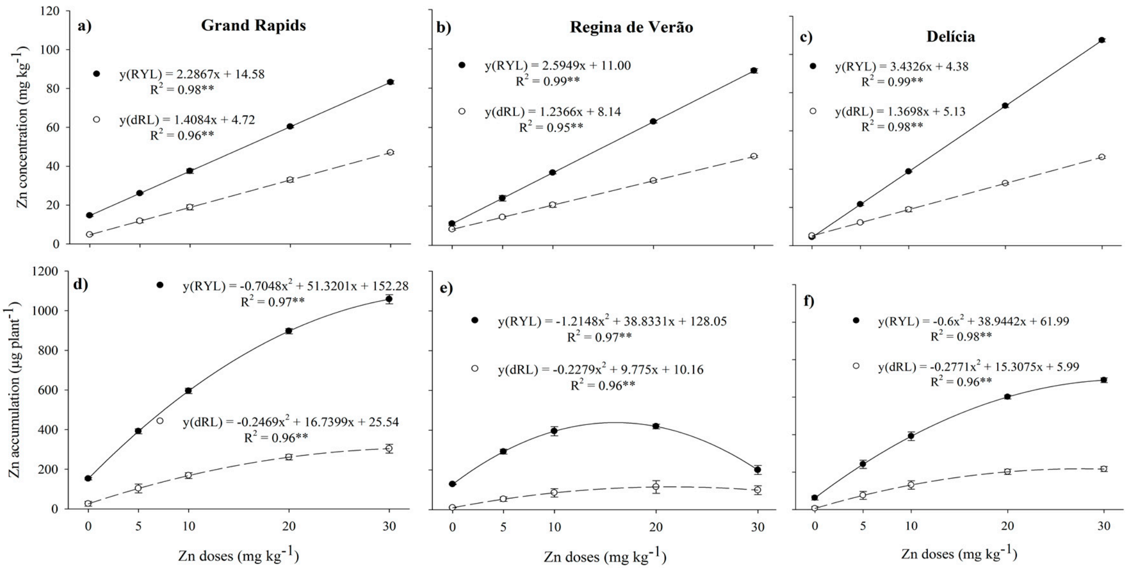

Figure 2. Zn concentration and accumulation in the shoot dry matter of three lettuce genotypes grown in Red-Yellow Latosol (RYL) and Dystroferric Red Latosol (dRL) with different Zn doses. (**a**,**d**) presents the values in 'Grand Rapids'; (**b**,**e**) presents the values in 'Regina de Verão'; and (**c**,**f**) presents the values in 'Delícia'. *, ** and ns means ignificance at 1%, 5% probability and not significant by F test, respectively.

Regardless of the soil, Zn concentrations in the three genotypes increased linearly in response to increased Zn supply (Figure 2a–c). All genotypes had the same leaf concentrations when not fertilized with Zn in dRL. By contrast, the Zn concentration in leaves of the 'Grand Rapids' (14.6 mg kg^{-1}) and 'Delícia' (4.4 mg kg^{-1}) genotypes were different when grown in RYL. Regarding the response amplitude to the Zn supply in RYL, the 'Delícia' genotype showed a 23-fold-increase compared to that observed in unfertilized plants while 'Grand Rapids' and 'Regina de Verão' had 5- and 6-fold increases compared to the control plants, respectively.

Among the lettuce genotypes, the 'Grand Rapids' grown in RYL had the highest Zn accumulation (1058.0 µg) in shoots (Figure 2d–f) after the highest Zn dose application. This is equivalent to a 246% increase in micronutrient absorption compared to the Zn accumulation (306.0 µg) in shoots fertilized with 30 mg kg^{-1} in dRL (Figure 2d). The 'Delícia' lettuce accumulated 690.3 µg of Zn in shoots at a dose of 30 mg kg^{-1}, i.e., a 3.2-fold greater accumulation when grown in the dRL using the same Zn treatment (Figure 2f).

The 'Regina de Verão' genotype had their highest Zn accumulation (439.6 µg) with the application of an estimated dose of 16.0 mg kg^{-1} in the RYL, which was 3.8-fold higher than the maximum accumulation obtained with the Zn dose of 21.4 mg kg^{-1} in the dRL (Figure 2e).

According to the correlation tests on the leaf Zn concentration and yield of SDM and RDM, lettuce genotypes responded differently to Zn fertilization in the two soils (Figure 3). A negative correlation was observed ($p < 0.001$) between RDM and Zn concentrations in 'Grand Rapids' ($r = -0.75$) grown in dRL. By contrast, the 'Regina de Verão' and 'Delícia' genotypes had negative correlations between leaf Zn concentration and RDM ($r = -0.78$; $r = -0.93$, respectively), and between leaf Zn concentration and SDM ($r = -0.94$; $r = -0$ 96, respectively) only when grown in the RYL.

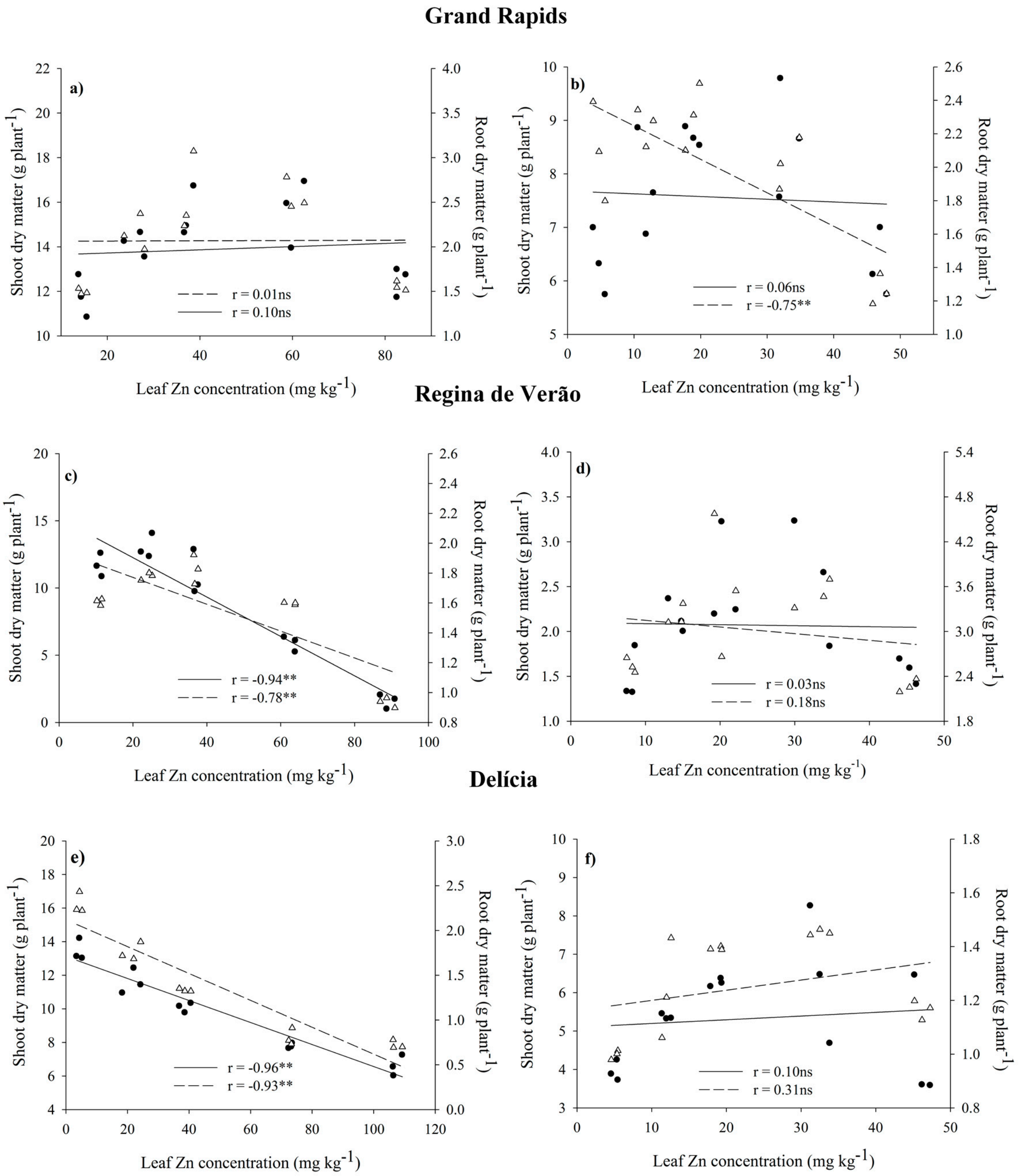

Figure 3. Correlation of leaf Zn concentration with shoot dry matter (●) and root dry matter (Δ) in three lettuce genotypes grown in Red-Yellow Latosol and Dystroferric Red Latosol with the application of different Zn doses. (**a**,**c**,**e**) presents the correlations obtained in the Red-Yellow Latosol; and (**b**,**d**,**f**) presents the correlations obtained in the Dystroferric Red Latosol. *, ** and ns means significance at 1%, 5% probability and not significant by F test, respectively.

4. Discussion

According to the results, all three genotypes of lettuce studied have a different ability to be biofortified in the same soil. By applying the dose for highest SDM yield, the 'Grand Rapids' (15.6 mg kg^{-1}), 'Regina de Verão' (4.4 mg kg^{-1}), and 'Delícia' (0 mg kg^{-1}) genotypes accumulated 781.3, 265.4, and 61.9 µg of Zn in the shoots of plants grown in RYL, respectively. Therefore, the 'Grand Rapids' genotype was enriched more with Zn without a loss of yield and presented a greater capacity for Zn biofortification. Micronutrient fertilization has been more successful in increasing the Zn concentration in the edible portion of leafy vegetables [13,16,23] than plants whose edible parts are reserve organs [17].

A similar linear increase of the leaf Zn concentration as in the three lettuce genotypes in both soils (Figure 2) was also found by White et al. [16] on genotypes of cabbage and broccoli grown in substrate. This is because the plants are natural extractors of minerals from the soil, without the distinction of how much is absorbed. This leads to phytotoxicity and consequently low crop yields, as found in our results (Figure 1). The increased availability of this micronutrient in soil coupled with its predominantly xylem transport causes a greater concentration in the organs (leaves) with s higher transpiratory rate [24]. However, the SDM yield was significantly reduced in all three genotypes at the dose of 30 mg kg^{-1}, because, although lettuce leaves were strongly Zn enriched, their biofortification capacity was limited by phytotoxicity.

Moreover, the Zn availability from fertilizer depends on the soil conditions or types. The soils used in our study (Table 1) differed regarding their ability to make Zn available to plants and consequently biofortify lettuce genotypes. The clay-textured dRL has a lower concentration of P and and Mn^{2+}, higher buffer capacity, higher Fe and Al oxide contents, and higher organic matter and gibbsite (35.9%) than RYL. These features increase Zn adsorption and decrease its availability to plants [23,25]. In addition, increasing pH by liming in both soils may also reduce the availability of this micronutrient, especially in dRL [26]. Pongrac et al. [23] found that soil types affected red cabbage growth more than phosphorus and Zn treatments. Therefore, these authors concluded that more studies in different types of soils are necessary to investigate their impact on the biofortification of the edible parts of crops. This suggests that the increases in the concentrations of essential mineral elements in the edible portions of crops are subject to the plant absorption capacity and to the restrictions imposed by the environment [16].

The biofortification methods should be used considering the genotype × soil interaction, since genotypes distinctly respond to different soils [10]. 'Regina de Verão' and 'Delicia' grown in RYL had the lowest tolerance to an increase in micronutrient concentrations, as demonstrated by the negative correlation found in these genotypes between biomass production and leaf Zn concentration (Figure 3c,e). Thus, we recommend biofortification with 7.8 and 3.7 mg kg^{-1} of Zn to the RYL and dRL, respectively, since higher doses resulted in a 10% loss of SDM yield of these genotypes. At these doses, we reached 31.2 and 17.1 mg kg^{-1} of Zn in the SDM of the 'Regina de Verão' and 'Delicia' genotypes, respectively. By contrast, the Zn dose of 15.6 mg kg^{-1} in the RYL maximized the SDM yield of 'Grand Rapids' and increased the leaf Zn concentration up to 50.3 mg kg^{-1}, almost a 250% higher micronutrient concentration than in plants not fertilized with Zn. If we consider the possibility of a 10% yield loss in SDM as in the other genotypes, 'Grand Rapids' would have its biofortification increased to 83.2 mg kg^{-1} Zn. On the other hand, 'Delícia' was the genotype with the highest Zn concentration (27.1 mg kg^{-1}) in SDM, followed by 'Grand Rapids' with 25.7 mg kg^{-1}, both grown in dRL soil.

The average concentration of Zn (43.8 mg kg^{-1}) from the highest leaf Zn concentrations were found for 'Regina de Verão' (31.2 mg kg^{-1}), 'Delicia' (17.1 mg kg^{-1}), and 'Grand Rapids' (83.2 mg kg^{-1}) without a loss of SDM yield. Therefore, if we assume that a daily serving size of the Zn-enriched lettuce is approximately 30 g fresh weight (or 3 g dry weight due to its 90% water content), consumption of the average Zn-containing lettuce (43.8 mg kg^{-1}) would result in the ingestion of 0.13 mg Zn, equivalent to 1.2% of the recommended dietary allowance for Zn (RDA; 11 mg day^{-1} Zn). Although these increments pose a small contribution to people's daily Zn intake, due to being the most widely produced and consumed leafy vegetable in the world, its biofortification will contribute to Zn intake.

Our results confirm the hypothesis of interaction between lettuce genotypes and soil conditions on the efficiency of agronomic biofortification supported by other studies [8,23]. Therefore, genetic and agronomic biofortification programs should be improved together, as their success depends on each other. Regardless of whether a new cultivar developed can absorb Zn, its success will depend on the availability of the Zn pool in the soil. In addition, plant breeding seems to be the most viable and economical way to reach rural populations with limited access to agronomically biofortified crops [14].

5. Conclusions

Lettuce genotypes responded differently to Zn application depending on the type of soil where they were grown.

Lettuce had better response to agronomic biofortification with Zn in a medium-textured Red-Yellow Latosol, with a lower buffer capacity and lower Zn adsorption than when grown in Dystroferric Red Latosol.

Author Contributions: Conceptualization, M.F.I. and A.E.F.N.; Formal analysis, M.F.I. and V.M.V.C.; Investigation, M.F.I. and H.J.d.A.; writing-original draft preparation, M.F.I. and H.J.d.A.; writing-review and editing, H.J.d.A., V.M.V.C., M.M. and A.B.C.F.; supervision, A.E.F.N.; project administration, M.F.I. and A.E.F.N.; funding acquisition, M.F.I. and H.J.d.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: To CNPq for granting the scholarship to the first and third authors, and to UFLA's Department of Soil Science for providing all the necessary conditions for the experiment.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Worldmeters. Available online: http://www.worldometers.info/world-population/#growthrate (accessed on 27 September 2019).
2. United Nations. *The Impact of Population Momentum on Future Population Growth*; United Nations: New York, NY, USA, 2017.
3. Sustainable Development Solutions Network. *Solutions for Sustainable Agriculture and Food Systems: Technical Report for the Post-2015 Development Agenda*; Sustainable Development Solutions Network: New York, NY, USA, 2015.
4. Godfray, H.C.J.; Beddington, J.R.; Crute, I.R.; Haddad, L.; Lawrence, D.; Muir, J.F.; Pretty, J.; Robinson, S.; Thomas, S.M.; Toulmin, C. Food Security: The challenge of feeding 9 billion people. *Science* **2010**, *327*, 812–818. [CrossRef] [PubMed]
5. FAO; WFP; IFAD. Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition. In *The State of Food Insecurity in the World 2012*; FAO: Rome, Italy, 2012.
6. Beal, T.; Massiot, E.; Joanne, E.; Arsenault, J.E.; Matthew, R.; Smith, M.R.; Hijmans, R.J. Global trends in dietary micronutrient supplies and estimated prevalence of inadequate intakes. *PLoS ONE* **2017**, *12*, e0175554. [CrossRef] [PubMed]
7. Wessells, K.R.; Brown, K.H. Estimating the global prevalence of zinc deficiency: Results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS ONE* **2012**, *7*, e50568. [CrossRef] [PubMed]
8. Hefferon, K. Biotechnological approaches for generating zinc-enriched crops to combat malnutrition. *Nutrients* **2019**, *11*, 253. [CrossRef] [PubMed]
9. Lopes, A.S.; Guilherme, L.R.G. A career perspective on soil management in the Cerrado region of Brazil. *Adv. Agron.* **2016**, *137*, 1–72.
10. Corguinha, A.P.B.; Carvalho, C.A.; de Souza, G.A.; de Carvalho, T.S.; Vieira, E.A.; Fialho, J.F.; Guilherme, L.R.G. Potential of cassava clones enriched with β-carotene and lycopene for zinc biofortification under different soil Zn conditions. *J. Sci. Food Agric.* **2019**, *99*, 666–674. [CrossRef] [PubMed]

11. Tittonell, P.; Giller, K.E. When yield gaps are poverty traps: The paradigm of ecological intensification in african smallholder agriculture. *Field Crop. Res.* **2013**, *143*, 76–90. [CrossRef]
12. Manzeke, M.G.; Mtambanengwe, F.; Nezomba, H.; Watts, M.J.; Broadley, M.R.; Mapfumo, P. Zinc fertilization increases productivity and grain nutritional quality of cowpea (*Vigna unguiculata* [L.] Walp.) under integrated soil fertility management. *Field Crop. Res.* **2017**, *213*, 231–244. [CrossRef]
13. Barrameda-Medina, Y.; Blasco, B.; Lentini, M.; Esposito, S.; Baenas, N.; Moreno, D.A.; Ruiz, J.M. Zinc biofortification improves phytochemicals and amino-acidic profile in *Brassica oleracea* cv. Bronco. *Plant Sci.* **2017**, *258*, 45–51. [CrossRef] [PubMed]
14. Bouis, H.E.; Saltzman, A. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Glob. Food Secur.* **2017**, *12*, 49–58. [CrossRef] [PubMed]
15. Poblaciones, M.J.; Rengel, Z. Soil and foliar zinc biofortification in field pea (*Pisum sativum* L.): Grain accumulation and bioavailability in raw and cooked grains. *Food Chem.* **2016**, *212*, 427–433. [CrossRef] [PubMed]
16. White, P.J.; Pongrac, P.; Sneddon, C.C.; Thompson, J.A.; Wright, G. Limits to the biofortification of leafy brassicas with zinc. *Agriculture* **2018**, *8*, 32. [CrossRef]
17. White, P.J.; Broadley, M.R. Physiological limits to zinc biofortification of edible crops. *Front. Plant Sci.* **2011**, *2*, 1–11. [CrossRef] [PubMed]
18. Maynard, D.N.; Hochmuth, G.J. *Knott's Handbook for Vegetable Growers*, 5th ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2007; pp. 179–181.
19. Padash, A.; Shahabivand, S.; Behtash, F.; Aghaee, A. A practicable method for zinc enrichment in lettuce leaves by the endophyte fungus *Piriformospora indica* under increasing zinc supply. *Sci. Hortic.* **2016**, *213*, 367–372. [CrossRef]
20. Faostat. Available online: http://www.fao.org/faostat/es/#data/QC (accessed on 29 December 2019).
21. Souza, R.F.; Faquin, V.; Fernandes, L.A.; Avila, F.W. Nutrição fosfatada e rendimento do feijoeiro sob influência da calagem e adubação orgânica. *Ciênc. Agrotec.* **2006**, *30*, 656–664. [CrossRef]
22. Barbosa, J.C.; Maldonado Júnior, W. *Experimentação Agronômica e Agroestat—Sistema Para Análises Estatísticas de Ensaios Agronômicos*, 1st ed.; Gráfica Multipress Ltda: Jaboticabal, Brazil, 2015.
23. Pongrac, P.; McNicol, J.W.; Lilly, A.; Thompson, J.A.; Wright, G.; Hillier, S.; White, P.J. Mineral element composition of cabbage as affected by soil type and phosphorus and zinc fertilization. *Plant Soil* **2019**, *434*, 151–165. [CrossRef]
24. Taiz, L.; Zeiger, E.; Moller, I.M.; Murphy, A. *Fisiologia e Desenvolvimento Vegetal*, 6th ed.; Artmed: Porto Alegre, Brazil, 2017; pp. 119–167.
25. Rafaey, Y.; Jansen, B.; Parsons, J.R.; Voogt, P.; Bagnis, S.; Markus, A.; El-Shater, A.-H.; El-Haddad, A.-A.; Kalbitz, K. Effects of clay minerals, hydroxides, and timing of dissolved organic matter addition on the competitive sorption of copper, nickel, and zinc: A column experiment. *J. Environ. Manag.* **2017**, *187*, 273–285. [CrossRef] [PubMed]
26. Broadley, M.; Brown, P.; Cakmak, I.; Rengel, Z.; Zhao, F. Function of nutrients: Micronutrients. In *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Marschner, P., Ed.; Academic Press: London, UK, 2012; pp. 191–248.