# Root morphology and kinetics of Zn absorption by roots of common bean influenced by Zn status of the root environment[1]

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## ABSTRACT

Understanding the kinetics of Zn absorption by roots and its effect on morphology of this organ is relevant for improving crop management, but still poorly studied for common beans. Therefore, an experiment was conducted in a hydroponic system with five initial concentrations of Zn (CZnI): 0.0; 1.0; 4.0; 16.0 and 48.0 μmol L$^{-1}$. The experiment was installed with plants at V3 stage of development and aliquots of the solution collected over 24 h. The maximum absorption rate (Vmax), Michaelis-Menten constant (Km) and the absorption power ($\alpha$) increased as a function of CZnI. The minimum concentration of Zn estimated for its absorption (Cmin) was at 0.0028 mg L$^{-1}$. The influx of Zn (Imax) was higher in higher CZnI, 16,0 μmol L$^{-1}$. Root length, root volume, root Zn content and Zn absorption efficiency increased with the increase of CZnI. Therefore, the increase of CZnI positively influenced kinetic parameters of root Zn absorption and common bean root morphology, characteristics that favor Zn absorption by roots and improves overall plant nutrition, favoring agronomical biofortification practices for Zn and other nutrients.

**Keywords:** root Zn inflow; absorption efficiency; kinetic parameters; Vmax; Cmin; Km.

## INTRODUCTION

Zinc (Zn) is an essential element for plant growth and development, which is absorbed primarily by the roots (Longnecker & Robson, 1993; Cakmak, 2008). Due to the metabolic functions of Zn, this nutrient is a plant growth promoter, regulating different morphological, physicochemical, molecular and metabolic processes in cultivated plants (Patel *et al*., 2018).

The absorption of Zn$^{2+}$ from the soil solution by the roots is a dynamic, complex and still poorly understood process. The accumulation of Zn in the roots over time is known to be biphasic, comprising the initial phase of rapid intake due to its binding on the negative charges present on the root cell walls, followed by the slower phase when the nutrient is transported through the plasmalemma (Hacisalihoglu *et al*., 2001; Meng *et al*., 2014). The absorption kinetics of Zn by wheat follows the Michaelis-Menten model and shows the rapid phase in the first 6 h and the slowest phase during the subsequent period (Hacisalihoglu *et al*., 2001). In bean plants, Zn absorption occurs predominantly fast, irreversibly and without metabolic energy expenditure (Joseph *et al*., 1971; Broughton *et al*., 2003).

Common beans, despite being one of the most consumed foods in underdeveloped countries in Latin America and Africa, few studies have been conducted on the absorption of Zn by the roots of the plant, especially when the interest is mineral nutrition for the biofortification of grains with Zn. Blair (2013) reviewed the advantages and needs of bean biofortification, however, his focus was on improving the development of new biofortified varieties, with a lesser approach to agronomic biofortification

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strategies. Cambraia *et al*. (2019), investigating the agronomic biofortification of beans with Zn, found that combined Zn in the soil and foliar application increases its concentration in beans. However, the processes related to the enrichment of the grains with Zn is still a gap, which deserves to be deepened so that it can understand how agronomic biofortification can be related to plant characteristics such as ion absorption kinetics and root system morphology.

The Zn concentration in the soil solution is one of the main factors related to the rate of Zn absorption by the roots. The ion concentration in the soil solutioninfluences ion absorption kinetics, including: Vmax (maximum absorption rate), Km (external ion concentration providing half of Vmax), Cmin (minimum ion concentration in the solution required for the nutrient to be absorbed) and Imax (inflow or ion absorption rate in a solution with low ion concentration); and in the morphology of plant root tissues (Epstein & Hagen, 1952; Nie *et al*., 2017).

Plants can change root morphology depending on the available Zn concentration in the medium. Coffee plants grown without Zn showed changes in root morphology caused by the deficiency of this nutrient, with bigger root stamen diameter, thicker epidermis, and bigger cross-sectional area of the cortex and stele (Rosolem *et al*., 2005). The increase in root cortex and stele diameter increased the surface area for nutrient absorption, which led to a lower Cmin (from 13.8 to 3.4 $\mu$mol $L^{-1}$) and higher Vmax (from 0.50 to 2.1 $\mu$mol $cm^{-2}$ $h^{-1}$) (Rosolem *et al*., 2005).

Identifying plant characteristics that increase plant's ability to accumulate Zn is important for plants and human nutrition. Even though common bean has not been the focus of biofortification studies yet, it is a staple food highly consumed by the low-income population of Latin America and certain African countries, which could definitely help to deliver Zn and keep people healthier. It is already a good source of Fe and, as other leguminous plants, it has a great potential for Zn and other metals accumulation in the grains, giving its high protein content.

Thus, it is important to understand Zn uptake to improve agronomic biofortification management practices and to guide plant breeding studies for genotypes that are more efficient in Zn absorption and accumulation. Therefore, the objective of this work was to evaluate the absorption kinetics of Zn by common bean roots and the influence of Zn status on the morphology of the root system at the V3 phenological stage, aiming to improve the agronomic biofortification of common bean grains.

## MATERIAL AND METHODS

### *Application of Zn concentrations*

For the experiment we used the common bean (*Phaseolus vulgaris L.)* cultivar BRSMG. The whole experiment was carried out in a greenhouse with temperature control (24 °C) and photoperiod of 13 h of light. Common bean seeds were germinated in washed sand, and after 10 d seedlings with fully expanded cotyledonary leaves were transplanted to a vessel covered with aluminum foil and with capacity for 150 mL of solution. Clark's solution with 1/8 ionic strength (Clark, 1977) was used and plants were kept in this condition until they reach V3 stage of development, which corresponds to the first pair of fully expanded leaves. Then, plants were transferred to 1/2 ionic strength Clark's solution, where they remained for 5 d. The solution received continuous aeration and the pH was adjusted daily to 6.0 (± 0.5).

After 3 d the nutrient solution was replaced by the solution with 0.2 mmol $L^{-1}$ of $CaSO_4$ and 12.5 $\mu$mol $L^{-1}$ of $H_3BO_3$ by 48 h, to ensure integrity of cell membranes and increase Zn absorption capacity by roots (Lee & Kathryn, 1985). After that plants were transferred to the solutions containing the treatments, which consisted of five initial concentrations of Zn in solution (CZnI) – 0.0; 1.0; 4.0; 16.0 and 48.0 µmol $L^{-1}$ of Zn as $ZnSO_4.7H_2O$, plus 0.2 mmol $L^{-1}$ of $CaSO_4$ and 12.5 µmol $L^{-1}$ of $H_3BO_3$.

### *Zn absorption kinetics*

Once the treatments were applied, samples of 2 mL of the solution were collected manually 11 times throughout the day (0, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24 h after treatments application), on which pH and Zn concentration were measured. At the end of 24 h, the remaining solution volume was quantified and stored. The roots were separated from the shoot, washed with 10% (v/v) alcohol and placed in 25% (v/v) alcohol and stored at 5 ºC for later evaluation of morphological characteristics in a professional Epson XL 10000 scanner using the software WinRHIZO Pro 2009.

### *Morphological evaluations and Zn content in roots and shoot*

The plants morphological evaluations were: root mean volume (Vroot), root mean length (Lroot), root mean area (Aroot), root mean diameter (Droot), root dry matter mass (DDNroot), Zn content in roots (ZCnroot), length of shoot (LS), leaf area (LA), dry matter mass of shoot (DMMS) and Zn content in shoot (ZCS). For root and shoot Zn quantification, samples were oven dried with forced air circulation at 65 ºC until reaching constant weight. Then the material was milled to a size smaller than 20 mesh in a Wiley sieve mill, the samples were opened using nitro perchloric acid digestion (4:1, v/v) and Zn subsequently quantified by atomic absorption spectrometry.

### *Zn Absorption Kinetic Parameters*

Data on the volume of the remaining solution, DDMroot and Zn concentration in the aliquots over time were used to obtain the values of the kinetic parameters: maximum absorption rate of Zn (Vmax); Michaelis-Menten constant (Km), and estimated minimum concentration of Zn for absorption (Cmin) to occur. In addition, we calculated: i) root Zn absorption power ($\alpha$) – root capacity to absorb $Zn^{2+}$ from the solution, which is the relationship between Vmax and Km; ii) Zn influx (Imax) value representing the influx equal to the nutrient efflux (Eq. 1); iii) efficiency of Zn absorption (EAZn), relationship between plant Zn content by root dry matter mass; iv) efficiency of translocation n (ETZn), relationship between the Zn content in the shoot by the Zn content in the plant; and v) Zn utilization efficiency in the production of shoot dry matter mass (EUZn), ratio of shoot dry matter mass to shoot Zn content.

$$\text{Imax} = \frac{[Vmax\ (Znl - Cmin)]}{[Km + (Znl - Cmin)]} \qquad \text{Eq. 1}$$

### *Mathematical graph method and statistical evaluation*

The estimated Vmax, Km and Cmin values were obtained by the mathematical graphical method using the “Kinetic” program (Ruiz, 1985; Ruiz & Fernandes Filho, 1992). The experimental design was randomized with three replications. The analysis of variance and regression for all variables were performed using the SAEG 9.0 (SAEG,2005) program, the graphs and tables were made in excel.

## RESULTS

The Zn depletion curve in the solution indicates that there was a decrease on Zn concentration in all CZnI (Figure 1). However, evidence that there was no Zn efflux by bean roots when the solution had no Zn. The pH of the solution, measured in every sample, did not change for all CZnI, kept on average at 6.1.

For CZnI of 1.0; 4.0 and 16.0 µmol $L^{-1}$; concentrations at the end of the 24 h reached 0.03; 0.04 and 0.24 mg $L^{-1}$ (Figure 1). While for CZnI of 48 µmol $L^{-1}$ the final Zn concentration after 24 h was close to 1.0 µmol $L^{-1}$ (Figure 1). The remaining volume of the solution at the end of the experiment was similar for all CZnI, averaging 104.5 mL.

The kinetic parameters, Vmax, Km and á, increased linearly as a function of CZnI (Figure 2). By the adjusted regression equations, the highest values of Vmax, Km and á were 2.27 µmol/ g h; 1.58µmol $L^{-1}$ and 0.69 L/ g h; obtained at the highest CZnI of 48 µmol $L^{-1}$ (Figure 2). Cmin values did not differ between CZnI. Cmin were very low, close to zero, with an average of 0.0028 mg $L^{-1}$ (Figure 2).

The estimated Imax of Zn by common bean roots differed between CZnI (Figure 3). For the two smallest CZnI; 1.0 and 4.0 µmol $L^{-1}$, the inflow did not reach the maximum, characterizing crescent line. For the two highest concentrations; 16.0 and 48.0 µmol $L^{-1}$; had a hyperbolic response. At a concentration of 48.0 µmol $L^{-1}$, Zn absorption saturation was reached at a concentration well below 48 µmol $L^{-1}$. While at a concentration of 16 µmol $L^{-1}$ Imax was reached close to µmol $L^{-1}$ (Figure 3). Imax curves overlapped in the lowest CZnI concentration ranges (up to 16 µmol $L^{-1}$), for all concentrations studied (Figure 3).

Morphological variables of root and shoot of common bean plants; Aroot, Droot, LS, ZCS, ETZn and EUZn did not respond to CZnI, averaged 1415.88 $mm^2$; 3.31 mm; 11.12 cm; 26.87 mg $kg^{-1}$; 0.59 and 0.02 (Table 1). Already Vroot, Lroot, DMMroot, ZCroot, LA, DMMS and EAZn were positively influenced by the increase of CZnI, the highest values of these variables were obtained in CZnI of 48.0 µmol $L^{-1}$ (Table 1).

## DISCUSSION

Studies of ion absorption kinetics and morphological data of bean roots influenced by the concentration of Zn available in the solution have not been reported in

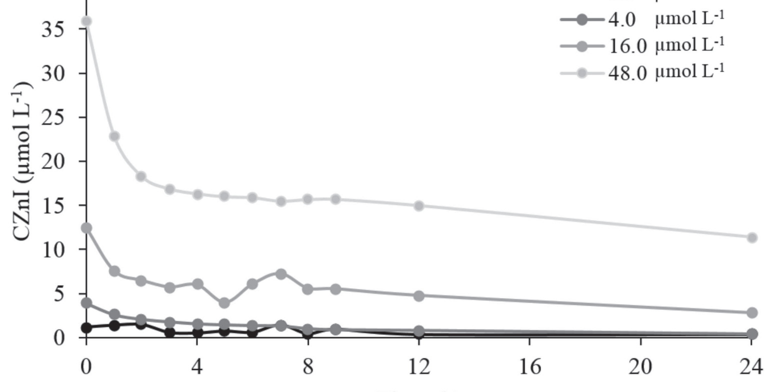

**Figure 1:** Zn depletion curve in the solution over 24 h as a function of the application of the initial concentrations of Zn in solution (CZnI) at the V3 stage of common bean development.

the literature. In this work, the kinetic parameters Vmax and Km have increased as a function of CZnI in solution, corroborated with Joseph, *et al*. (1971). As Cmin was low for all CZnI, it indicates that common bean has a high ability to absorb Zn from their roots (Nie *et al*., 2017).

In addition, high Vmax also favors root absorption as it is associated with low selectivity of $Zn^{2+}$ by the ion-transporting proteins (Epstein & Hagen, 1952; Fageria & Baligar, 1997; Hafeez *et al*., 2013). High Vmax and low Cmin are very interesting for Zn absorption. When common beans are grown in dilute solutions, when the soil has low levels of available Zn, as occurs naturally in most agricultural areas, including those in Brazil plants are still able to absorb Zn due to the low Cmin (Barber, 1995; Malavolta *et al*., 1997). On the other side, when Zn is applied as fertilizers, high Vmax also helps plants absorbing high amounts of the nutrient, favoring their accumulation in edible parts of the plant.

Zn absorption by bean roots was possibly performed by low and high affinity systems, as was observed for crops such as rice and wheat (Hacisalihoglu *et al*., 2001; Meng *et al*., 2014). The evidence that high affinity systems were active is that Cmin was very low. Besides that, Km values between 0.6 and 2 µmol $L^{-1}$ activate these transporters active (Hacisalihoglu *et al*., 2001) and in this work, the highest Km value was 1.58 µmol $L^{-1}$.

Absorption of Zn by common bean roots by low affinity transporters may have been mediated by ion channels and facilitated by the physical or physicochemical connection of $Zn^{2+}$ to the cell wall or free space components (Rathore, 1970; Joseph *et al*., 1971). In fertilizer management aimed at agronomic biofortification of the common bean with Zn, ion absorption by ion channels is a highly favorable feature. This is because ion channels allow a high influx of ions in a short period of time (Robson, 1993).

EAZn, á and Imax are characteristics of plants that measure the ability of roots to absorb ions. The higher the

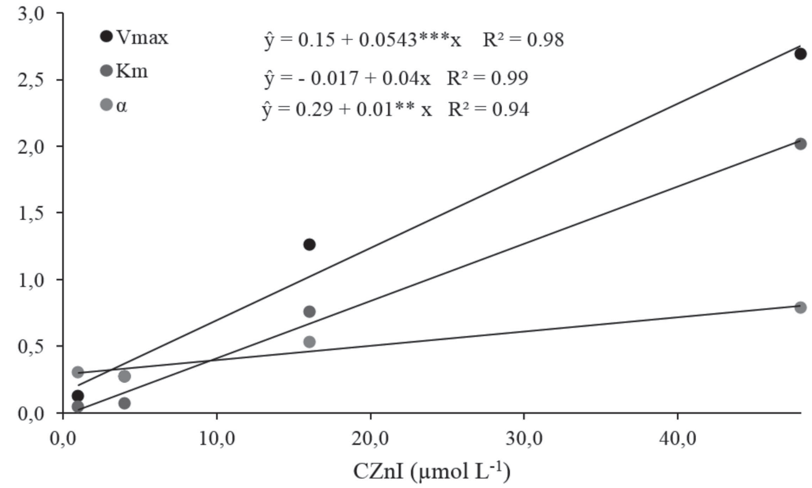

**Figure 2:** Regression equations adjusted for the kinetic parameters, Vmax, Km and á, as a function of the initial concentrations of Zn in solution (CZnI). Vmax (µmol/g h) - maximum ion absorption velocity, Km (µmol $L^{-1}$) - Michaelis-Menten constant, and (L/g h) - ion absorption power. **, ***, significant at 1 and 10% probability.

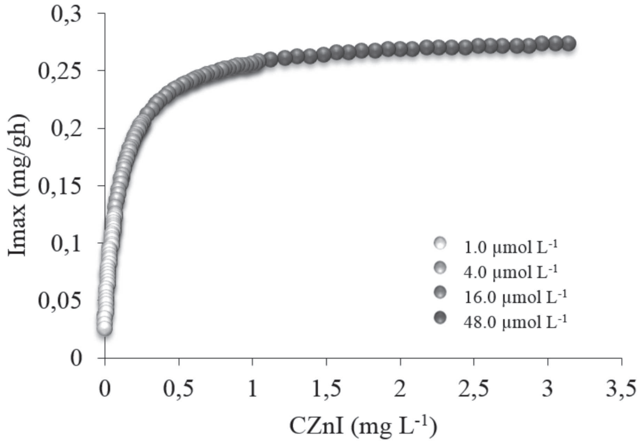

**Figure 3:** Estimated Zn Influx – Imax (mg/gh), as a function of the initial concentrations of Zn in solution (CZnI).

á value, the higher the rate of ion absorption per root unit and consequently the higher the nutrient acquisition by the plant (Nye & Tinker, 2000; Smith & Mullins, 2001; Sanes *et al*., 2013). The common bean presented higher EAZn and á when it increased CZnI, which may be related to the efficiency of the common bean in responding to Zn fertilization in the soil and enriching the grains when agronomic biofortification techniques are applied in the crop (Cambraia, 2019; Ram *et al*., 2016; Figueiredo *et al*., 2017).

Imax is obtained when all ion transport sites are loaded and ion absorption reaches a plateau. In the lowest CZnI, 1.0 and 4.0 $\mu$mol L$^{-1}$, inflow did not peak, a condition in which high affinity carriers can establish (Hacisalihoglu *et al*., 2001; Glass *et al*., 2002; Pedas *et al*., 2005). On the other hand, in CZnI 16.0 and 48.0 $\mu$mol L$^{-1}$ the curve was characteristic of this variable, a hyperbole. There was saturation of Zn absorption at the concentration from 16.0 µmol L$^{-1}$, so it is likely that at the concentration of 48.0 µmol L$^{-1}$ deluxe accumulation of Zn occurred, and that the CZnI of 16.0 µmol L$^{-1}$ s the interesting concentration to study the absorption of Zn by bean roots.

The concentration of Zn in the medium may alter root morphology, as well as root system morphological characteristics may explain the difference between genetic materials in nutrient absorption, translocation and accumulation (Sanes *et al*., 2013; Pinto & Nazareth, 2016). Because Zn is one of the components responsible for indole acetic acid (IAA) biosynthesis and activity in plants, it is expected to find differences in root systems of Zn deficient plants compared to non-deficient plants in Zn (Schäfer *et al*., 2016).

**Table 1:** Regression equations adjusted for the variables; Vroot, Lroot, MMSroot, TZnroot, Aroot, Droot, LAP, FA, MMSAP; TZnAP, EAZn, ETZn, EUZn; as a function of the initial concentrations of Zn in solution (CZnI), with the coefficients of determination

| **Variables** | **Ajusted equations** | **R$^2$** |
|---|---|---|
| Vroot (mm$^3$) | $\hat{y} = 13.10 + 974.74^{**}$ CZnI | 0.72 |
| Lroot (mm) | $\hat{y} = 246.56 + 89.97^{*}$ CZnI | 0.53 |
| MMSroot (g) | $\hat{y} = 0.047 + 0.0027$ CZnI | 0.90 |
| TZnroot (mg kg$^{-1}$) | $\hat{y} = 33.01 + 99.93^{*}$ CZnI | 0.91 |
| Aroot (mm$^2$) | $\hat{y} = \bar{y} = 1415.88$ | - |
| Droot (mm) | $= \bar{y} = 3.31$ | - |
| LAP (cm) | $= \bar{y} = 11.12$ | - |
| FA (cm$^2$) | $= 15.97 + 10.33^{**}$ CZnI | 0.95 |
| MMSAP (g) | $\hat{y} = 0.076 + 0.079^{*}$ CZnI | 0.91 |
| TZnAP (mg kg$^{-1}$) | $\hat{y} = \bar{y} = 26.87$ | - |
| EAZn | $= 136.78 + 35.038^{*}$ CZnI | 0.80 |
| ETZn | $\hat{y} = \bar{y} = 0.59$ | - |
| EUZn | $= \bar{y} = 0.02$ | - |

Vroot – root mean volume, Lroot - root mean length, Aroot - root mean area, Droot - root mean diameter, DMMroot - dry matter mass of root, ZCnroot - Zn content in roots, LS - length of shoot, LA – leaf area, DMMS - dry matter mass of shoot, ZCS - Zn content in roots, EAZn - Zn absorption efficiency, ETZn - Zn transport efficiency, EUZn - Zn utilization efficiency. ** and *significant at 1 and 5% probability.

The root morphology variables, Aroot and Droot, did not respond to CZnI, this may have occurred because the minimum tissue Zn concentration achieved in this experiment was not low enough to decrease the auxin level to interfere with the cell elongation (Riseman & Craig; 2000). While Vroot, Lroot and DMMroot increased with CZnI. Nie *et al*., 2017 observed that Zn concentrations in solution interfered with Lroot, Aroot, Vroot and Droot when the mineral wassupplied to the plants of. More Lroot and Aroot indicate that the plants can exploit better the soil, increasing its capacity to absorb nutrients (Batista *et al*., 2016). This is interesting especially for less mobile nutrients in the soil, which are transported primarily by diffusion such as P, K, Zn and Mn (Barber, 1995; Zonta *et al*., 2006). In addition, as the root system represents the biggest carbon (C) input for soil organic matter formation, Zn must be included in the fertilization practices in order to increase root system development and C input to the soils.

ETZn and therefore EUZn were not replied to the CZnI because ZCS did not change. Thus, the absorbed Zn was more concentrated in the root than in the shoot, possibly due to the short experiment period and root supply of Zn, only 24 h. Thus, the experiment conduction time was not sufficient for activation of compounds related to the requirement, mobility and activation of fundamental Zn transporters for Zn transport and utilization, such as carbonic anhydrases, alcoholic dehydrogenases, nicotianamine, metallothionein, glutathione, among others (Wilcox & Fageria, 1976; Sadeghzadeh & Rengel, 2011; Gupta *et al*., 2016; Patel *et al*., 2018).

The information generated in this study shows that common beans responded to the increase of Zn concentration in the solution by adjusting ion absorption kinetic parameters and altering root morphology, favoring Zn absorption. These common bean characteristics are fundamental to assist studies of agronomic and genetic biofortification with Zn, considered a worldwide challenge and important alternative to improve the nutritional quality of food and ensure a balanced diet (Pedraza, 2017; Cakmak & Kutman, 2018; Balk *et al*., 2019).

## CONCLUSIONS

The common bean responded to the increase in the concentration of Zn in the solution, adjusting kinetic parameters of ion absorption and changing the root morphology to favor the absorption of Zn.

Common beans have an efficient form of Zn absorption, with low Cmin and high Vmax and Km. Low Cmin ensures the absorption of Zn even at very low concentrations of Zn in the solution, which is important for plant nutrition. On the other hand, high Km and Vmax guarantee a high Zn intake when the concentration of Zn in the soil solution is high, as when Zn is added in fertilization practices, for example. This high Zn intake is highly appreciated for biofortification purposes, since translocation of Zn from the roots to the edible parts of the plants is a very difficult process.

Interestingly, there were also the rapid morphological responses of the bean roots to zinc in the external solution. Although the experiment lasted only 24 h, the volume, length and biomass of the root increase with the availability of Zn, showing the importance of this nutrient for the development of the bean root, influencing the absorption of water and nutrients and the C cycle itself.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in carrying the research and publishing this manuscript.

## REFERENCES

Balk J, Connorton JM, Wan Y, Lovegrove A, Moore KL, Uauy C & Sharp PA (2019) Improving wheat as a source of iron and zinc for global nutrition. Nutrition Bulletin, 44:53-59.

Barber SA (1995) Soil Nutrient Bioavailability: A Mechanistic Approach. 2º ed. New York, John Wiley & Sons. 46p.

Batista RO, Eduardo A, Neto F, Fernanda S, Deccetti C & Viana CS (2016) Root morphology and nutrient uptake kinetics by australian cedar clones. Revista Caatinga, 29:153-162.

Blair WM (2013) Mineral Biofortification Strategies for Food Staples: The Example of Common Bean. Journal of Agricultural and Food Chemistry, 61:8287-8294.

Broughton WJ, Hernacutendez G, Blair M, Beebe S, Gepts P & Vanderleyden J (2003) Beans (Phaseolus spp.) endash; model food legumes. Plant and Soil, 252:55-128.

Cambraia TLL, Fontes RLF, Vergütz L, Vieira RF, Neves JCL, Netto PSC & Dias RF N (2019) Agronomic biofortification of common bean grain with zinc. Pesquisa Agropecuária Brasileira, 54:e01003.

Cakmak I (2008) Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant soil, 302:01-17.

Cakmak I & Kutman UB (2018) Agronomic biofortification of cereals with zinc: a review. European journal of soil science, 69:172-180.

Cambraia TL, Fontes RLF, Vergütz L, Vieira RF, Neves JCL, Corrêa Netto OS & Dias RFN (2019) Agronomic biofortification of common bean grain with zinc. Pesquisa Agropecuária Brasileira, 54, e01003.

Clark A (1997) Being there: Putting brain, body, and world together again. Cambridge, MIT Press. 292p.

Figueiredo MA, Boldrin PF, Hart JJ, de Andrade MJB, Guilherme LRG, Glahn RP & Li L (2017) Zinc and selenium accumulation and their effect on iron bioavailability in common bean seeds. Plant Physiology and Biochemistry, 111:193-202.

Epstein E & Hagen CE (1952) A kinetic study of the absorption of alkali cations by barley roots. Plant physiology, 27:457-474.

Fageria NK & Baligar CV (1997) Response of common bean, upland rice, corn, wheat, and soybean to soil fertility of an Oxisol. Journal of Plant Nutrition, 20:1279-1289.

Glass ADM, Britto DT, Kaiser BN, Kinghorn JR, Kronzucker J, Kumar A, Okamoto M, Rawat S, Siddiqi MY, Unkles E & Vidmar JJ (2002) The regulation of nitrate and ammonium transport systems in plants. Journal of Experimental Botany, 53:855-864.

Gupta N, Ram H & Kumar B (2016) Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. Reviews in Environmental Science and Bio/Technology,15:89-109.

Hacisalihoglu G, Hart JJ & Kochian L V (2001) High and Low Affinity Zinc Transport Systems and Their Possible Role in Zinc Efficiency in Bread Wheat. Plant Physiology, 125:456-463.

Hafeez B, Khanif YM & Saleem M (2013) Role of Zinc in Plant Nutrition- A Review. American Journal of Experimental Agriculture, 3:374-391.

Joseph C, Rathore VS, Bajaj YPS & Wittwer SH (1971) Mechanism of Zinc Absorption by Intact Bean Plants. Annals of Botany, 35:683-686.

Lee RB & Kathryn AR (1985) Effects of Nitrogen Deficiency on the Absorption of Nitrate and Ammonium by Barley Plants. Oxford Journals, 57:471-486.

Longnecker NE & Robson AD (1993) Distribution and Transport of Zinc in Plants. In: Robson AD (Ed.) Zinc in Soils and Plants. Developments in Plant and Soil Sciences. Dordrecht, Springer. p.79-91.

Malavolta E, Vitti GC & Oliveira AS (1997) Avaliação do estado nutricional das plantas: princípios e aplicações. Piracicaba, Associação Brasileira para Pesquisa da Potassa e do Fosfato. 319p.

Meng F, Liu D, Yang X, Shohag MJI, Yang J, Li T, Lu L & Feng Y (2014) Zinc uptake kinetics in the low and high-affinity systems of two contrasting rice genotypes. Journal of Plant Nutrition and Soil Science, 177:412-420.

Nie Z, Zhao P, Wang J, Li J & Liu H (2017) Absorption Kinetics and Subcellular Fractionation of Zinc in Winter Wheat in Response to Nitrogen Supply. Frontiers in Plant Science, 8:01-10.

Nye P & Tinker P (2000) The uptake properties of the root system. In: Press NYOU. Solute movement in the rhizosphere, 448p.

Patel PJ, Trivedi GR, Shah RK & Saraf M (2018) Selenorhizobacteria: As biofortification tool in sustainable agriculture. Biocatalysis and Agricultural Biotechnology, 14:198-203.

Pedas P, Hebbern CA, Schjoerring JK, Holm PE & Husted S (2005) Differential capacity for high-affinity manganese uptake contributes to differences between barley genotypes in tolerance to low manganese availability. Plant physiology,139:1411-1420.

Pedraza DF (2017) Estudos realizados no Brasil sobre a deficiência e a suplementação de zinco: ênfase em crianças. Revista Brasileira de Saúde Materna e Infantil, 17:233-249.

Pinto GM & Nazareth R (2016) Green synthesis and characterization of zinc oxide nanoparticles. Journal of Chemical and Pharmaceutical Research, 6:427-432.

Ram H, Rashid A, Zhang W, Duarte AP, Phattarakul N, Simunji S, Kalayci M, Freitas R, Rerkasem B, Bal RS, Mahmood K, Savasli E, Lungu O, Wang ZH, Barros VLNP, Malik SS, Arisoy RZ, Guo JX, Sohu VS, Zou CQ & Cakmak I (2016) Biofortification of wheat, rice and common bean by applying foliar zinc fertilizer along with pesticides in seven countries. Plant and Soil, 403:389-401.

Rathore VS, Wittwer SH, Jyung WH, Bajaj YPS & Adams MW (1970) Mechanism of Zinc Uptake in Bean (Phaseolus vulgaris) Tissues. Physiologia Plantarum, 23:908-919.

Ribeiro Júnior JI(2001) Análises estatísticas no SAEG. Viçosa, UFV. 300p.

Rosolem CA, Sacramento LVS & Oliveira DMT (2005) Kinetics of zinc uptake and anatomy of roots and leaves of coffee trees as affected by zinc nutrition. Journal of Plant Nutrition, 28:2101-2112.

Riseman A & Craig R (2000). Physiological and morphological traits associated with zinc efficiency in Exacum. Plant Soil, 219:41-47.

Ruiz HA (1985) Estimativa dos parâmetros cinéticos Km e Vmax por uma aproximação gráfico-matemática. Revista Ceres, 32:79-84.

Ruiz HA & Fernandes Filho EI (1992) Cinética: Software para estimar as constantes Vmáx, Km da equação de Michaelis-Menten. In: Reunião brasileira de fertilidade do solo e nutrição de plantas. Anais, Piracicaba, Sociedade Brasileira de Ciência do Solo. p.124-125.

SAEG (2005) Sistema para análises estatísticas, Versão 9.0. Viçosa, Fundação Arthur Bernardes/UFV.CD-ROM.

Sadeghzadeh B & Rengel Z (2011) Zinc in Soils and Crop Nutrition. In: Hawkesford MJ & Barraclough P (Eds.) The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops. Oxford, Wiley-Blackwell. p.335–375.

Sanes FSM, Castilhos RMV, Scivittaro WB, Vahl LC & Morais JR (2013) Morfologia de raízes e cinética de absorção de potássio em genótipos de arroz irrigado. Revista Brasileira de Ciências do Solo, 37:688-697.

Schäfer M, Brütting C, Baldwin IT & Kallenbach M (2016) High-Throughput quantification of more than 100 primary- and secondary-metabolites, and phytohormones by a single solid-phase extraction based sample preparation with analysis by UHPLC – HESI – MS / MS. Plant Methods, 12:01-18.

Smith KA & Mullins CE (2001) Soil and environmental analysis: Physical methods. New York, Marcel Dekker. 637p.

Wilcox EG & Fageria NK (1976) Deficiências nutricionais do feijão, sua identificação e correção. Brasília, Embrapa Arroz e Feijão. 22p. (Boletim técnico, 5)

Zonta E, Brasil FC, Goi SR & Rosa MMT (2006) O sistema radicular e suas interações com o ambiente edáfico. In: Fernandes MS (Ed.) Nutrição mineral de plantas. Viçosa, Sociedade Brasileira de Ciência do Solo. p.8-52.