Chapter

# Micronutrients for Production and Biofortification of Cassava

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## Abstract

Micronutrients are strategic elements for increasing the production of crops and improving the quality of harvested products. Despite this, they have been neglected in cassava (*Manihot esculenta* Crantz), one of the most important root vegetables in the tropics. This chapter aims to show how micronutrients can increase yield and improve nutritional quality of cassava. Research results covering the influence of micronutrients on yield and biofortification of roots of the plant are reviewed in detail. Boron, Cu, Mn, or Zn fertilization can increase root yield. Genetic biofortification is efficient in increasing Fe and Zn concentrations in cassava roots. The increase in Zn concentration can also be achieved by agronomic biofortification. A combination of genetic and agronomic strategies to biofortify cassava needs to be studied. In addition, further studies are needed to establish criteria for the large-scale application of micronutrient fertilizers in cassava. Use of genetically biofortified cultivars and judicious application of micronutrients are essential to increase production and improve the nutritional quality of cassava and thus ensure food security in the tropics.


**Keywords:** boron, copper, fertilization, iron, manganese, *Manihot esculenta*, mineral nutrition, root crop, zinc

## 1. Introduction

Plants require 14 mineral nutrients to grow [1]. These nutrients are divided into macronutrients and micronutrients. The macronutrients comprise nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). The micronutrients include boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn). The difference between these two classes of nutrients is in the amount required by plants. Macronutrients are required in large amounts, and micronutrients in small amounts. Despite their lower requirement, micronutrients are as important as macronutrients for plant growth. However, little attention has been given to micronutrient nutrition in some crop plants. One of these plants is cassava (*Manihot esculenta* Crantz).

Macronutrient nutrition of cassava has been extensively studied [2]. On the other hand, there are few studies on its micronutrient nutrition [3]. The reasons for this scarcity of studies have not been determined yet. Nonetheless, some causes are suggested here. The first is the focus of research on N, P, and K nutrition, probably motivated by the high cost of fertilizers used to provide these nutrients for the crop.

The second is the assumption that the high colonization of cassava roots by mycorrhizal fungi observed in several studies [4–6] is sufficient for the acquisition of the amounts of micronutrients required by the plant. The third is that cassava cultivation is predominantly carried out in very acidic soils where there is higher availability of micronutrients and thus greater capacity to meet the plant's needs. The fourth is the low demand of farmers for research with micronutrients, since they have little knowledge about the effects of these nutrients on the plant.

However, studies on micronutrients in cassava have increased in recent years, probably due to the need to better understand the role of such nutrients in the production of this crop. Another reason is the interest in biofortifying cassava with micronutrients, particularly with Fe and Zn. Biofortification refers to the increase in micronutrient concentration in the edible parts of food crops through breeding or fertilization [7–9]. Both strategies have been successfully used to biofortify cassava root, which is the main edible part of the plant [10, 11]. Thus, productive and nutritional aspects have motivated research on micronutrients in the crop.

Cassava is an essential vegetable crop for food security in the tropics. It is estimated that this crop contributes to the nutrition of more than 800 million people distributed mainly in South America, sub-Saharan Africa, and Asia [12, 13]. It is also strategic to strengthen agriculture as a livelihood in rural areas [14] and to produce food in an uncertain agricultural scenario due to climate change [15]. In this context, micronutrients can play an important role in the production and biofortification of cassava, providing nutritious food in sufficient amounts to help feed future generations.

This chapter aims to show how micronutrients can increase yield and improve the nutritional quality of cassava.

## 2. Responses to micronutrients

An overview of micronutrients in soils and plants is provided for B, Cu, Mn, and Zn, including total and available concentrations in soil, uptake, and functions in plant growth. In the sequence, symptoms and critical concentrations of deficiency and toxicity as well as responses of cassava to fertilization are provided.

### 2.1 Boron

The total concentration of B in the surface layer of tropical acid soils is quite variable. Surveys carried out in some municipalities of Brazil, for example, show a variation from 10 to 38 mg $kg^{-1}$ [16, 17]. The concentration of hot water soluble B, which is a measure of B availability to plants, also varies greatly in Brazil, ranging from 0.01 to 10.6 mg $dm^{-3}$ for soils of the estate of São Paulo and from 0.01 to 8.25 mg $dm^{-3}$ for soils of other Brazilian states, with relatively low mean and median values (0.32 and 0.23 mg $dm^{-3}$ and 0.31 and 0.24 mg $dm^{-3}$, respectively) [18]. Low concentrations of soluble B in soils restrict the absorption of B by plant roots.

Boron is absorbed from the solution of acid soils as undissociated boric acid ($H_3BO_3$) and its uptake rate is influenced by several factors, including (i) external concentration of B and other elements, (ii) soil pH, (iii) soil moisture, as well as (iv) characteristics of root system, (v) growth stage, (vi) transpiration and (vii) nutritional status of plants [19, 20]. For cassava, B uptake was shown to be also dependent on the temperature in the root zone, being higher in the range from 26 to 33°C [21].

Once absorbed, B is transported *via* xylem to organs that are growing rapidly. Boron can also be remobilized to other organs, but only in some plant species, where it is mobile in the phloem [22]. In most species, however, B is immobile in the phloem [22], and thus it cannot be remobilized. Cassava is included in this last group of plants, where B is immobile in the phloem [23]. Because of the B immobility, symptoms of B deficiency appear first in younger leaves, and those of B toxicity in older leaves.

The functions of B in plants are not well understood yet. However, it is well accepted that the primary role of B is to stabilize the cell wall structure [24, 25]. Other roles would be secondary and linked to the primary role. Secondary roles include (i) plasma membrane integrity and function, (ii) phenol and phytohormone metabolism, (iii) root elongation and shoot growth, and (iv) formation and viability of reproductive structures [25]. Boron can also improve important processes within the plant such as photosynthesis and water use [26–32]. When the supply of B is insufficient, these functions are impaired, and deficiency symptoms, reduced growth, and loss of yield may occur in plants.

Symptoms of B deficiency in cassava were described for the first time by Krochmal and Samuels [33]. Plants grown in sand culture without added B were dwarfed in the initial stages of growth, and younger leaves were slightly chlorotic and reduced in size. However, plant height and fresh weight of shoot were not affected by the deficiency. Later, Forno et al. [21] expanded the description of symptoms for plants grown in solution culture. The first symptoms reported by the authors occurred in the root system. The roots of B-deficient plants were short, swollen at the tip, and often blackened. At the plant top, symptoms included sequentially stunted growth, shortened internodes, younger leaves reduced in size, curved sideways, and with fine chlorotic spots, as well as longitudinal cracks on the stem and petioles. Finally, dry matter yields of tops, roots, and whole plants were drastically reduced by B deficiency. Although the critical concentration for B deficiency could not be calculated precisely in this study, the authors suggested that it would be around 17 mg $kg^{-1}$ at the top of plants with 40 days of growth. Subsequently, Howeler et al. [34] accurately determined the critical B deficiency concentration in the youngest fully expanded leaf (YFEL) blades of plants grown in solution culture for 9 weeks, obtaining a value of 35 mg $kg^{-1}$. Concentrations below 17 mg $kg^{-1}$ at the plant top or 35 mg $kg^{-1}$ in YFEL blades indicate B deficiency in young plants. Boron deficiency in field-grown cassava does not seem to be as uncommon as reported in the literature [34], since it has occurred in Colombia, Vietnam, and China [35]. In areas subject to deficiency, the crop may respond to B application.

Field experiments conducted to determine cassava responses to B application are reduced in number but distributed in different parts of the world. In Australia, application of up to 3.6 kg B $ha^{-1}$ to a soil replaced after bauxite mining did not significantly affect root yield [36]. In this study, B concentration in fully expanded leaves ranged from 22 to 38 mg $kg^{-1}$. On the other hand, positive and significant responses were obtained in an experiment in Thailand [37]. Some results of this study are shown in **Table 1**. Application of 1 kg B $ha^{-1}$ as boric acid to an Ultisol increased the fresh tuberous root yield regardless of Ca supply. The highest yield (36.8 t $ha^{-1}$) occurred in combination with the application of 400 kg Ca $ha^{-1}$. This combination also resulted in a high yield of starch (10.1 t $ha^{-1}$) and a concentration of B in YFEL (21.6 mg $kg^{-1}$) higher than that in the control (17.5 mg $kg^{-1}$). Application of 2 kg B $ha^{-1}$ to the soil, foliar application (1 or 2 kg B $ha^{-1}$), and mixed application (1 or 2 kg B $ha^{-1}$, half in soil and half in leaves) did not provide consistently better productive results.

| **Soil-applied Ca rate** (1) | **Soil-applied B rate** (2) | **Leaf-applied B rate** (2) | **Fresh tuberous root yield** | **Starch yield** | **B in leaf**(3) |
|---|---|---|---|---|---|
| kg ha$^{-1}$ | | | t ha$^{-1}$ | | mg kg$^{-1}$ |
| 0 | 0 | | 26.5 no(4) | 7.1 k | 12.4 h-k |
| | 1 | | 35.1 a-d | 10.9 a | 20.5 e |
| | 2 | | 31.4 f-k | 8.9 e-i | 10.6 kl |
| | | 1 | 27.5 mno | 7.9 jk | 29.2 a |
| | | 2 | 27.1 mno | 8.1 ij | 13.3 hij |
| | 0.5 | 0.5 | 33.9 b-g | 10.0 a-d | 21.1 e |
| | 1 | 1 | 36.0 ab | 10.4 abc | 22.7 de |
| 100 | 0 | | 29.3 j-n | 8.4 g-j | 21.2 e |
| | 1 | | 28.1 l-o | 7.2 k | 13.7 g-j |
| | 2 | | 34.1 a-f | 9.7 b-e | 14.0 ghi |
| | | 1 | 34.4 a-e | 9.7 b-e | 18.0 f |
| | | 2 | 33.7 b-g | 8.8 e-i | 25.3 bc |
| | 0.5 | 0.5 | 33.0 c-h | 9.3 d-g | 14.3 ghi |
| | 1 | 1 | 34.5 a-e | 9.2 d-h | 20.3 f |
| 200 | 0 | | 25.8 o | 7.0 k | 9.3 l |
| | 1 | | 31.6 e-j | 9.2 d-h | 16.1 fg |
| | 2 | | 31.3 f-k | 9.4 def | 12.3 h-k |
| | | 1 | 30.0 i-m | 9.0 e-i | 23.7 cd |
| | | 2 | 28.7 k-n | 8.6 f-j | 11.6 i-l |
| | 0.5 | 0.5 | 29.2 j-n | 7.8 jk | 15.8 gh |
| | 1 | 1 | 32.5 d-i | 9.4 def | 11.3 jkl |
| 400 | 0 | | 29.7 i-m | 8.2 hij | 17.5 fg |
| | 1 | | 36.8 a | 10.1 a-d | 21.6 de |
| | 2 | | 32.4 d-i | 9.1 d-h | 22.0 de |
| | | 1 | 30.7 h-l | 8.4 f-j | 23.8 cd |
| | | 2 | 30.1 g-k | 9.3 d-g | 21.0 e |
| | 0.5 | 0.5 | 36.0 ab | 9.6 cde | 21.8 de |
| | 1 | 1 | 35.7 abc | 10.6 abc | 27.1 ab |

(1)*Calcium applied as limestone before planting.*
(2)*Boron applied as boric acid 2 months after planting.*
(3)*Leaf sampled 4 months after planting.*
(4)*Means followed by a common letter within a column are not significatively different by the Duncan's multiple range test ($p < 0.05$).*
*Source: Adapted from Bowichean et al. [37].*

**Table 1.**
*Influence of Ca and B fertilization for cassava on fresh tuberous root and starch yield as well as B concentration in youngest fully leaves in Thailand.*

The positive response to B fertilization in this study occurred even at a not very low hot water soluble-B concentration (0.43 mg kg$^{-1}$ at the 0–30 cm soil layer), suggesting that cassava may be more responsive to B supply than previously thought. The responsiveness of cassava to B was also observed in a study carried out in Brazil [38]. However, the response of the plant was dependent on the cultivar. Application of 1 or 2 kg B ha$^{-1}$ as boric acid to an Ultisol with a low hot water soluble-B concentration (0.15 mg dm$^{-3}$ at the 0–20 cm soil layer) significantly increased the tuberous root yield of the cultivar BRS 399. Application of 1 kg B ha$^{-1}$, however, was insufficient to increase the yield of the cultivar IAC 576-70, which required 2 kg B ha$^{-1}$. The cultivars BRS 399, Palito, and Precoce did not respond positively to B. In the specific case of the cultivar Palito, application of 2 kg B ha$^{-1}$ reduced the tuberous roots yield, indicating that there was B toxicity with the highest rate of B applied. These findings led the authors to conclude that B application should be cultivar specific.

Although reduced in number, these studies suggest that B fertilization may benefit the production of B-responsive cassava cultivars grown in soils limited in available B.

The concern with B toxicity should be as great as the concern with B deficiency because both nutritional disorders can reduce crop growth and yield. Boron toxicity in cassava is more likely to occur in naturally high B soils or due to accidental application of a high amount of B fertilizer [3, 39]. Symptoms of B toxicity in cassava have been described in the literature. Forno et al. [21] described light-brown necrotic spots in older leaves as initial symptoms in plants grown in solution culture. Subsequently, interveinal areas in these leaves became chlorotic. Finally, a reduction in dry matter yield was observed. The critical B toxicity concentration was suggested to be about 140 mg kg$^{-1}$ at the top of plants with 40 days of growth. A slightly lower critical concentration (100 mg kg$^{-1}$) was calculated by Howeler et al. [34] for youngest fully expanded leaf (YFEL) blades of plants grown in solution culture for 9 weeks. The most recent description seems to be that of Silva [3], who observed symptoms in cassava grown for 68 days in pots filled with soil in a greenhouse. This author has described that the symptoms began with a mild chlorosis in the tips and margins of older leaves. The chlorosis increased in size over time. In the sequence, chlorotic tissues became necrotic, and some darker halos appeared on leaf blades. In an advanced stage, tips and margins of older leaves were curved downwards. An old leaf with symptoms is shown in **Figure 1**. Despite the symptoms, there was no significant reduction in plant dry matter. Consequently, it was not possible to calculate the critical B toxicity concentration. However, toxicity thresholds were suggested based on the appearance of visual symptoms. The thresholds in younger leaf blades and older leaf blades were 99.9 and 130.7 mg kg$^{-1}$, respectively. These thresholds corresponded

**Figure 1.**
*Symptoms of B toxicity in an old leaf of cassava. Photo by Alysson Roberto Baizi e Silva.*

to the respective thresholds for hot water soluble B concentration of 0.875 and 0.891 mg $dm^{-3}$. Concentrations above these thresholds in both leaf blades and soil could indicate suspected B toxicity symptoms.

## 2.2 Copper

The total Cu concentration in the surface layer of tropical soils varies considerably with the parent material. In São Paulo state, Brazil, Valadares [40] found mean concentrations in the A horizon of 189.5 mg $kg^{-1}$ for soils derived from basic rocks, 27.3 mg $kg^{-1}$ for soils derived from alluvial and colluvial materials, 22.5 mg $kg^{-1}$ for soils derived from modern sediments, 8.1 mg $kg^{-1}$ for soils derived from modern sandy sediments, and 5.2 mg $kg^{-1}$ for soils derived from sandstone. This study suggests that Cu reserves are high in soils derived from basic rocks and very low in soils derived from sandstone.

The concentration of plant-available soil Cu can be assessed by different extractants. The most common ones are DTPA and Mehlich-1. A comprehensive study conducted in Brazil revealed DTPA-extractable Cu concentrations ranging from 0.1 to 106 mg $dm^{-3}$ for soils from the state of São Paulo and from 0.1 to 56.2 mg $dm^{-3}$ for soils from other Brazilian states, with respective means and medians of 2.5 and 1.4 mg $dm^{-3}$ and 2.3 and 1.3 mg $dm^{-3}$ [18]. The solubility of Cu in soil is mainly controlled by pH and organic matter. Increasing the pH value and organic matter content increases the retention of Cu in the soil [41, 42], decreasing the availability of Cu for uptake by plant roots.

Copper is absorbed as $Cu^{2+}$, but under low Cu availability, $Cu^{2+}$ is reduced to $Cu^{+}$ and subsequently taken up by specific transporters [25]. Copper plays several roles in plant physiology, including involvement in photosynthesis, respiration, defense against oxidative stress, carbohydrate, lipid and nitrogen metabolism, and lignification. It is present in enzymes such as plastocyanin, superoxide dismutase, cytochrome c oxidase, ascorbate oxidase, diamine oxidases, and polyphenol oxidases [25]. For cassava, a study showed that the expression and activity of superoxide dismutase increased with increasing temperature, alleviating the damage caused by heat-induced oxidative stress [43]. Under Cu deficiency, the functioning of this enzyme would be inefficient, and the plant would suffer more with the increased temperature.

Symptoms of Cu deficiency in cassava have been described in the literature. In solution culture, plants exhibited chlorosis and deformation in younger leaves, often with necrosis at the leaf tip, leaf margins cupped upwards, and reduction in number of lobes under severe deficiency [39]. Critical Cu deficiency concentration was calculated to be 6 mg $kg^{-1}$ in YFEL blades of plants grown in solution culture for 9 weeks [34]. Copper deficiency has not been observed in the field, except in organic soils [34], which retains Cu in forms not absorbable by plants. Under such conditions, cassava can respond positively to Cu fertilization.

Two field experiments conducted in peat soils of Malaysia confirm the productive benefits of Cu application for cassava [44]. In the first experiment, effects of B, Cu, Fe, Mn, Mo, and Zn application were tested. Of all these micronutrients, cassava responded only to Cu. A single application of 20 kg $ha^{-1}$ of copper sulfate in the first year increased by about 3-fold the tuberous root dry matter yield and starch yield in two consecutive seasons (**Table 2**). In the second experiment, three application rates of copper sulfate were tested. Application of 10 kg $ha^{-1}$ of copper sulfate increased tuberous root dry matter yield and starch yield by about 3-fold in the first season and

| | Tuberous root dry matter | | Starch | |
|---|---|---|---|---|
| **Copper sulfate (Cu)** | **1973/74** | **1974/75** | **1973/74** | **1974/75** |
| kg $ha^{-1}$ | ——— t $ha^{-1}$ ——— | | ——— t $ha^{-1}$ ——— | |
| 0 (0) | 4.44 | 3.98 | 3.16 | 2.88 |
| 20 (5) | 14.72 | 13.96 | 9.72 | 8.42 |
| LSD[(1)] ($p$ = 0.05) | 3.64 | 2.42 | 3.01 | 2.29 |
| CV[(2)] (%) | 27.6 | 18.5 | 27.9 | 19.6 |

*[(1)] Least significant difference.*
*[(2)] Coefficient of variation.*
*Source: Adapted from Chew et al. [44].*

**Table 2.**
*Effect of a single application of copper sulfate for cassava cv. Black Twig grown in a peat soil on tuberous root dry matter yield and starch yield in two seasons in Malaysia.*

about 6-fold in the second season (**Table 3**). There was no additional benefit in applying 20 kg $ha^{-1}$ of copper sulfate, except for starch yield in the second season. The Cu concentration in the first fully open leaf associated with the increase in productivity provided by the application of 10 kg $ha^{-1}$ of copper sulfate was around 14 mg $kg^{-1}$ in both seasons (**Table 3**). Control plants (no Cu application) exhibited Cu deficiency symptoms, described as chlorosis and wrinkling of younger leaves, upturned leaf margins, leaf blade forming a right angle with the petiole, leaf drop, die-back of defoliated shoot, tuberous roots reduced in number and size, and bushy and stunted

| **Copper sulfate (Cu)** | **Tuberous root dry matter** | **Starch** | **Cu in leaf[(3)]** |
|---|---|---|---|
| kg $ha^{-1}$ | ——— t $ha^{-1}$ ——— | | mg $kg^{-1}$ |
| | First season | | |
| 0 (0) | 4.1 | 2.7 | 7.3 |
| 10 (2.5) | 12.2 | 7.9 | 14.4 |
| 20(5) | 13.1 | 8.5 | 15.4 |
| LSD[(1)] ($p$ = 0.05) | 4.9 | 3.2 | 6.8 |
| CV[(2)] (%) | 25 | 5 | 26.6 |
| | Second season | | |
| 0 (0) | 2.0 | 1.3 | 6.5 |
| 10 (2.5) | 9.4 | 9.4 | 13.9 |
| 20 (5) | 10.2 | 6.6 | 14.7 |
| LSD[(1)] ($p$ = 0.05) | 3.4 | 2.2 | 6.6 |
| CV[(2)] (%) | 19 | 18 | 25.1 |

*[(1)] Least significant difference.*
*[(2)] Coefficient of variation.*
*[(3)] Leaf sampled 3 months after planting.*
*Source: Adapted from Chew et al. [44].*

**Table 3.**
*Effect of a single application of copper sulfate for cassava cv. Black Twig grown in a peat soil on tuberous root dry matter yield, starch yield, and Cu concentration in the first fully open leaf in two seasons in Malaysia.*

plant. The Cu concentrations in the first fully open leaf of the control plants were 7.3 mg $kg^{-1}$ in the first season and 6.5 mg $kg^{-1}$ in the second season (**Table 3**). In conclusion, fertilization with 10 kg $ha^{-1}$ of copper sulfate (or 2.5 kg Cu $ha^{-1}$) for cassava grown in peat soils of Malaysia was suggested by the authors of this study.

Copper toxicity has not been reported in the literature. However, it may occur in specific situations. One of these situations is the cultivation of cassava in high-Cu soils due to repeated applications of Cu-based pesticides to previous crops [39]. No description of symptoms of Cu toxicity for cassava was found. For plants in general, the symptoms are very generic, consisting of chlorosis and necrosis, stunting, and inhibition of root and shoot growth [45]. Howeler et al. [34] determined a critical Cu toxicity concentration in YFEL blades of plants grown in solution culture for 9 weeks. The value calculated by the authors was 15 mg $kg^{-1}$. Concentrations above this limit may indicate Cu toxicity in young cassava plants.

### 2.3 Manganese

The total Mn concentration in tropical soils seems to vary according to soil texture. Catani and Gallo [46] determined total Mn in the surface layer of some soils in the state of São Paulo, Brazil. The highest concentration was found in a clayey soil (2000 mg $kg^{-1}$) and the lowest in a sandy soil (514 mg $kg^{-1}$). This difference reflects the Mn concentration in parent material and suggests lower reserve of Mn in coarse-textured soils.

As for Cu, DTPA and Mehlich-1 also are extractants used to evaluate soil Mn available to plants. In the survey carried out in Brazil by Abreu et al. [18], DTPA-extractable Mn concentrations ranged from 0.1 to 325 mg $dm^{-3}$ for soils from the state of São Paulo and from 0.1 to 315 mg $dm^{-3}$ for soils from other Brazilian states, with respective means and medians of 16.1 and 8.8 mg $dm^{-3}$ and 14.9 and 8.3 mg $dm^{-3}$. Several factors affect Mn availability in soils, such as soil moisture, temperature, depth and pH [47]. Soil pH seems to be the main factor controlling the available Mn concentration, which decreases with increasing pH [48]. The decrease in Mn availability due to reduced soil acidity compromises Mn uptake by plants.

Manganese is absorbed only as $Mn^{2+}$, and $Mn^{2+}$ can be oxidized to $Mn^{3+}$ and $Mn^{4+}$ within the plant [25]. Manganese plays an important role in photosynthesis, metabolism of proteins, carbohydrates and lipids, cell division and extension, and in the composition or activation of enzymes, many of which are related to the defense system against oxidative stress [25]. These functions are impaired when Mn supply is insufficient, and symptoms of Mn deficiency may appear in plants. The most common symptoms are described as interveinal chlorosis in younger leaves, brown necrotic spots in older leaves, and decreased plant growth and yield [25]. For cassava, critical Mn deficiency concentration of 50 mg $kg^{-1}$ in YFEL blades of plants grown in solution culture for 9 weeks was determined by Howeler et al. [34].

Symptoms of Mn deficiency in cassava have been reported in Brazil. Santos and Tupirambá [49] reported these symptoms in several cassava growing areas in the state of Sergipe, northeastern Brazil. The symptoms include interveinal leaf chlorosis with poor root yield. Interveinal chlorosis was confirmed as a symptom of Mn deficiency in 15 plots of an experiment conducted by the authors in an Ultisol. Such nutritional disorder occurred when Mn concentration in middle leaves was below 20 mg $kg^{-1}$ and soil pH in water above 5.8, as well as at exchangeable Ca and exchangeable Ca + Mg concentrations above 3.8 and 5.2 $cmol_c$ $dm^{-3}$, respectively. These values give an idea of some conditions under which this deficiency may arise. In a cassava crop grown in a medium-textured soil (Oxisol) in the state of Minas Gerais, southeastern Brazil, interveinal chlorosis in younger leaves associated with Mn deficiency was identified by Malavolta et al. [50]. In this case study, the deficiency concentrations in the leaf blade and petiole were respectively 16 and 24 mg $kg^{-1}$, at the onset of symptoms, and 12 and 10 mg $kg^{-1}$, at the most advanced stage of symptoms. Symptoms of Mn deficiency have also been observed in commercial cassava fields in the state of Pará (**Figure 2**). The occurrence of Mn deficiency opens the possibility of obtaining positive responses to Mn application.

Several Mn fertilization treatments for cassava under organic management in the Federal District, Brazil, were tested in a field experiment conducted by Marchi et al. [51]. Application of 4.28 kg Mn $ha^{-1}$ as Mn sulfate banded in a clayey Oxisol with a Mehlich-1-extractable Mn concentration of 29 mg $dm^{-3}$ did not result in an increase in tuberous root yield (**Table 4**). In contrast, Mn applied at stem cutting or spraying Mn on leaves significantly increased root yield (**Table 4**). Soil application was not

**Figure 2.**
*Symptoms of Mn deficiency in younger leaf of cassava in a commercial field in the municipality of Tracuateua, state of Pará, Brazil. Photos by Alysson Roberto Baizi e Silva.*

| Mn treatment | Fresh tuberous root yield |
|---|---|
| | t $ha^{-1}$ |
| T1: Control (no Mn) | 3.6 b[(5)] |
| T2: 4.28 kg Mn $ha^{-1}$ applied to soil[(1)] | 3.9 b |
| T3: Mn applied to stem[(2)] | 16.6 a |
| T4: T3 + T2 | 18.1 a |
| T5: T3 + T2 + $S^{0}$[(3)] | 19.5 a |
| T6: Leaf Mn spraying[(4)] | 19.4 a |

*(1)Manganese sulfate banded before planting.*
*(2)Stem cutting immersed in a 5% Mn sulfate solution for 15 minutes.*
*(3)$S^0$: elemental sulfur banded before planting.*
*(4)Spraying of a 0.5% Mn sulfate solution on leaves.*
*(5)Means followed by a common letter within a column are not significantly different by the Tukey's test ($p < 0.05$).*
*Source: Adapted from Marchi et al. [51].*

**Table 4.**
*Effect of different Mn treatments on cassava cv. IAC 576–70 under organic management on fresh tuberous root yield in the Federal District, Brazil.*

efficient in this case probably due to the high soil pH in water (6.75), which may have reduced the availability of applied Mn. The mean Mn concentrations in the fourth fully expanded leaf collected 4 months after plant emergence and in leaves collected at harvest (1 year after planting) did not adequately reflect the Mn treatments and were very low (≤ 12 mg $kg^{-1}$). Furthermore, plants in all treatments showed interveinal chlorosis and uniform chlorosis in upper and middle leaves as symptoms of Mn deficiency. This study shows how preventing or correcting a Mn deficiency in cassava can be difficult.

Manganese toxicity is not so common in cassava, perhaps because it is a plant tolerant to soil acidity factors, including excess Mn. Symptoms of Mn toxicity in cassava include small dark spots concentrated along the veins on older leaves, followed by yellowing of these leaves [39]. Howeler et al. [34] obtained a critical Mn toxicity concentration in YFEL blades of plants grown in solution culture for 9 weeks. This concentration was calculated to be 250 mg $kg^{-1}$. Manganese toxicity in young cassava plants would be more likely to occur in concentrations above this value.

## 2.4 Zinc

The total Zn concentration in the surface layer of tropical soils also varies with the parent material. Valadares [52] analyzed total Zn in 227 samples from 28 soil profiles representative of the state of São Paulo, Brazil. Total Zn concentrations in the A horizon ranged from 87 to 315 mg $kg^{-1}$ for soils derived from basic rocks, from 53 to 84 mg $kg^{-1}$ for soils derived from alluvial and colluvial materials, from 29 to 65 mg $kg^{-1}$ for soils derived from modern sediments, from 16 to 30 mg $kg^{-1}$ for soils derived from sandstone, and from 1 to 17 mg $kg^{-1}$ for soils derived from modern sandy sediments. Thus, Zn reserves tend to be higher in soils derived from basic rocks and lower in soils derived from modern sandy sediments.

Plant-available soil Zn is often assessed by DTPA and Mehlich-1 extractants. In Brazil, a survey showed DTPA-extractable Zn concentrations ranging from 0.1 to 453 mg $dm^{-3}$ for soils from the state of São Paulo and also from 0.1 to 453 mg $dm^{-3}$ for soils from other Brazilian states, with respective means and medians of 4.8 and 1.6 mg $dm^{-3}$ and 4.4 and 1.6 mg $dm^{-3}$ [18]. Soil pH is the most important factor controlling Zn solubility in soils. Numerous studies show that the available Zn concentration decreases with increasing soil pH [53]. When Zn availability decreases, Zn uptake by plant roots is reduced.

Zinc is mainly absorbed by plants as $Zn^{2+}$ [25]. Zinc is a component and activator of many enzymes (e.g., alcohol dehydrogenase, carbonic anhydrase, superoxide dismutase). Therefore, it plays several physiological roles in plants, such as (i) protein synthesis, (ii) carbohydrate metabolism, (iii) tryptophan and indole acetic acid (IAA) synthesis, and (iv) membrane integrity and lipid peroxidation [25]. As a component of superoxide dismutase together with Cu, Zn also plays an important role in reducing damage caused by oxidative stress in plants including cassava, as Lee et al. [43] demonstrated.

Cassava is very susceptible to Zn deficiency [25]. This nutritional disorder has been observed in several parts of the world and can be induced by liming [39]. Typical symptoms of Zn deficiency in cassava include chlorosis in younger leaves, small leaves, narrow leaf lobes, and necrotic leaf tips [39]. Critical Zn deficiency concentration in YFEL blades of plants grown in solution culture for 9 weeks was calculated to be 30 mg $kg^{-1}$ [34]. Concentrations below this value may suggest Zn deficiency. Because of its susceptibility to Zn deficiency, cassava has been considered a plant responsive to Zn application.

Positive responses of cassava to Zn fertilization have been observed under quite different conditions. In Australia, application of 8 kg Zn $ha^{-1}$ as Zn sulfate broadcasted to a soil replaced after bauxite mining significantly increased fresh tuberous root yield from 5.7 (control) to 16.2 t $ha^{-1}$ [36]. Also in Australia, a study showed that prior soaking of stem cuttings in Zn sulfate solutions varying from 17.4 to 348 mmol Zn $L^{-1}$ improved initial growth and increased Zn concentration in leaf blades of cassava grown in pots filled with a lateritic soil [54]. In Brazil, Silva et al. [55] grew cassava in large plastic boxes filled with a low-Zn Oxisol (DTPA-extractable Zn concentration of 0.4 mg $dm^{-3}$) subjected to Zn sulfate rates ranging from 0 to 6 g per plant. Zinc sulfate application increased root dry matter yield up to an estimated rate of 2.5 g per plant. For field application, Zn rates of 5–10 kg $ha^{-1}$ as banded Zn sulfate at planting or by incorporation of ZnO before planting have been suggested [39].

Zinc toxicity in cassava under field conditions has not been reported in the literature. Howeler et al. [34] determined a critical Zn toxicity concentration in YFEL blades of plants grown in solution culture for 9 weeks. The critical concentration was calculated to be 120 mg $kg^{-1}$. Zinc concentrations above this value would indicate Zn toxicity.

## 3. Biofortification

Micronutrient deficiencies affect a large proportion of the world's population [56]. These deficiencies occur mainly in infants, children, and women living in developing countries [56]. Among the most common deficiencies are those of Fe and Zn [57, 58]. The main disorder caused by Fe deficiency is anemia [59]. Other clinical manifestations of Fe deficiency include reduced growth and low immunity [59]. Zinc deficiency also reduces growth, and it can cause serious skin lesions, including the formation of rashes [59].

Cassava root-based diets have been a risk factor for Fe and Zn deficiency in children. A survey conducted in Kenya and Nigeria revealed that rural children aged 2–5 years who consumed cassava as a staple food rich in calories had low intakes of these micronutrients [60]. The intakes of Fe and Zn were below their minimum limits in 78 and 69% of the children surveyed in Kenya and 43 and 59% of the children surveyed in Nigeria, respectively. These high proportions of children subjected to mineral malnutrition were associated with low concentrations of these micronutrients in the tuberous roots of cassava, when expressed per unit of calories (1.6 μg $kcal^{-1}$ for Fe and 0.2 μg $kcal^{-1}$ for Zn). In fact, cassava roots have been shown to be very low in Fe (19 mg $kg^{-1}$) and Zn (10 mg $kg^{-1}$) [61].

Supplementation, food fortification, and dietary diversification are considered traditional methods of combating mineral malnutrition. However, these methods have not been fully effective due to poor logistics, inconsistent policies, inadequate social infrastructure, and a lack of continued investment [8]. In this context, biofortification emerges as an alternative method to combat malnutrition. Biofortification consists of increasing micronutrient concentrations in the edible parts of food crops through breeding or fertilization [7–9]. Both strategies have been used to biofortify cassava.

Biofortification of cassava with Fe has been based on genetic transformation. Ihemere et al. [62] introduced a gene from an alga into a cassava cultivar. The gene is *FEA1,* and it was taken from the algae *Chlamydomonas reinhardtii*. This gene has the specific function of facilitating Fe uptake. As a result of the introduction of the *FEA1* gene, the transgenic cassava accumulated more Fe in its roots. The Fe concentration in the tuberous roots of the modified cultivar was 36 mg $kg^{-1}$, whereas the nontransgenic cultivar had an Fe concentration of only 10 mg $kg^{-1}$. This genetic modification increased the Fe level in roots by almost four times. This increase was sufficient to meet the minimum daily requirement for Fe in a 500 g meal.

In another example of genetic biofortification, Narayanan et al. [63] overexpressed the gene *AtVIT1* in cassava. *AtVIT1* encodes a vacuolar Fe transporter (VIT1) in *Arabidopsis thaliana*. Under greenhouse conditions, transgenic cassava containing the *AtVIT1* gene had three to four times more Fe in tuberous roots than nontransgenic cassava. Later Narayanan et al. [64] confirmed the efficiency of cassava biofortification with the *AtVIT1* gene under field conditions. The authors found 3–7 times higher concentrations in the tuberous roots of transgenic cassava than in nontransgenic cassava. In the same study [64], the co-expression of two genes was tested. The Fe transporter *IRT1* and ferritin *FER1* genes were taken from *A. thaliana* and introduced into cassava. Under field conditions, the concentrations of Fe and Zn in the tuberous roots of transgenic cassava with *IRT1* + *FER1* were respectively 7–18 and 3–10 times higher than those in the tuberous roots of nontransgenic cassava. These increases could provide 40–50% of the Fe requirement and 60–70% of the Zn requirement of 1- to 6-year-old children.

These results show that biofortification based on genetic engineering is very efficient. Traditional breeding techniques, such as simple selection, can also be used, since there is great genetic variability for Fe and Zn concentrations in cassava [65]. Inacio et al. [66] evaluated 20 cassava genotypes in Nova Andradina, Mato Grosso do Sul state, Brazil. The genotypes MS018, DG014, and DG839 were very productive and presented the highest concentrations of Fe (above 10 mg $kg^{-1}$) and Zn (above 8 mg $kg^{-1}$). The authors suggested the inclusion of these three selected genotypes in biofortification programs.

Genetic biofortification relies on plant breeding, which is an expensive and time-consuming process, even with the use of modern genetic engineering techniques. On the other hand, agronomic biofortification, based on soil or plant fertilization, is a simpler alternative to increase the concentration of micronutrients in cassava roots. For Zn, studies have shown interesting results. In Nigeria, Binang et al. [67] found an increase of 79% in Zn concentration in fresh roots and root processing products (89% for *gari* and 79% for *lafun*) by foliar application of 3 kg Zn $ha^{-1}$ as Zn sulfate. Zinc fertilization resulted in Zn concentrations of 18.3, 19.5, and 15.9 mg $kg^{-1}$ for fresh roots, *gari,* and *lafun*, respectively. In the control (no Zn application), fresh roots, *gari,* and *lafun* had lower Zn concentrations (10.2, 10.3, and 8.9 mg $kg^{-1}$, respectively). In Brazil, Corguinha et al. [10] found clone-specific responses to total Zn concentration in soils. This led the authors to suggest a combination of genetic and agronomic strategies to biofortify cassava with Zn.

## 4. Research needs

This study shows promising results for micronutrient fertilization in cassava. However, there is a lack of information to guide better this practice. Response curve experiments conducted under field conditions are needed to determine rates of application and critical concentrations of micronutrients in soil and plants. Opportunities exist for experiments with Cu in mineral soils. In northern Brazil, for example, there is an important cassava-producing region whose soils have low concentrations of DTPA-extractable Cu (0.1 mg $dm^{-3}$) [68]. In this region, a Cu response curve experiment could produce important results to improve the crop's yield. Organic wastes and other strategic sources of micronutrients for cassava [69, 70] should be evaluated for agronomic efficiency. The role of microorganisms in the acquisition of micronutrients by the plant needs to be further studied. Comparison between soil application and foliar application of micronutrients is crucial to define the most appropriate method to biofortify cassava.

## 5. Conclusion

The results presented in this chapter have shown that B, Cu, Mn, or Zn fertilization can increase the tuberous root yield of cassava, thus refuting the widespread assumption that the plant is capable of obtaining the required amounts of these micronutrients without fertilizer application. Genetic biofortification based on transgenics is efficient in increasing Fe and Zn concentrations in cassava roots. The increase in Zn concentration can also be achieved by agronomic biofortification, based on the use of fertilizers. Combination of genetic and agronomic strategies to biofortify cassava needs to be studied. In addition, further studies are needed to establish criteria for large-scale application of micronutrient fertilizers in cassava. Use of genetically biofortified cultivars and judicious application of micronutrients are essential to increase production and improve the nutritional quality of cassava and thus ensure food security in the tropics.

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## Conflict of interest

The author declares no conflict of interest.

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