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Potential of Cassava Clones for Iron, Zinc, and Selenium Biofortification

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Abstract: Cassava (*Manihot esculenta* Crantz) is a crucial staple food in South America, providing starchy storage roots that contribute to the sustenance of millions. To address deficiencies in iron (Fe), zinc (Zn), and selenium (Se), a global initiative is underway to identify plant species and genotypes that naturally accumulate these nutrients for human consumption, such as cassava. In this way, this study aims to identify potential cassava genotypes for biofortification in Fe, Zn, and Se, while also improving the overall cassava yield. We evaluated the accumulation potential of Fe, Zn, and Se in 20 South American cassava genotypes under traditional growing conditions, concurrently examining their photosynthetic and growth characteristics. Cassava roots exhibited Zn content ranging from 3.20 to 8.56 mg kg^{-1}, Fe content from 2.20 to 10.73 mg kg^{-1}, and Se content from 1.20 to 9.43 µg kg^{-1} (expressed on a dry basis). Genotypes MS018, DG014, and DG839 emerged as promising candidates for biofortification programs, displaying elevated levels of Fe, Zn, and Se, coupled with superior photosynthetic capacity. These genotypes, recommended for biofortification programs, also demonstrated increased yield potential. The findings from this study contribute to the development of cassava genotypes with enhanced agronomic biofortification and elevated yield potential.

Keywords: *Manihot esculenta*; gas exchange; human malnutrition

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1. Introduction

The number of malnourished individuals worldwide has been on the rise, reaching an alarming figure of 820 million in recent years [1]. Malnutrition results from the consumption of staple foods that lack in essential minerals and vitamins, and is more common in developing countries, such as those in Latin America [2]. Among the nutrients that are commonly deficient in human nutrition, iron (Fe), zinc (Zn), and selenium (Se) stand out.

Iron (Fe) is an essential element for both plants and humans [3]. In plants, this micronutrient is a constituent of heme and non-heme proteins (Fe-S) that participate in the redox system, which is responsible for detoxifying cells by reducing hydrogen peroxide (H_2O_2) into water and oxygen [4]. In animals, Fe is a component of hemoglobin, the oxygen-transporting protein in red blood cells [5]. Iron deficiency in humans is the most widespread nutritional deficiency globally, affecting approximately two billion people and causing one million deaths annually [6]. This condition adversely affects the cognitive development of children, the work capacity of adults, and the immune system of individuals [5].

Zinc (Zn) is an essential element in numerous biochemical processes, as it is a component of proteins and enzymes involved in energy production, the maintenance of membrane structural integrity, nucleic acid synthesis (DNA and RNA), auxin metabolism, and cell growth and differentiation [7]. In humans, zinc is a component of approximately 300 enzymes and the only metal to participate in all six enzyme classes [oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases]. It is estimated that about 17% of the global population is deficient in zinc [8]. The primary consequences of this deficiency in the human body include impaired brain function, a weakened immune system, and stunted physical growth [9].

Selenium (Se) is an essential element for human health, just like Fe and Zn. Organisms deficient in Se become more susceptible to various diseases, such as hyperthyroidism, cardiovascular diseases, and cancer [10]. It is estimated that one billion people worldwide suffer from Se deficiency [11]. There is strong evidence of Se deficiency in nutrition in Brazil, primarily due to the low Se levels in the soils used for Brazilian agriculture [12]. In contrast to Brazil, in other countries, Se deficiency in humans is a public health issue. In Finland, since 1984, the addition of selenate to NPK fertilizers has been an effective method to increase Se levels in the population. However, there are challenges that need to be understood for the proper selection of plants for the biofortification of Fe, Zn, and Se in edible components [2].

These challenges result from the homeostatic mechanisms that regulate the absorption and redistribution of elements in plants (e.g., Fe, Zn, and Se), reducing their accumulation in edible sections [13]. Thus, these challenges can be overcome through the selection of genotypes with potential for nutrient accumulation. In addition to the widespread consumption in the Brazilian population, cassava (*Manihot esculenta*) biofortification presents itself as an alternative to reduce Fe, Zn, and Se deficiencies in humans. However, there is an increasing importance in identifying cassava genotypes with both high nutrient accumulation in the roots and a strong photosynthetic capacity. These traits may serve as valuable targets for genetic improvement programs [14,15]. However, genetic advancements rely on comprehending the existing diversity associated with a desired trait within the available germplasm. In the context of bioengineering strategies, it is crucial to grasp the limitations of the target trait, allowing for the design of appropriate approaches to address and overcome the identified constraints. While the diversity in the steady-state photosynthesis of South American cassava cultivars has been assessed [16,17], there is currently no available data on the photosynthetic capacity that is associated with genotypes holding potential for biofortification.

Cassava is one of the main products in Brazilian agriculture and is consumed throughout the country. Moreover, cassava cultivation is closely linked to the rural development of family agriculture, particularly in the state of Mato Grosso do Sul, where productivity reaches around 20 t ha^{-1}, compared to the national average of 15 t ha^{-1}. Although higher than the national average, this productivity falls short of the crop's potential, which can reach 80 t ha^{-1} in the absence of stressors [18]. Therefore, there is a need to focus on research to improve nutrient absorption in the Brazilian population, as well as to increase productivity [19]. Here, we quantify the concentration of Fe, Zn, and Se in roots (the edible part) of 20 Brazilian cassava genotypes under traditional growing conditions, in addition to describing their photosynthetic potential and growth characteristics. In this way, the present study aimed to identify genotypes with the potential for Fe, Zn, and Se biofortification programs for human consumption and to increase the yield of cassava.

2. Materials and Methods

2.1. Location and Characterization of the Experimental Area

The experiment was conducted for two growing seasons (2021/2022 and 2022/2023) at the Institute of Federal Education, Science, and Technology of Mato Grosso do Sul farm, located in the city of Nova Andradina, Mato Grosso do Sul. The soil in the experimental area was classified as typic quartzipsamment [20]. In summary, a "typic quartzipsamment" is a soil classification that represents a typical example of a sandy soil with a high quartz content. These soils are often well-draining due to their coarse texture, but may require additional organic matter or nutrients for optimal fertility. To determine the soil's chemical properties, on the 30th of April, 2022, soil subsamples were randomly collected from the top 20 cm of the experimental area. Subsamples were mixed, homogenized, and evaluated according to Raij [21]. The soil pH was assessed in a 1:2.5 soil–water mixture. Soil organic matter was determined using the potassium dichromate (K_2Cr2O_7) method, involving oxidizing a 0.5 g soil aliquot with a $K_2Cr_2O_7$ + H_2SO_4 solution at 160 °C. Calcium (Ca) and magnesium (Mg) were extracted by mixing a 10 cm^3 volumetric soil aliquot with 100 mL 1 mol L^{-1} KCl at room temperature overnight. Available soil phosphorus (P) and potassium (K) were extracted using 100 mL Mehlich-I solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) reacted with a 10 mL soil sample. Copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were extracted using 100 mL diethylenetriaminepentaacetic acid extractable (DTPA) [22]. Concentrations of Ca, Mg, Fe, Mn, Zn, and Cu in the extracted solutions were determined using a flame atomic absorption spectrometer (F-AAS; AAnalyst™ 800, PerkinElmer, Waltham, MA, USA), with certified or Sigma-Aldrich single element AAS standards for QA/QC. The available K was measured by flame photometry, and the available P was determined by colorimetry. High-purity reagents, sourced from either Sigma-Aldrich or Merck, were employed in all procedures, and the HNO_3 underwent distillation before being utilized in the digestions. To validate the accuracy of the analytical results, standard reference materials from the National Institute of Standards and Technology (NIST—SRM 1573a Tomato Leaves and SRM 2710 Montana Soil) were utilized. For quality control, blank and certified reference samples were systematically analyzed in conjunction with each digestion batch.

The soil's chemical characteristics were as follows: pH 4.4; phosphorus, boron, copper, iron, manganese, and zinc 1.21, 0.40, 0.30, 15, 13, and 0.50 mg dm^{-3}, respectively; potassium (resin), calcium (resin), magnesium (resin), and cation exchange capacity: 0.51, 6, 4, 12, and 22.5 $mmol_c$ dm^{-3}, respectively; base saturation: 47%. The concentrations of total and exchangeable selenium (Se) in the soil were 40 μg kg^{-1} and 4 μg kg^{-1}, respectively.

2.2. Experimental Setup

The experimental design followed a randomized complete block design with twenty treatments and five replications (for each treatment). The study focused on the accumulation of Fe, Zn, and Se, as well as the productivity of 20 cassava genotypes (Table 1). The DG genotypes originate from EMBRAPA's germplasm bank, while the MS genotypes come from the germplasm bank of IFMS Nova Andradina.

The land preparation was mechanized, and the planting of cassava cuttings (manivas) took place in June 2021 and 2022, manually, in holes that were previously opened with a hoe at a depth of 10 cm. The holes were fertilized with 30 kg ha^{-1} of potassium chloride, 35 kg ha^{-1} of single superphosphate, and 70 kg ha^{-1} of urea [19]. One cassava cutting (maniva-semente) was placed per planting hole, with a spacing of 1 m between rows and 1 m between plants. Each plot consisted of 10 m × 4 m (40 m^2) with four rows per plot. Each row contained 10 plants, and the central part of the plot (16 central plants) was considered the useful area. In this way, analyses were carried out on 16 plants in each plot. All analyses were performed in triplicates. The plots were separated from each other by a row of plants on the sides (previously tested growing conditions).

Emergence began 10 days after planting (DAP). No additional sources of Fe, Zn, and Se were added, meaning that the results would reflect the potential accumulation of these nutrients from the typical Cerrado soil. Cultural practices followed the recommendations for cassava cultivation in the Cerrado region. It is worth noting that there was no need for the use of agricultural pesticides. The roots were harvested in July 2022 and 2023 by hand after 12 months, and then washed with distilled water, peeled, and stored in a cold chamber (4 °C) for subsequent analysis. The yield was calculated by multiplying the fresh weight of the roots by the plant density.

Table 1. Identification of the genotypes chosen for the experiment.

Identification	State of Origin in Brazil	Region or City Collected	Classification
DG014	São Paulo	Iguape	Brave
DG125	São Paulo	Iguape	Brave
DG203	Pará	Belém/Embrapa	Sweet
DG707	Amazonas	Boa Esperança	Brave
DG745	Minas Gerais	Conceição dos Ouros/Ouros Velho	Brave
DG768	Minas Gerais	Conceição dos Ouros/Ouros Velho	Sweet
DG839	Minas Gerais	Conceição dos Ouros/Pintos	Sweet
DG848	Minas Gerais	Conceição dos Ouros/Sertãozinho	Sweet
MS018	Mato Grosso do Sul	Bodoquena	Sweet
MS019	Mato Grosso do Sul	Miranda	Sweet
MS053	Mato Grosso do Sul	Bela Vista	Sweet
MS055	Mato Grosso do Sul	Rio Verde	Sweet
MS077	Mato Grosso do Sul	Chapadão do Sul	Sweet
MS119	Mato Grosso do Sul	Cassilândia	Sweet
MS127	Mato Grosso do Sul	Bonito	Sweet
MS132	Mato Grosso do Sul	Bonito	Sweet
MS250	Mato Grosso do Sul	Novo Horizonte do Sul	Sweet
MS260	Mato Grosso do Sul	Antônio João	Sweet
MS298	Mato Grosso do Sul	Ponta Porã	Sweet
MS317	Mato Grosso do Sul	Ponta Porã	Sweet

The leaf area index (LAI), specific leaf area (SLA), and starch content for each plot were observed from six sampled plants at harvest. The subsamples of fresh leaves were used to measure the leaf area using a leaf area meter (LI-Cor 3100, LI-COR Inc., Lincoln, NE, USA). The LAI values were calculated as the ratio of canopy leaf area–ground area, and the values for the SLA were recorded as the ratio of leaf area per leaf dry weight [23]. The starch contents of storage roots were determined by using the specific gravity method [24], and starch yields were calculated based on the starch contents and the weights of the storage roots.

2.3. Determination of Chlorophyll Content

The leaf tissue was macerated in 80% acetone; an aliquot of 200 µL of the extract was then collected and added to 1.8 mL of 80% acetone. A spectrophotometer (SP-220, bioespectro™, Biospectro, São Paulo, Brazil) was used to take measurements at the following wavelengths (absorbance): 647, 653, 663, and 665 nm [25]. The results from the sum of chlorophyll (total chlorophyll) were expressed in µg kg^{-1}.

2.4. Digestion and Mineral Analysis

Subsamples (0.20 g) of dried, milled leaves and grain materials were weighed (exact weights recorded) and digested in digestion tubes of a perfluoroalkoxy (PFA) liner material containing 2 mL 70% Trace Analysis Grade HNO_3, 1 mL Milli-Q water, and 1 mL H_2O_2. Prior to Se and nutrients [phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn)] analysis, the digestates were diluted using milli-Q water at a rate of 1:10. Data processing was undertaken using Qtegra™ software Version 2.8 (Thermo Fisher Scientific, Waltham, MA, USA) as previously described by Santos [12]. The nitrogen concentration was determined through the micro-Kjeldahl analytical method, following the sulfuric acid digestion of plant material. The concentrations of other mineral elements were assessed using an inductively coupled plasma optical emission spectroscope (ICP-OES; iCAP 7000 Series, Thermo Fisher Scientific, Waltham, MA, USA), following the nitric acid digestion of plant material. The accurately weighed samples of dried plant material (200 mg) were digested in a closed-vessel microwave oven (ETHOS 1600©, Milestone, Italy) using HNO_3 and H_2O_2. The quality control for the analytical procedures involved the use of certified reference materials 1515 Apple Leaves and 1568 Rice Flour (National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.5. Gas Exchange Parameters

Gas exchange parameters were performed with an infrared gas analyzer (LI-6400XT, LICOR, Lincoln, NE, USA) between 08:00 and 10:00 h at a photon flux density (PPFD) of 1700 µmol m^{-2} s^{-1} and an air CO_2 concentration of 380 µmol mol^{-1} on the same day that the plants were harvested. The leaf stomatal conductance (gS), transpiration (E), and the net photosynthesis rate (*A*) were measured according to Santos [26].

2.6. Statistical Analysis

The results were analyzed by the F test ($p < 0.05$), and significant differences among means were determined by the Scott–Knott test ($p < 0.05$). The principal component analyses (PCA) were applied to visualize the responses of each species in terms of treatments, using R [27]. The cluster analysis was measured with the Euclidean distance of similarity to identify groups among species. The software packages SAS version 9.1 [28] and R version 3.5.1 [27] were used to statistical analyses. The data variability was indicated with the standard error and shown graphically using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA).

3. Results

The ANOVA results are summarized in Tables S1 and S2. Significant differences, due to all sources of variation, were observed. In both growing seasons, a significant effect of the genotypes was observed. The growth and yield results of cassava exhibited consistent patterns across both growing seasons (Tables 2 and 3). The MS250 genotype achieved the shortest plant height (1.07 m), while DG014 had the tallest (2.73 m). For the stem diameter, the results ranged from 3.20 mm for genotypes DG125 and DG848 to 8.56 mm for DG014. The chlorophyll accumulation results varied between 1.20 µg kg^{-1} for genotype DG848 and 9.92 µg kg^{-1} for DG014 (Tables 2 and 3).

There were statistically significant differences in the yields of the genotypes, ranging from 19.00 to 21.15 t ha^{-1} in 2021/2022 and from 18.93 to 22.33 t ha^{-1} in 2022/2023. Notably, the genotypes in this study demonstrated an average yield that was higher (20 t ha^{-1}) than the Brazilian average (17 t ha^{-1}) [18]. The genotypes DG014, DG839, and MS018 exhibited the highest yields in both growing seasons. Furthermore, the DG014, DG839, and MS018 genotypes exhibited stem heights and diameters approximately twice as large as those of the other genotypes. Furthermore, these specific genotypes demonstrated an eightfold increase in the accumulation of total chlorophyll compared to genotypes with lower levels of this photosynthetic pigment (DG125, DG745, DG848, MS127, MS132, MS250, MS260, MS298, and MS317). The genotypes DG014, DG839, and MS018 also showed the highest results of LAI, SLA, and starch content in both growing seasons.

No differences were observed in the shoot nutrient concentration (Tables S3 and S4); however, the plants demonstrate variations for the concentrations of Fe, Zn, and Se in the roots (Table S2). All cassava genotypes exhibited no differences in Fe, Zn, and Se accumulation across different growing seasons. However, the ANOVA results indicated non-significant differences in the interactions between genotypes and the growing season. There are no differences between genotypes when comparing the concentrations of Fe, Zn, and Se in cassava roots that were grown in different growing seasons (Table S2).

Table 2. The results of height (H), diameter (D), and chlorophyll content (CHL) of 20 cassava genotypes in two growing seasons (2021–2022 [1] and 2022–2023 [2]).

Genotypes	H [1] (m)	H [2] (m)	D [1] (mm)	D [2] (mm)	CHL [1] (μg kg^{-1})	CHL [2] (μg kg^{-1})
DG014	2.73 Aa	2.76 Aa	8.56 Aa	8.43 Aa	9.85 Ba	9.92 Aa
DG125	1.20 Ab	1.24 Ab	3.20 Bc	3.94 Ac	1.24 Ae	1.20 Ae
DG203	1.60 Ab	1.62 Ab	4.31 Ac	4.13 Bc	7.25 Ab	7.23 Ab
DG707	1.50 Bb	1.63 Ab	4.13 Ac	4.06 Ac	6.15 Ac	6.12 Ac
DG745	1.21 Bb	1.43 Ab	8.40 Aa	8.02 Ba	2.35 Ae	2.33 Ae
DG768	1.30 Bb	1.55 Ab	3.41 Bc	3.74 Ac	8.34 Ab	8.31 Ab
DG839	2.42 Ba	2.67 Aa	8.47 Aa	8.49 Aa	9.83 Aa	9.81 Aa
DG848	1.20 Bb	1.31 Ab	3.20 Ac	3.22 Ac	1.22 Ae	1.20 Ae
MS018	2.31 Ba	2.41 Aa	8.32 Aa	8.36 Aa	9.94 Aa	9.90 Aa
MS019	1.32 Bb	1.47Ab	4.47 Ac	4.52 Ac	7.86 Ab	7.81 Ab
MS053	1.42 Ab	1.47 Ab	8.47 Aa	8.41 Aa	4.86 Bd	5.03 Ad
MS055	1.42 Ab	1.36 Ab	3.47 Bc	4.01 Ac	4.91 Ad	4.81 Ad
MS079	1.32 Ab	1.21 Bb	3.47 Bc	3.91 Ac	4.93 Ad	4.81 Ad
MS119	1.44 Ab	1.26 Bb	8.25 Aa	8.17 Aa	4.94 Ad	4.13 Bd
MS127	1.43 Ab	1.26 Bb	8.01 Aa	8.03 Aa	2.17 Ae	2.14 Ae
MS132	1.08 Bb	1.39 Ab	6.47 Ab	6.31 Bb	2.22 Ae	2.01 Ae
MS250	1.07 Bb	1.29Ab	5.13 Ab	5.03 Bb	2.06 Ae	2.03 Ae
MS260	1.13 Ab	1.14 Ab	4.33 Ad	4.34 Ad	2.42 Ae	2.41 Ae
MS298	1.51 Ab	1.56 Ab	5.09 Ab	5.04 Ab	2.07 Ae	2.02 Ae
MS317	1.41 Ab	1.46 Ab	4.01 Ac	3.96 Ac	2.08 Ae	2.03 Ae

Different uppercase letters show statistically significant differences when comparing growing seasons, and different lowercase letters indicate statistically significant differences when comparing genotypes, according to the Scott–Knott test (5%).

Table 3. The results of yield, leaf area index (LAI), specific leaf area (SLA), and starch content of 20 cassava genotypes in two growing seasons (2021–2022 [1] and 2022–2023 [2]).

Genotypes	Yield [1] (t ha^{-1})	Yield [2] (t ha^{-1})	LAI [1] (cm^2)	LAI [2] (cm^2)	SLA [1] (cm^2 g^{-1})	SLA [2] (cm^2 g^{-1})	Starch [1] (t ha^{-1})	Starch [2] (t ha^{-1})
DG014	21.15 Aa	22.33 Aa	1.43 Aa	1.40 Aa	185.25 Aa	184.45 Aa	14.91 Aa	14.20 Aa
DG125	19.05 Bd	19.55 Ad	0.81 Bc	0.75 Ac	156.05 Bc	155.65 Ac	6.33 Ad	6.20 Ac
DG203	20.05 Ab	20.25 Ab	1.19 Ab	1.10 Ab	164.95 Ab	164.45 Ab	11.05 Ab	11.45 Ab
DG707	20.15 Ab	20.55 Ab	0.83 Ac	0.90 Ac	153.25 Ac	152.45 Bc	10.56 Ab	10.96 Ab
DG745	19.47 Bc	20.37 Ab	0.77 Ac	0.72 Bc	151.31 Ac	150.91 Bc	6.81 Ad	6.01 Ac
DG768	19.42 Bd	19.92 Ac	1.17 Ab	1.12 Bb	165.85 Ab	165.05 Ab	11.33 Ab	10.63 Ab
DG839	21.11 Ba	21.91 Aa	1.45 Aa	1.41 Aa	184.23 Aa	184.13 Aa	14.82 Aa	14.02 Aa
DG848	19.05 Ad	19.25 Ac	0.78 Ac	0.74 Ac	150.21 Ab	150.10 Ac	6.71 Ad	6.31 Ac
MS018	21.10 Aa	21.31 Aa	1.48 Aa	1.39 Aa	184.05 Aa	183.15 Ba	14.33 Aa	14.10 Aa
MS019	19.51 Bc	20.41 Ab	1.17 Ab	1.12 Ab	162.55 Ab	162.45 Ab	10.74 Ab	10.94 Ab
MS053	19.42 Ac	19.92 Ab	0.81 Ac	0.82 Ac	160.44 Ab	160.14 Ab	8.95 Ac	8.45 Ac
MS055	20.51 Ab	20.81 Ab	0.84 Ac	0.82 Ac	159.15 Ab	152.45 Bc	8.75 Ac	8.35 Ac
MS079	20.03 Ab	20.43 Ab	0.87 Ac	0.80 Ac	153.25 Ac	152.45 Ac	9.03 Ac	8.23 Ac
MS119	20.21 Ab	20.41 Ab	0.86 Ac	0.80 Ac	151.66 Ac	151.46 Ac	8.81 Ac	8.01 Ac
MS127	20.12 Ab	20.52 Ab	0.79 Ac	0.70 Ac	151.55 Ac	151.45 Ac	6.93 Ad	6.13 Ad
MS132	20.51 Ab	20.21 Ab	0.71 Ac	0.70 Ac	151.66 Ac	151.46 Ac	7.11 Ad	6.31 Ad
MS250	20.41 Ab	20.21 Ab	0.72 Ac	0.70 Ac	151.87 Ac	151.37 Ac	6.73 Ad	6.03 Ad
MS260	19.51 Ac	19.71 Ac	0.78 Ac	0.70 Ac	152.11 Ac	151.21 Ac	7.61 Ad	6.91 Ad
MS298	19.00 Ad	18.93 Ad	0.81 Ac	0.71 Ac	151.77 Ac	151.37 Ac	6.85 Ad	6.05 Ad
MS317	19.01 Bd	19.91 Ac	0.78 Ac	0.70 Ac	151.73 Ac	151.03 Ac	6.58 Ad	6.38 Ad

Different uppercase letters show statistically significant differences when comparing growing seasons, and different lowercase letters indicate statistically significant differences when comparing genotypes, according to the Scott–Knott test (5%).

In the concentration of Fe (Figure 1), the evaluated genotypes ranged from 2.20 to 10.73 mg kg^{-1} in roots, with notable performances from DG014, MS055, MS079, DG839, MS018, and MS019, as all of them contained above 10.0 mg kg^{-1} and did not statistically differ from each other. Plants serve as the primary origin of dietary iron, whether consumed directly in the form of staple crops and vegetables, or indirectly through animal feed. Enhancing the nutrient content of edible plant components, a process referred to as biofortification, is considered a sustainable strategy to combat iron deficiency, a significant global health concern [29]. Considering that the average annual cassava consumption per person in the state of Mato Grosso do Sul is 25 kg [30], these genotypes would result in an additional intake of 270 mg of Fe. This represents 72.5% of the micronutrient's requirement for a child, 7.25% for men, and 4.83% for women [5].

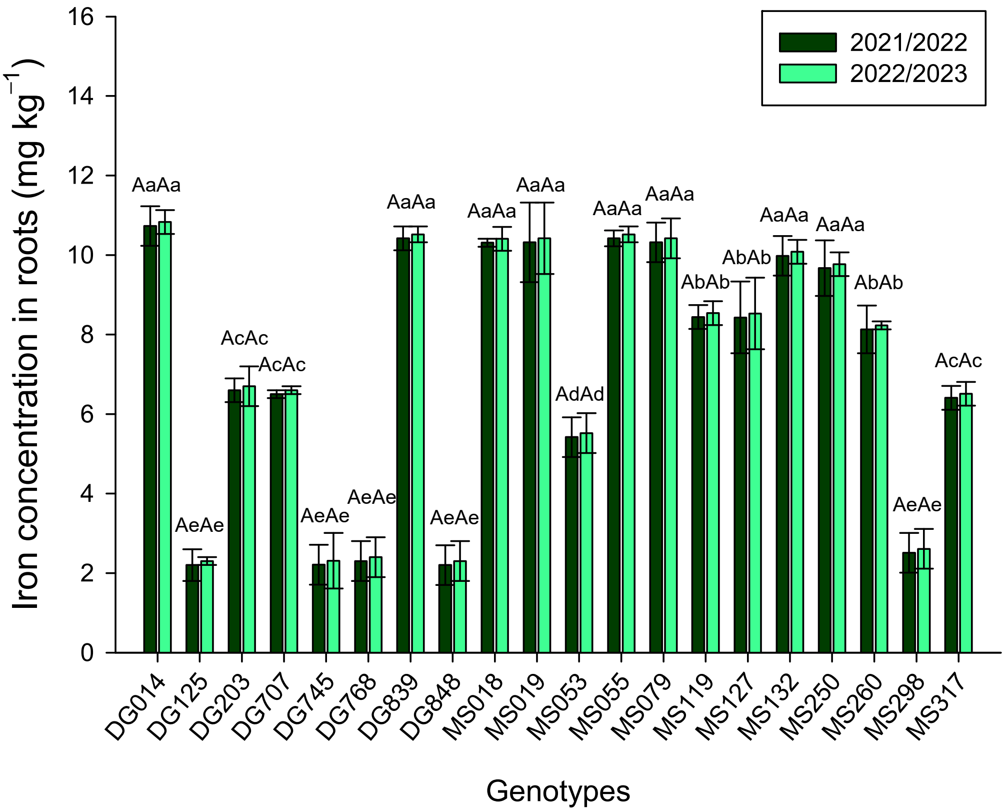

Figure 1. The Iron (Fe) concentration in the roots of 20 cassava genotypes from South America (2021/2022 and 2022/2023). Different uppercase letters show differences when comparing growing seasons, and different lowercase letters indicate differences when comparing genotypes, according to the Scott–Knott test (5%).

The concentration of Zn (Figure 2) in the cassava roots ranged from 3.21 to 8.56 mg kg^{-1}, with the genotypes DG014, DG745, DG839, MS018, MS053, MS119, and MS127 showing concentrations above 8.0 mg kg^{-1} of Zn, and there were no statistical differences among them. Silva [31], in their study on the Zn content in cassava roots, observed an increase of more than 40% in nutrient accumulation with fertilization up to 2.8 g kg^{-1} of zinc sulfate, along with an increase in dry matter and productivity. It is worth noticing that the recommended daily intake of Zn is 400 mg/year for women, 5500 mg/year for men, and 7000 mg/year for pregnant and lactating women [9]. Therefore, the consumption of 25 kg of cassava per person per year could meet 5%, 4%, and 3% of the requirements for women, men, and pregnant women, respectively.

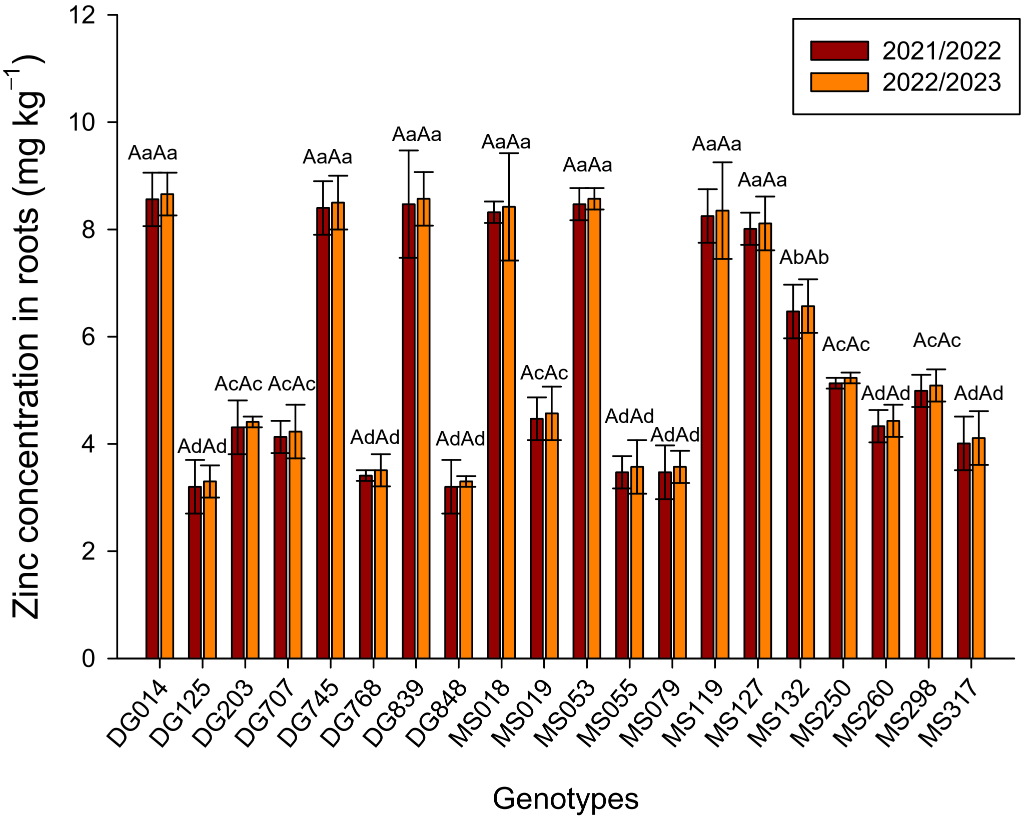

Figure 2. The Zinc (Zn) concentration in the roots of 20 cassava genotypes from South America (2021/2022 and 2022/2023). Different uppercase letters show differences when comparing growing seasons, and different lowercase letters indicate differences when comparing genotypes, according to the Scott–Knott test (5%).

For the concentration of Se (Figure 3), the genotypes ranged from 1.20 to 10.92 μg kg^{-1}, with concentrations above 10.0 μg kg^{-1} observed in DG014, MS018, DG839, MS260, MS132, MS250, MS298, and MS317, which did not differ statistically from each other. The minimum daily recommended intake of Se is 55 μg, and no adverse effects are observed with a dosage of up to 400 μg per day [11]. Therefore, the annual consumption of 25 kg of the roots from genotypes DG014, MS018, DG839, MS260, MS132, MS250, MS298, and MS317 would be sufficient to meet 10% of the annual Se consumption requirement.

No statistically significant variances were observed for the net photosynthesis rate (*A*), transpiration (*E*), and stomatal conductance (*gs*), as observed in the growth data (Table S2). Notably, genotypes DG014, DG839, and MS018 demonstrated the highest values for *A*, *E*, and *gs* (Figure S1). The genotypes DG014, DG839, and MS018 showed *A* values twice as high as the genotypes with the lowest *A* values (DG125, DG745, and DG848). In terms of the hydraulic driving capacity (*E* and *gs* value), the genotypes DG014, DG839, and MS018 demonstrated a 30% increase in relation to the average of the evaluated genotypes (DG125, DG745, and DG848).

According to the cluster analyses (Figure 4a), three main groups were formed within the plant species. Group 1 was represented by DG014, DG839, and MS018; Group 2 by DG125, DG848, DG745, DG707, MS055, MS079, MS119, MS127, MS132, MS250, MS260, MS317, and MS298; and Group 3 by DG203, MS019, DG768, and MS053. The analysis of principal components indicates a clear difference of genotype response regarding the nutrients concentration, growth, leaf gas exchange, and chlorophyll. The total variance was 83%, with the principal components 1 and 2 (PC1 and PC2) explaining 69% and 14%, respectively (Figure 4b). MS018, DG014, and DG839 had a high height, leaf gas exchange, and productivity, while DG745, MS119, and MS127 showed the highest values of Zn and diameter. The opposite was observed in MS019, MS055, MS079, MS260, MS317,

MS250, MS298, DG768, DG203, DG707, DG848, and DG125, resulting in lower values of Zn, diameter, height, leaf gas exchange, and productivity. Moreover, the species MS132 displayed average values.

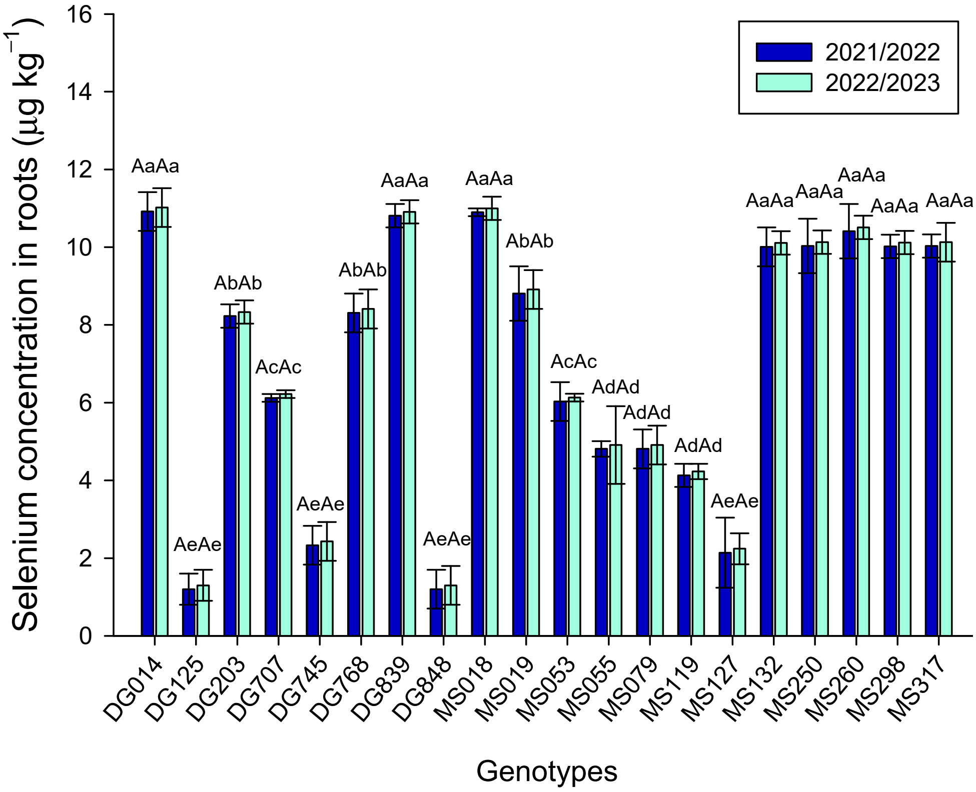

Figure 3. The Selenium (Se) concentration in the roots of 20 cassava genotypes from South America (2021/2022 and 2022/2023). Different uppercase letters show differences when comparing growing seasons, and different lowercase letters indicate differences when comparing genotypes, according to the Scott–Knott test (5%).

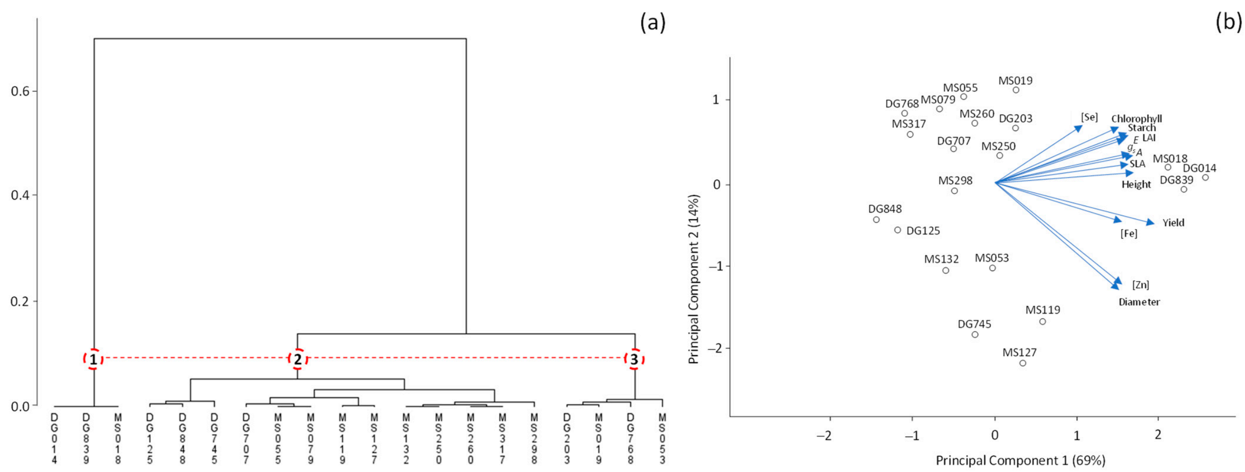

Figure 4. Hierarchical cluster analyses (**a**) and principal component analysis (**b**) were conducted on 20 cassava genotypes from South America for the years 2021/2022 and 2022/2023. The numerical designations in the hierarchical cluster analyses indicate distinct groups of genotypes with similar concentration of iron, zinc, and selenium, as well as growth and physiological responses in the two growing seasons.

Cluster analysis reveals that Group 1 (MS018, DG014, and DG839) comprises genotypes that are characterized by high productivity and the significant accumulation of Fe, Zn, and Se in cassava roots (Figure 4a). This observation bears significance for human biofortification strategies, suggesting that the genotypes in Group 1 hold promise for the development of nutritionally enhanced cassava varieties. The potential implications of these characteristics underscore their relevance in addressing specific micronutrient deficiencies that are prevalent in populations that rely on cassava as a staple food source.

4. Discussion

Micronutrient deficiency presents a global health concern, with approximately 161 million children under the age of 5 experiencing stunted growth [32]. This condition is, in part, attributed to hidden hunger, a phenomenon arising from the insufficient presence of essential vitamins and minerals in foodstuffs. Around 800 million people globally rely on the consumption of the tropical root crop cassava [33]. The genotypes DG014, DG839, and MS018 also showed the highest results of Fe, Zn, and Se accumulation, as well as yield, LAI, SLA, and starch content in both growing seasons. The optimal LAI value proves advantageous for canopy photosynthesis and, consequently, enhances crop yield [23].

Several studies use the LAI and SLA indices to select cassava genotypes with high productivity [34–36]. However, the results of this study revealed a positive correlation between elevated LAI and SLA values and increased root yield in the cassava genotypes, along with higher concentrations of Fe, Zn, and Se in the roots (Figure 4b). Recognizing the pivotal role of leaves in crop development and yield, the maximum individual leaf area emerges as a potential supplementary criterion for better elucidating the adaptability of cassava in both the yield and biofortification trials. Furthermore, a comprehensive assessment of the entire canopy's leaf area can offer clearer insights into crop behavior [37]. Nonetheless, exploring the feasibility of non-intrusive methods to determine the leaf area without disturbing the plants is crucial [38], especially given the limited number of plants available during the early stages of the cassava biofortification trial. Our results indicate that the cassava genotype with the potential for the biofortification of Fe, Zn, and Se have the highest LAI and SLA indices. The LAI and SLA indices in this study were similar to those observed by Mwamba [39].

The genotypes with higher levels of Fe, Zn, and Se also showed high levels of starch in the roots. Starch, the primary carbohydrate source for plant species, consists of two crucial components: amylose and amylopectin [40]. The content and structures of these components contribute to starch's distinct properties, essential for applications in food processing and various industries [23]. Cassava, a starchy root crop, serves as a staple food in tropical and sub-tropical regions, and finds applications in diverse industrial processes [33]. The starch biosynthesis in cassava is regulated by multiple isoforms of enzymes expressed during root development. Significant efforts have been dedicated to comprehending the mechanisms governing starch biosynthesis and its regulation [40]. The genotypes with the highest starch accumulation also demonstrate high accumulations of Fe, Zn, and Se (Figures 1–3). However, there was no difference in the concentrations of Fe, Zn, and Se in cassava roots grown in different years. This finding bears significance and holds promise in the realm of cassava genetic enhancement for biofortification purposes, with respect to Fe, Zn, and Se. The uniformity in the elemental accumulation, despite differing growing seasons, underscores the robustness of genotypic traits, affirming their consistent capacity to accumulate Fe, Zn, and Se. Yabuta [14] demonstrated a high correlation between cassava growth and yield with an increase in the gas exchange rate. In the present study, it was shown that, in addition to growth, cassava plants with a greater capacity to accumulate Fe, Zn, and Se also exhibit enhanced photosynthetic capacity.

In cassava, there is no information available regarding how photosynthesis correlates with the potential accumulation of Fe, Zn, and Se. This information is crucial for developing strategies to enhance carbon gain and water use efficiency in this crop. In addition to physiological measurements, correlating these parameters with the potential for biofortification provides a means to test hypotheses that are related to different dynamic behaviors in vivo, offering a broader view and a guide to quantitatively assess the value of various individual characteristics that affect photosynthetic efficiency. Previous model predictions determined potential routes for improvements in photosynthesis [41,42], which were later successfully translated into increases in yield [15,43]. This approach is employed here, integrating physiological aspects and the accumulation of Fe, Zn, and Se in order to identify genotypes suitable for use in agronomic biofortification programs.

Cassava utilizes C3 photosynthesis with leaf photoassimilates apoplasmically loaded into the phloem [44]. Fibrous roots, originating from the nodes on the planted cassava stem segments, undergo a secondary growth to transform into storage roots. These storage roots exhibit a well-organized vascular cambium, situated between the phloem and xylem. Longitudinal vascular ray cells, derived from the cambium, form a bridge between these two cell types, facilitating the exchange of water, nutrients, and carbohydrates [45]. The unloading of photoassimilates in storage roots follows a symplasmic route to the storage parenchyma cells, facilitated by vascular rays [46]. The vascular cambium's alternating ray initial cells and fusiform initial cells give rise to vascular ray cells and xylem/phloem cells, respectively. Unlike ray initial cells, which are connected to the root symplast, fusiform initial cells receive nutritional support through apoplastic transport. Presumably, genotypes DG014, DG839, and MS018 possess genetic adaptations that enable efficient starch accumulation when compared to other genotypes, as observed by Sonnewald [45]. The results of the present work suggest that these same mechanisms can enhance the accumulation of Fe, Zn, and Se in cassava roots.

Cassava, while serving as a significant energy source, typically lacks in nutritional quality [47]. Despite this, root crops are recognized for their higher Fe and Zn content, especially when compared to cereal grains [19,48]. Consequently, investigations centered on enhancing the nutritional profile of cassava roots can contribute to the development of effective techniques for the biofortification of cassava. Tagliapietra [49], in their study on the nutritional quality and sensory acceptance of biofortified cassava, found higher levels of carotenoids in the biofortified cultivars. Additionally, the sensory analysis achieved an approval rate of 78.7%, demonstrating good potential for incorporating these roots into school meals. It is important to note that there are other food sources of Fe, Zn, and Se consumed in the human diet [2]. Furthermore, food crops biofortified with iron, zinc, selenium, and pro-vitamin A provide satisfactory levels of these micronutrients in the diets of populations that previously suffered from deficiencies, especially in developing regions [50].

There were no differences in the concentrations of nutrients in the cassava aerial segment. Thus, it is evident that, for the genotypes evaluated, the accumulation of Fe, Zn, and Se in the roots was primarily influenced by the genetic effect rather than any potential nutritional interaction, as observed by Corguinha [19]. Genetic factors significantly impact the nutritional quality of crops. The plant's capacity to absorb and accumulate nutrients varies based on its genotype, emphasizing that variability is a fundamental prerequisite in developing genotypes with elevated nutrient content in its edible sections, which is completed via genetic biofortification approaches [51].

Deficiencies in Fe, Zn, and Se can have severe consequences for human health, including conditions such as anemia, compromised immune function, and cognitive impairments [2]. The research, which identifies cassava genotypes that naturally accumulate these nutrients, has the potential to make a substantial positive impact on global public health. Agronomic biofortification is the process of increasing the nutrient content of food crops through breeding and agronomic practices [12,52,53]. Cassava, a widely consumed staple crop in many parts of the world, is an excellent candidate for biofortification [19]. By identifying cassava genotypes with higher nutrient content, this research contributes to the broader efforts that improve the nutritional quality of staple crops. Our emphasis on easily accessible plant species and genotypes is crucial for addressing nutrient deficiencies, especially in low-income and food-insecure regions. Cassava is a source of sustenance for millions of people, particularly in tropical and subtropical regions [33,54,55]. Enhancing its nutrient content can have a direct and positive impact on the nutritional status of vulnerable populations.

Considering environmental influences, the incorporation of agronomic practices can serve as a valuable complement to ensure an adequate nutritional balance in plants. As suggested by Corguinha [19], agronomic biofortification, achieved through the application of fertilizers (either on the soil or as foliar spray) or the use of NPK fertilizers that are enriched with Fe, Zn, or Se, could be an efficient and practical approach to increase the absorption and accumulation of these nutrients in cassava.

In addition to addressing nutrient deficiencies, our results also highlight the importance of increasing cassava productivity. This is significant for ensuring food security, especially in areas where cassava is a primary food source. Higher yields of nutrient-rich cassava genotypes can help reduce malnutrition and improve the overall well-being of communities. While this study primarily focuses on cassava, its findings and methodology can be applied to other crops as well. The identification of plant genotypes with elevated nutrient content can serve as a model for addressing nutrient deficiencies in a variety of staple crops, which is vital for achieving global food security.

5. Conclusions

The findings of this study underscore the suitability of cassava genotypes for inclusion in biofortification programs, specifically MS018, DG014, and DG839. These genotypes exhibited elevated concentrations of Fe, Zn, and Se in their edible components, coupled with high productivity, making them promising candidates for enhancing the nutritional quality of cassava. Notably, our results emphasize the importance of fertilization with these elements to maximize the absorption and accumulation of Fe, Zn, and Se in cassava genotypes under traditional cultivation conditions. It is recommended that biofortification initiatives prioritize the use of genotypes with inherently higher contents of these essential elements, as demonstrated by MS018, DG014, and DG839. However, it is crucial to acknowledge the potential for enhancing Fe, Zn, and Se levels in other genotypes through targeted fertilization strategies. Further investigations are warranted to comprehensively assess the impact of varying the doses and forms of Fe, Zn, and Se fertilization on different cassava genotypes, thereby contributing valuable insights for the optimization of biofortification practices in cassava cultivation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14020268/s1, Table S1: ANOVA for height (H), diameter (D), chlorophyll content (CHL), yield, leaf area index (LAI), specific leaf area (SLA) of 20 cassava genotypes from South America for growing seasons; Table S2: ANOVA for Iron (Fe), Zinc (Zn), Selenium (Se) concentration; and net photosynthesis rate (A), transpiration (E), and stomatal conductance (gS) of 20 cassava genotypes from South America for growing seasons; Table S3: Concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), cupper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and selenium (Se) in the shoot of cassava genotypes grown in 2021/2022; Table S4: Concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), cupper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and selenium (Se) in the shoot of cassava genotypes grown in 2022/2023. Figure S1: Net photosynthesis rate (A) (a), transpiration (E) (b), and stomatal conductance (gs) (c) in 20 cassava genotypes from South America (2021/2022 and 2022/2023). Different uppercase letters show differences when comparing growing seasons, and different lowercase letters indicate differences when comparing genotypes according to the Scott-Knott test (5%).

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