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Physiological traits, cadmium bioaccumulation and biomass distribution in seven cocoa genotypes

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Introduction: Cocoa plants tend to accumulate significant amounts of cadmium (Cd) in their beans and derived products due to their high affinity for Cd, which can pose challenges for commercialization. Research on the variations in Cd absorption capability among cocoa genotypes is still limited.

Aims: This study assessed physiological traits, soil pH, soil electrical conductivity (EC), macronutrient concentrations, Cd bioaccumulation, and biomass distribution in seven cocoa genotypes (CCN-51, EET-103, IMC-67, POUND-12, EET-399, EET-95 and EET-400) grown in greenhouse.

Methods: The genotypes were arranged in a randomized block design with three replicates, assessing variables such as chlorophyll content, leaf gas exchange, pH, soil EC, macronutrient concentration, Cd uptake and transfer, Cd bioaccumulation, and dry root and shoot biomass.

Results and discussion: Results showed no significant differences in chlorophyll content and gas exchange between genotypes, although EET-103 exhibited a higher transpiration rate. A significant reduction in gas exchange occurred 80 days after sowing, associated with decreases in chlorophyll content and stomatal conductance. The soil displayed a strongly acidic pH (≤ 5.5) and high EC (6 dS m⁻¹) across all genotypes, with no significant inter-genotype differences observed. Cadmium accumulation was nine times higher in the shoot than in the roots, with EET-95 showing a reduced Cd uptake capacity, and EET-399 demonstrating limited Cd transfer from root to shoot.

Conclusion: The genotype with the highest Cd absorption and shoot accumulation was CCN-51. Conversely, POUND-12 had the lowest Cd levels in both shoot and root, suggesting its potential as a reference genotype to limit Cd entry to aboveground tissues, especially in soils with elevated Cd concentrations.

KEYWORDS

Theobroma cacao, heavy metals, soils, absorption, physiology

Introduction

Cadmium (Cd) is a trace element found in the earth's crust and is considered a natural pollutant in agricultural soils. In the aqueous phase of the soil, Cd is easily absorbed by plants and bioaccumulated in different tissues, which makes it easier for it to enter the food chain (Zhao et al., 2020). The soil-to-plant transfer rate of Cd in cocoa cultivation is greater than 1 (Oliva et al., 2020), which leads to bean Cd concentration above 10 mg kg⁻¹, which is a serious concern for chocolate production (Argüello et al., 2019). Consumed Cd is harmful is known to cause serious health issues, such as cardiovascular disease, bone disorders, renal dysfunction, and DNA damage and, consequently, cause cancer (Davidova et al., 2024).

The European Union (EU) has set a maximum permissible limit of 0.80 mg kg⁻¹ of Cd in cocoa by-products to reduce risks to its citizens (Florida Rofner, 2021). However, countries like the Russian Federation, New Zealand, and Australia have established more stringent restrictions of 0.50 mg kg⁻¹ of Cd in chocolate products (Meter et al., 2019). Currently, in Ecuador, Peru, and Colombia there is no set limit for Cd in cocoa beans, although high levels have been found in cocoa derivatives (Argüello et al., 2019; Bravo et al., 2014; Chávez et al., 2015; Florida et al., 2018; Mite et al., 2010). Ecuador's El Oro, Manabí, and Esmeraldas provinces are the most affected by high levels of Cd contamination in cocoa-growing soils, reaching critical values ≥ 2 mg kg⁻¹ (Argüello et al., 2019; Mite et al., 2010). Ecuador's coastal regions rely heavily on cocoa for high-value chocolate production, but increased Cd contents could hinder global trade, especially under EU rules (Morales-Rodriguez et al., 2025).

Cocoa plants are affected by heavy metals like Cd, which disrupt various biological functions such as metabolism, leaf gas exchange, chlorophyll synthesis, cell division, electron transfer rate, and photochemical efficiency in the light reactions of photosynthesis (Haider et al., 2021; Borjas-Ventura et al., 2022; Vera Pinargote et al., 2025). Plants lacking Cd tolerance experience higher toxicity, leading to decreased leaf water content, reduced stomatal conductance (g_s), and increased reactive oxygen species production, necrosis, and cell damage. CCN-51 seedlings exposed to different Cd concentration showed altered mineral nutrient absorption, chlorophyll content, photosynthetic apparatus damage and reduced quantum yield, and the expression of the *psbA* gene was also disrupted, biomembrane rupture occurring in root and leaf cells (Pereira de Araújo et al., 2017).

It has been recently reported that the cultivation of the BN-34 genotype grafted onto the CCN-51 rootstock shows a high tolerance to Cd, which was associated with a high activity of ascorbate peroxidase, a protein that removes free radicals from plant cells, suggesting that some combinations of cocoa scions and rootstocks may be used to mitigate Cd toxicity (Almeida et al., 2023). Also, the EET-62 genotype showed strong tolerance to Cd content at 6 and 12 mg kg⁻¹; while PA-46 and IMC-67 genotypes had lower Cd accumulation, suggesting their potential use to limit Cd buildup in cocoa tissues (Galvis et al., 2023). Juvenile cocoa plants grown in soils with high Cd levels or combined Mn + Cd levels exhibit

impaired photosynthesis, oxidative stress, metabolic alterations, and increased Cd absorption, transport, and accumulation in roots and leaves (Barroso et al., 2023).

Genetic differences in Cd tolerance were also noted among cocoa rootstocks, with the EET-103 genotype exhibiting lower Cd extraction and accumulation capacity compared to CCN-51 (Reyes-Perez et al., 2023a). Genetic variations in cocoa hybrids during seedling growth exhibit differences in Cd partitioning, biomass accumulation, and photosynthetic efficiency, suggesting that cocoa responses to Cd stress are primarily determined by genetic variations (Borjas-Ventura et al., 2022; Vera Pinargote et al., 2025). Previous research findings highlight the role played by genetic enhancement programs in obtaining genotypes that accumulate less Cd and achieve better physiological performance, growth, and development of cocoa plants. Agronomic strategies aim to reduce Cd transfer rates by applying organic and inorganic amendments to precipitate or adsorb Cd, decreasing its bioavailability in soil solution, and leveraging interspecific variations in Cd phytoextraction and accumulation among cocoa genotypes (Meter et al., 2019; Engbersen et al., 2019).

However, limited documentation exists on the Cd absorption capacity of commercial cocoa genotypes from Ecuador. We hypothesize that: 1. The genotypic variation among the cocoa genotypes studied will result in significant differences in physiological and agronomic characteristics, and 2. there will be significant differences among the genotypes of cocoa in Cd absorption, transfer, and bioaccumulation. Therefore, this study aimed to evaluate the physiological characteristics (SPAD values, gas exchange, macronutrient concentrations in the shoot, and Cd bioaccumulation) and biomass distribution in seedlings of seven cocoa genotypes (CCN-51, EET-103, IMC-67, POUND-12, EET-399, EET-95, and EET-400) cultivated in greenhouse conditions.

Materials and methods

Study location

This research was conducted in the greenhouse and laboratory of the Soil and Water Management Department at the Pichilingue Tropical Experimental Station (EETP), part of the National Institute of Agricultural Research (INIAP). The station is located at 79°27'W longitude and 1°06'S latitude, at an altitude of 75 meters above sea level.

Genotypes

The study included the following cocoa genotypes: IMC-67 and POUND-12 (from the Iquitos genetic group), EET-399 and EET-400 (Curaray group), EET-103 and EET-95 (National group), and CCN-51 (CCN-51 genotype, a hybrid of IMC-67 $\times$ ICS-95) (Motamayor et al., 2008; Morillo et al., 2023; Thomas et al., 2024). The first four genotypes are resistant to machete sickness (*Ceratocystis cacaofunesta*) and are recommended by INIAP for use

as rootstocks (Suárez Capello et al., 1993; Vera Barahona et al., 1984); whereas, EET 103 is tolerant to *C. cacaofunesta* and recommended for cultivation in the province of Manabí. Additionally, EET-95 is tolerant to *C. cacaofunesta* (Quiroz Vera, 2000). Although the CCN-51 clone is susceptible to this disease, it remains the most widely used rootstock by propagators and was therefore selected as the control for this study.

Physical and chemical characteristics of the soil

The clay loam soil had the following chemical characteristics: ammonium (NH_4): 22 mg kg^{-1} ; phosphorus (P): 23 mg kg^{-1} ; potassium (K): 0.49 meq 100 mL^{-1} ; calcium (Ca): 19 meq 100 mL^{-1} ; magnesium (Mg): 2.8 meq 100 mL^{-1} ; sulfur (S): 6 mg kg^{-1} ; zinc (Zn): 8.2 mg kg^{-1} ; Cd: 1.53 mg kg^{-1} ; pH: 6.2; and electrical conductivity: 0.31 dS m^{-1} .

Experimental design

The study involved seven cocoa genotypes (treatments) arranged in a randomized block design with three replicates. Each experimental unit consisted of four plants, resulting in a total of 84 plants. The experiment lasted 120 days, comprising 90 days of plant growth, management, and evaluation under greenhouse conditions, followed by 30 days of laboratory analyses.

Experimental management

Soil for the experiment was collected from Río Negro Parish, Santa Rosa canton, El Oro province. It was dried in a greenhouse, disaggregated using a glass roller, and sieved through a 2 mm mesh. Polyethylene bags ($5 \times 8''$) were filled with 700 g of prepared soil.

The plants were propagated from seeds obtained through assisted pollination to ensure genetic purity. Ripe fruits were harvested, and seeds were removed, cleaned of their testa, and soaked in deionized water for 24 hours. Seeds were then planted individually in pots, positioned vertically with the narrower tip facing upward. Before planting, soil moisture was adjusted to field capacity using deionized water, which was maintained through periodic irrigation.

The seedlings were grown in a greenhouse covered with a 65% polyshade screen, providing an average light intensity of $210 \pm 30 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 12 hours of natural light daily. Greenhouse temperatures ranged from 23.3 to 33.5 °C, with relative humidity levels between 52% and 82%.

Fertilization was based on soil analysis and the nutritional requirements of cocoa during the nursery stage, tailored for containers with 700 g of soil (Table 1). Nutrients were applied as follows: Nitrogen (N) and S were divided into three applications at 21, 42, and 63 days after sowing (DAS). K, Mg, and Ca were applied in two fractions at 42 and 63 DAS, while P was applied as a single dose at planting. The fertilizers used were Urea (46% N), diammonium phosphate (18% N, 46% P), potassium chloride (60% K), ammonium sulfate (24% S, 21% N), magnesium sulfate (27% MgO, 16% S), and calcium nitrate (15% N, 26% CaO). All fertilizers, except phosphorus, were dissolved in water and applied as a soil drench at 20 mL per plant.

Variables evaluated

pH and EC of the soil

Soil samples were collected individually for each plant after removing the casings. Each sample was weighed (20 g) on an analytical balance (A&D Weighing, model HR200, Japan) and placed into a plastic cup. Then, 50 mL of deionized water was added to create a 1:2.5 P:V ratio. The mixture was stirred for 5 minutes and left to settle for 1 hour (Henríquez et al., 1998). pH readings were recorded using a potentiometer (HACH model SensION™ MM340, Germany), and EC was measured with a conductivity meter (HACH model SensION™ EC71, Germany).

Chlorophyll index

The chlorophyll content was assessed using a SPAD-502 Plus chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan). SPAD values are directly proportional to the total chlorophyll content in leaves (da Cunha et al., 2015). Measurements were taken every two weeks from 40 to 80 DAS between 9:00 a.m. and 1:00 p.m., on the same leaves used for gas exchange measurements.

Leaf gas exchange

Gas exchange parameters were measured at 40 and 80 DAS between 9:00 a.m. and 1:00 p.m. on six plants per treatment ($n = 6$). Measurements were performed with an infrared gas analyzer

TABLE 1 The nutritional requirements of cocoa determined by soil analysis and fertilization at the nursery stage are shown.

Fertilizers dose (g kg^{-1} of soil ⁻¹)											Applications (DAS)
N	P ₂ O ₅	S	MgO	CaO	Urea	(NH ₄) ₂ SO ₄	Ca(NO ₃) ₂	DAP	MgSO ₄	KCl	
1.02	2.58	0.41	—	—	—	1.71	—	5.62	—	—	21
0.68	—	0.41	0.20	—	—	1.24	1.65	—	0.71	1.40	42
1.38	—	0.41	0.20	0.42	1.48	1.24	1.65	—	0.71	1.40	63

Nutritional plan used in the development of cocoa genotypes.

DAP, diammonium phosphate; DAS, days after sowing; KCl, Potassium chloride.

(CIRAS 2, PP Systems, Hitchin, UK) to determine g_s , A , E , water use efficiency ($WUE = A/E$), and intercellular CO_2 concentration (C_i). These measurements were taken from the mid-section of leaf No. 4, considered a fully expanded, healthy adult leaf. Experimental conditions included: ambient CO_2 concentration (C_a) of $415 \pm 10 \mu\text{mol mol}^{-1}$, 21% O_2 , temperature of $28 \pm 1^\circ\text{C}$, a photon flux density (PPFD) of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ to saturate photosynthesis, and a leaf-air water vapor gradient (DPV) of 1.0–1.5 kPa.

Dried shoot and root biomass

Plants harvested at 90 DAS were rinsed with tap water and sequentially immersed in hydrochloric acid (3%), distilled water, magnesium sulfate (0.001 mol L^{-1}), and deionized water. The plants were then sectioned into shoot (leaves and stems) and roots. Each section was placed into labeled kraft paper sleeves and dried in an oven (Memmert, GmbH 450, Germany) at 65°C for 72 hours. The dry biomass was weighed on an analytical balance (A&D Weighing, model HR200, Japan).

Macronutrient concentration in the shoot

N concentration was determined using the Kjeldahl method (KjeltecTM 8400 TecatorTM Line Foss, Jinan, China) (Carrillo Zenteno et al., 2019). K, Ca, and Mg concentrations were analyzed using an atomic absorption spectrophotometer (AA-6800, Kyoto, Japan). S and P concentrations were quantified via the colorimetric method (Spercord 210 Plus, Jena, Germany) (Remache et al., 2017).

Determination of Cd in tissues

Dried plant tissues were ground using a Willey-type mill (IKA, model A11 basic, USA). To prevent contamination, the mill was cleaned with absorbent towels, diluted aqua regia (10%), and deionized water before each use. Cd was extracted through nitric-perchloric acid mineralization (8 mL HNO_3 and 2 mL $HClO_4$; Carrillo, 2003). Cd concentrations were measured with an atomic absorption spectrophotometer (Perkin Elmer, model AAnalyst 800, Japan) equipped with a graphite furnace, at a wavelength (λ) of 228.8 nm.

Cd content in root and shoots

The Cd content in roots and shoots was calculated using the following formula:

$$\text{Cd content } (\mu\text{g}) = \text{Biomass (g)} \times \text{Cd } \mu\text{g g}^{-1}$$

Cd extraction capacity

The efficiencies of Cd absorption (1) and translocation (2) were determined using equations described by Wang et al. (2007):

1. Absorption efficiency ($\mu\text{g g}^{-1}$) = $\frac{\text{Total Cd content in the plant}}{\text{Root dry mass}}$.
2. Translocation efficiency = $\frac{\text{Cd content in stem and leaves}}{\text{Cd content in roots}}$.

Statistical analysis

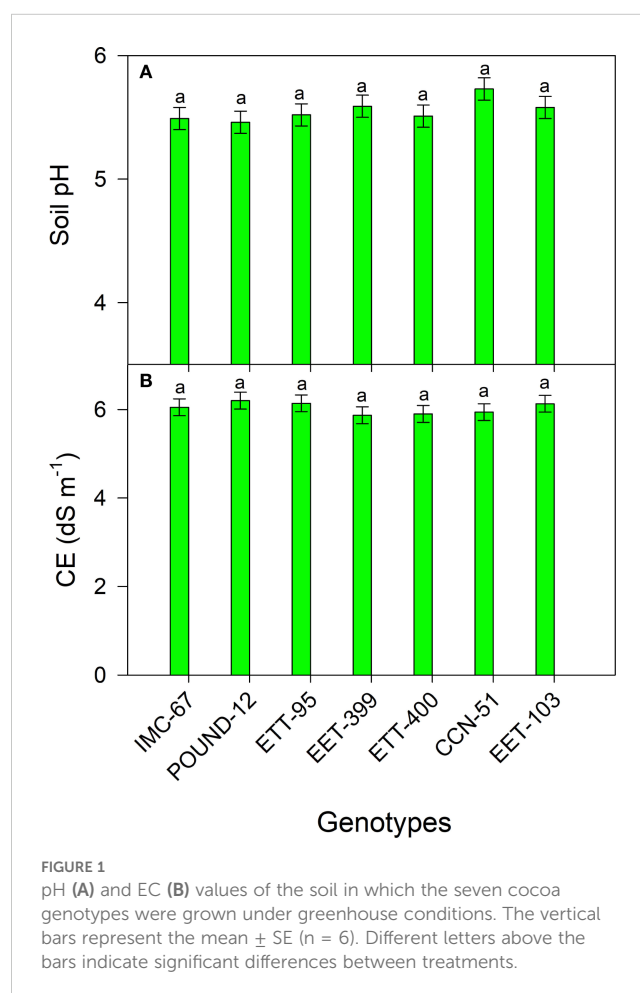
Data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Bartlett tests, respectively. An analysis of variance (ANOVA) followed by a Tukey's test was conducted at a 0.05 significance level. Statistical analyses were performed using Minitab version 19, and graphs were created using SigmaPlot 12 (Systat Software, San Jose, CA, USA).

Results

There were no significant differences in soil pH and EC among the genotypes. However, all genotypes exhibited strongly acidic soil pH values (≤ 5.5) and high EC levels (6 dS m^{-1}) (Figure 1).

Chlorophyll concentrations, measured at 40, 55, and 80 DAS, showed no statistically significant differences between genotypes (Figure 2). Nevertheless, a significant reduction in SPAD units, amounting to 31.5%, was observed at 80 DAS.

In terms of leaf gas exchange, no significant differences were found between genotypes at 40 and 80 DAS, except for transpiration, which was statistically significant at 80 DAS. The



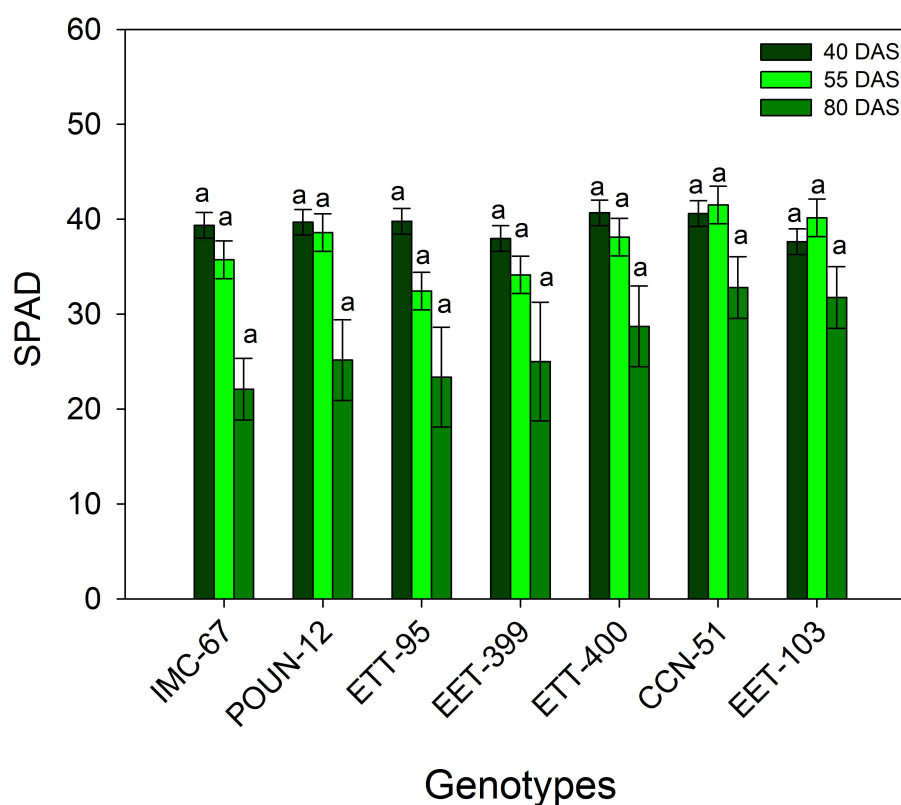


FIGURE 2

Chlorophyll concentration was evaluated in the seven cocoa genotypes used as standards at 40, 55, and 80 DAS. The vertical bars represent the mean $\pm$ SE ($n = 6$). Different letters above the bars indicate significant differences between treatments, as determined by the Tukey test ($p < 0.05$).

EET-103 genotype showed the highest E at $0.39 \text{ mmol m}^{-2} \text{ s}^{-1}$ (Figure 3). At 80 DAS, a reduction of 94% in A , 37% in E , 45% in g_s , and 71% in WUE was observed, while C_i increased by 61%.

There were no significant differences between cocoa genotypes in the production of dry biomass in shoots and roots (Figure 4). However, for aerial dry matter, IMC-67 produced 5% more biomass than CCN-51, while the clones POUND-12, EET-103, EET-399, EET-95, and EET-400 produced 36%, 27%, 22%, 25%, and 19% less biomass than CCN-51, respectively.

Similarly, in root dry weight, IMC-67 exhibited 5% higher biomass compared to CCN-51. In contrast, the POUND-12 and EET-400 genotypes produced 29% less biomass than CCN-51, followed by EET-103 (14% less), EET-399 (10% less), and EET-95 (5% less).

Macronutrient concentrations in the shoot (Figure 5) showed statistically significant differences only for N, P, and S. Among the genotypes, the highest concentrations were observed in CCN-51 for N (6.1 dag kg^{-1}), EET-399 for P (0.54 dag kg^{-1}), and IMC-67 for S (0.61 dag kg^{-1}).

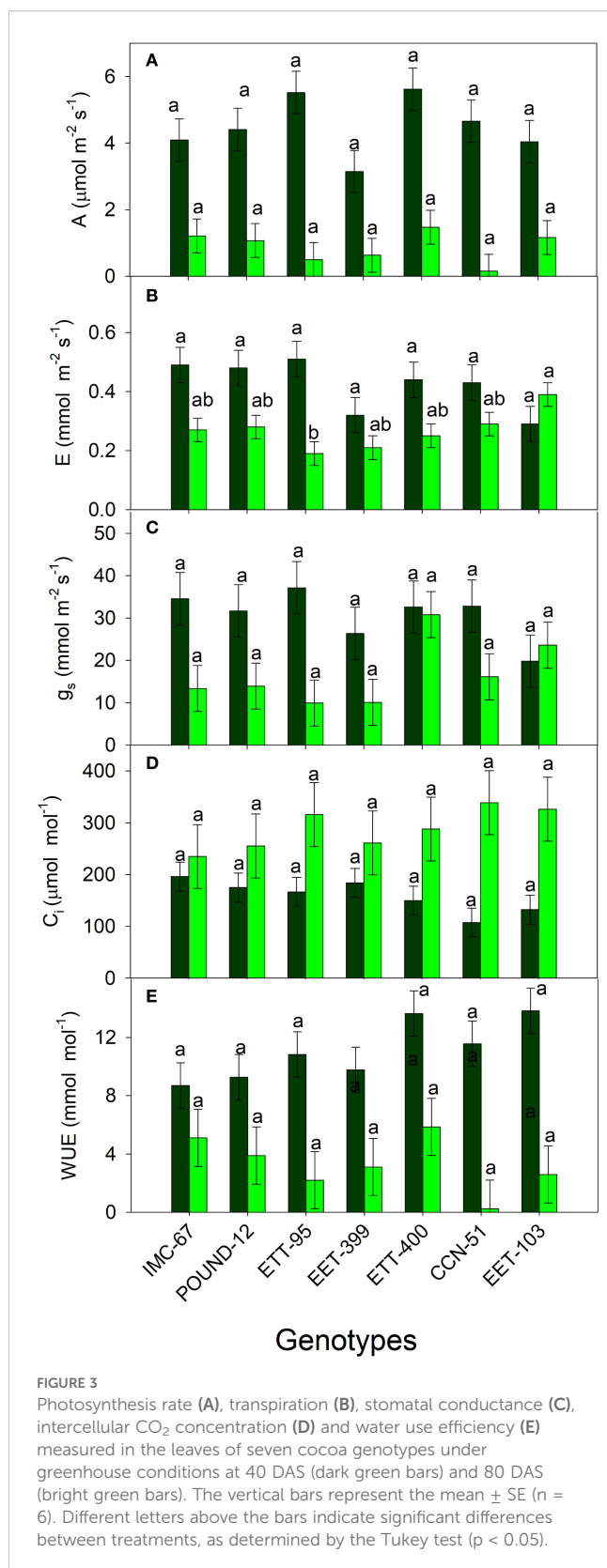
Cocoa genotypes did not exhibit significant differences in Cd absorption and translocation efficiencies or Cd content in shoots, except for root Cd accumulation, which was statistically significant (Figure 6). Among the genotypes, EET-95, POUND-12, and EET-399 absorbed 26%, 24%, and 22% less Cd, respectively, compared to CCN-51, the control and the genotype with the highest Cd absorption (Figure 6A). Cadmium uptake efficiency ranged from

68.7 to $79.7 \mu\text{g Cd g}^{-1}$ of dry root matter, with the lowest value found in EET-95 and the highest average in CCN-51, respectively.

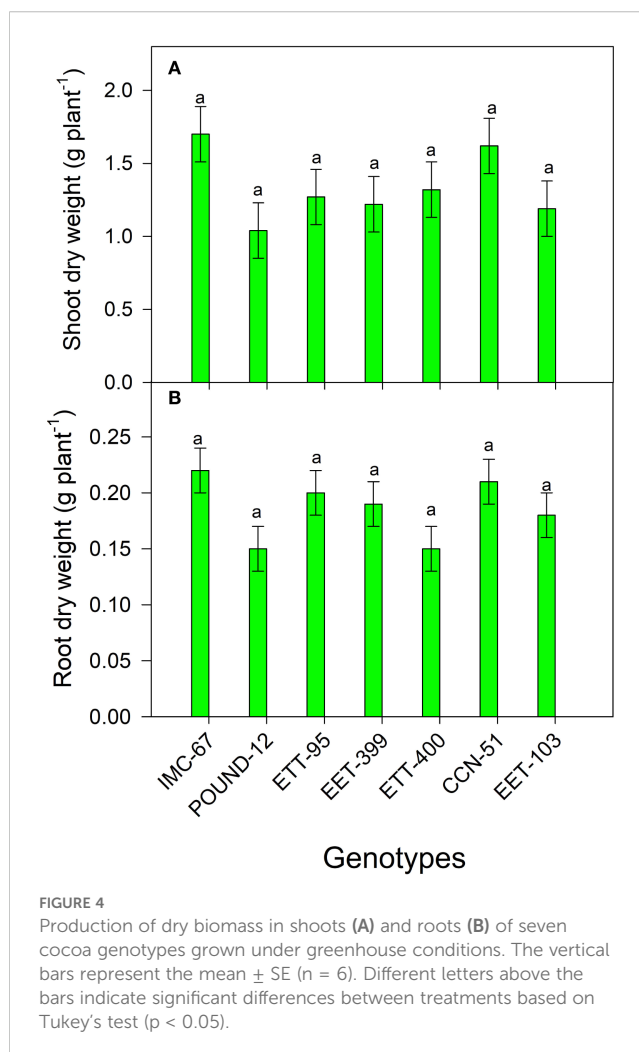
In Cd translocation, values ranged from 5.8 to 9.5, with the lowest average obtained in EET-399 and the highest in CCN-51, respectively. In terms of Cd translocation efficiency from roots to shoots, EET-399, EET-103, and EET-95 exhibited 39%, 23%, and 17% lower translocation rates, respectively, than CCN-51 (Figure 6B). Additionally, the POUND-12 genotype bioaccumulated 49% less Cd in the shoot compared to CCN-51, followed by EET-399 (39%) and EET-400 (32%).

Discussion

The study revealed no significant differences among the evaluated cocoa genotypes in physiological variables or biomass distribution, thereby rejecting Hypothesis 1. In contrast, significant genetic differences were evident in Cd absorption efficiency, translocation, and content, supporting Hypothesis 2. Notably, POUND-12 genotype producing the least biomass and exhibited reduced Cd accumulation in shoots. This genetic effect in Cd bioaccumulation aligns with findings by Galvis et al. (2023) and Reyes-Pérez et al. (2023a), who identified similar trends in genotypes with lower biomass production, such as PA-46 and EET-103, respectively.



At the end of the experiment, chlorophyll reduction correlated with decreased leaf gas exchange. This was likely due to salt stress induced by high soil salinity (6 dS m^{-1}). A similar response was



reported by Reyes-Pérez et al. (2023a) under high EC conditions (5 dS m^{-1}), which led to reduced chlorophyll (90 DAS), and gas exchange (80 DAS), along with reddish-brown leaf margins. This leaf margin color was also observed in our study. Comparable reductions in A , g_s , E have also been observed in juvenile cocoa plants under water stress and Cd stress (Ortiz-Álvarez et al., 2023; Pereira de Araújo et al., 2017).

Among physiological parameters, only E at 80 DAS showed significant genetic variation, with EET-103 exhibiting the highest transpiration rate ($0.39 \text{ mmol m}^{-2} \text{s}^{-1}$). In fact, most cocoa genotypes showed low E due to small g_s , probably in response to the salt stress experienced by the cocoa seedlings. Higher transpiration values were reported in cocoa genotypes by Reyes-Pérez et al. (2023a). The physiological performance of the genotypes that were evaluated in this study is not documented in the literature under the same conditions. However, the greater transpiration in IMC-67 observed by Ortiz-Álvarez et al. (2023) under water stress, linked to higher Cd translocation, was not corroborated under the present study. The physiological traits of 18 cocoa genotypes under irrigation effects, found higher gas exchange performance (A , g_s and E) in genotypes such as CP-41, CP-43, and CCN-51 (Araújo et al., 2024).

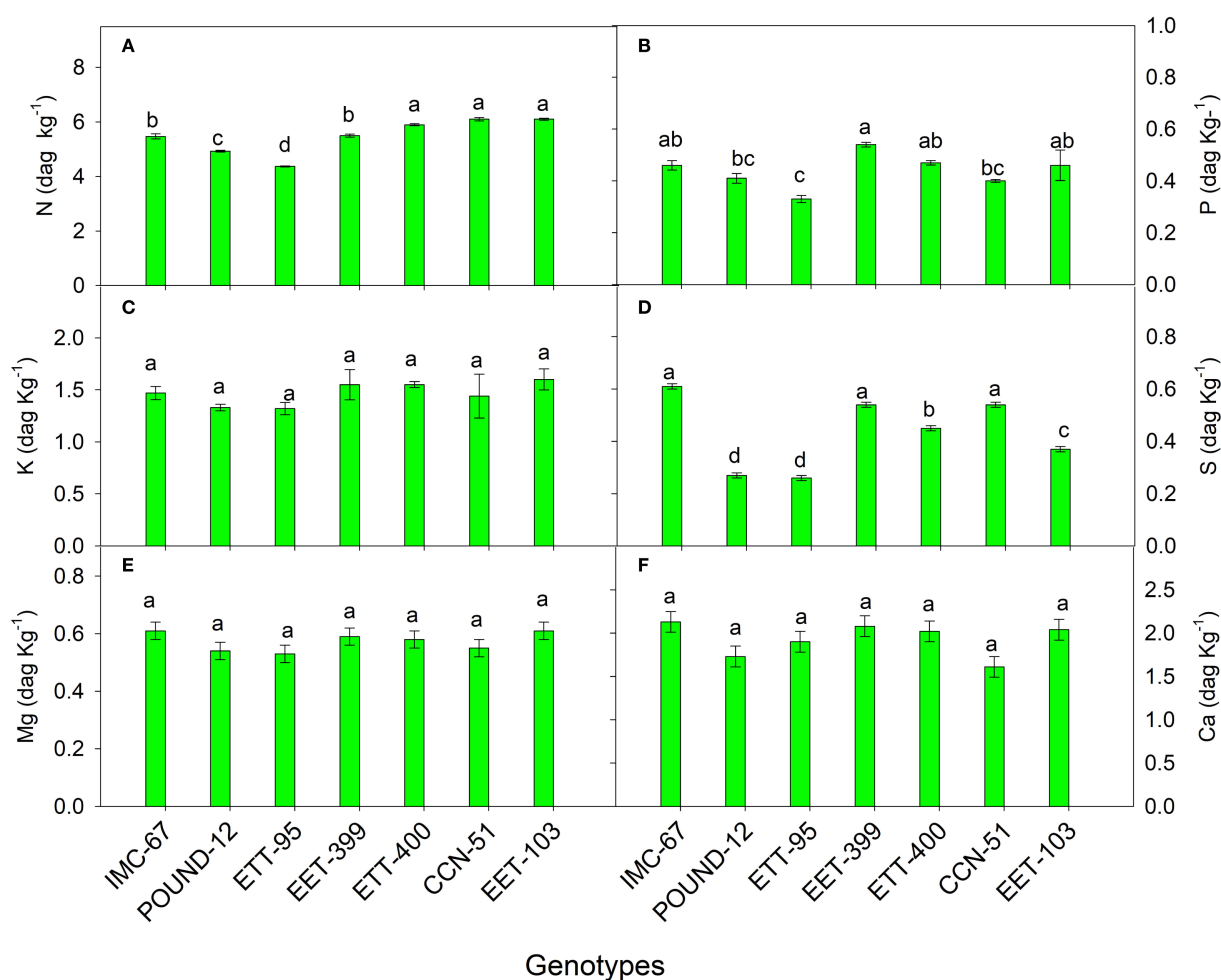


FIGURE 5

Concentrations of nitrogen (A), phosphorus (B), potassium (C), sulfur (D), magnesium (E), and calcium (F) in the stems of seven cocoa genotypes. The vertical bars represent the mean $\pm$ SE ($n = 6$). Different letters above the bars indicate significant differences between treatments after the Tukey test ($p < 0.05$).

Dry biomass is a key indicator of plant growth, directly linked to water content and the synthesis of primary metabolites (Huang et al., 2020). In this study, the IMC-67 and CCN-51 genotypes demonstrated the highest accumulation of dry biomass in both shoot and root compared to the other genotypes evaluated (EET-95, EET-399, EET-103, EET-400, and POUND-12). These findings are consistent with those reported by Reyes-Pérez et al. (2023a, 2023b), who observed that CCN-51 produces greater biomass than domestic cocoa genotypes like EET-103 and EET-801.

However, contrasting results were noted in the work of Borjas-Ventura et al. (2022), where POUND-7 outperformed CCN-51 in shoot biomass production. This variation highlights the influence of environmental adaptability and genetic plasticity on biomass outcomes. Studies by Héctor Ardisana et al. (2018) and Palacio-López and Rodríguez-López (2007) suggest that biomass variation among genotypes is often driven by their ability to adapt to specific climatic conditions, which, in turn, affects the expression of phenotypic traits such as leaf plasticity.

Although this study was conducted with juvenile plants, the Cd content in the shoot may be related to the findings of Lewis et al.

(2018), who observed a 2:1 relationship between Cd concentrations in the leaves (2.15 mg kg^{-1}) and beans with controls (1 mg kg^{-1}) in adult plants of 100 cocoa genotypes from eight genetic groups. This relationship was supported by a significant correlation coefficient ($r = 0.61$; $P < 0.05$). Furcal-Beriguete and Torres-Morales (2020) reported a similar pattern, with Cd concentrations of 3.44 mg kg^{-1} in leaves and 2.31 mg kg^{-1} in beans, showing a correlation coefficient of $r = 0.90$. Therefore, regardless of the plant's age, genetic load, or edaphoclimatic conditions, it is considered that the beans accumulate less than 50% of the Cd found in the leaves.

Ortiz-Álvarez et al. (2023) found that increases in dry matter, biomass output, leaf area or proliferation, and Cd bioaccumulation in terms of content are all linked to Cd accumulation under greenhouse conditions. These authors also showed that when transpiration increases, there is greater Cd accumulation because sap flow carries more Cd to the leaves and shoot (Sterckeman, 2025), but it is diluted in terms of concentration (Ortiz-Álvarez et al., 2023). These explanations are reinforced by the results reported in POUND-12, IMC-67, and CCN-51. The first genotype had lower dry matter and Cd content, and the other

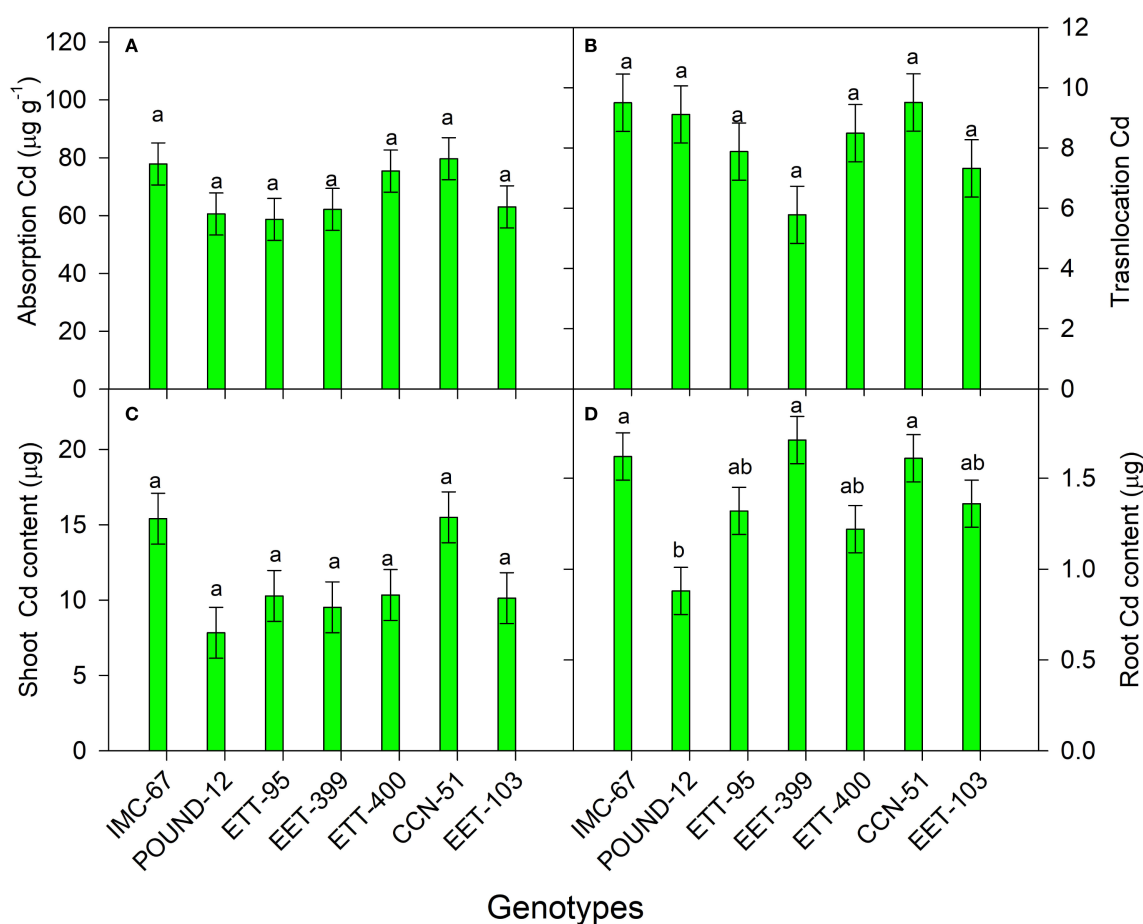


FIGURE 6

Absorption (A) and translocation of Cd (B), Cd contents in the shoot (C) and root (D), of seven cocoa genotypes grown under greenhouse conditions. The vertical bars represent the mean $\pm$ SE ($n = 6$). Different letters above the bars indicate significant differences between treatments after the Tukey test ($p < 0.05$).

two clones were superior in terms of shoot dry matter production and Cd bioaccumulation. In cacao seedlings, was found in CCN-51 had a greater capacity to produce dry matter and absorb nutrients and Cd (Arias-Contreras et al., 2024).

In the present study, there were differences in root Cd bioaccumulation, with the highest accumulation found in the roots of EET-399, IMC-67, and CCN-51 and the lowest allocation in POUND-12. Similarly, significant variations in Cd bioaccumulation were observed in the roots of young plants cultivated in greenhouses; the PA-121 x IMC-67 progeny exhibited the highest accumulation (Fernandez-Paz et al., 2021). Growing in a greenhouse with soil Cd concentrations of 12 mg kg^{-1} , PA-121 was shown to have the maximum Cd allocation in root tissues at 30 and 60 DDS, in comparison to the genotypes EET-61, EET-62, IMC-60, IMC-67, PA-150, PA-46, SCC-85, and SCC-86 (Galvis et al., 2023).

All cocoa genotypes had Cd translocation > 1 , but EET-399, compared to the other clones (EET-103, IMC-67, EET-95, CCN-51, EET-400, and POUND-12) had the lowest translocation. This result is consistent with the higher Cd accumulation in the roots of this genotype, showing that it restricts Cd translocation to the aerial parts by capturing it in the root system. Reyes-Perez et al. (2023a)

also detected a similar phenomenon: the EET-103 clone had higher Cd accumulation in the roots and lower Cd translocation to the shoots, compared to CCN-51, which obtained the opposite response. Also, were found a genotypic variations in Cd accumulation in the roots of five fine aroma cocoa genotypes, detecting the highest amounts of Cd ($156.75 \text{ mg kg}^{-1}$) and lower translocation to the leaves in the INDES-38 genotype (Meléndez-Mori et al., 2023).

It was also observed that the Cd content in the shoot was nine times higher than that in the root. Similarly, Castro et al. (2015) found the highest accumulations of Cd in the offspring of two cocoa progenies (CCN-10 x SCA-6 and Catongo x Catongo) at the juvenile stage. In contrast, Oliva et al. (2020) reported that in mature trees, the leaves accumulate 123 times more Cd than the roots. However, other studies involving juvenile plants (Calva Jiménez et al., 2022) and adult trees (Llatance et al., 2018) found that the roots accumulated 4.84 and 3.36 times more Cd than the leaves, respectively. These findings demonstrate that Cd accumulation in cocoa plants varies between organs, influenced by age, genotype, and environmental conditions that affect Cd migration from one organ to another.

Of the seven genotypes studied, CCN-51 exhibited the highest absorption and accumulation of Cd in the offspring. In Ecuador, this genotype is widely used as a rootstock, covering 75% of the planted area (Latin American Center for Rural Development (RISMIP), 2023). These findings support the results of Reyes-Pérez et al. (2023a), who reported that the EET-103 genotype accumulates less Cd than CCN-51. Additionally, due to its resistance to machete sickness, EET-103 was recommended as a standard.

The POUND-12 genotype exhibited the lowest Cd content in both shoot and root. Due to its resistance to *C. cacaofunesta*, it is proposed as a key genetic rootstock strategy to mitigate Cd in soils with high metal concentrations. Additionally, EET-399 and EET-400 are viable alternatives, as they demonstrate low Cd accumulation, resistance to *C. cacaofunesta* (Suárez Capello et al., 1993), and, according to Jaimez et al. (2021), tolerance to water stress.

In contrast, Arévalo-Hernández et al. (2021) in Peru reported results that differ from the present study, finding that CCN-51, with a Cd concentration of 1.56 mg kg⁻¹, accumulated less Cd in the shoots compared to EET-400 (18.56 mg kg⁻¹). Despite its susceptibility to root diseases, CCN-51 was included in a list of 11 genotypes recommended as rootstocks for Cd mitigation, alongside AYP-22, PAS-105, UGU-126, ICT-1026, ICT-1087, ICT-1189, ICT-1292, PH-17, ICS-39, and TSH-565. Furthermore, Borjas-Ventura et al. (2022) found in the Central Jungle of Peru that CCN-51 bioaccumulates more Cd in the shoots compared to genotypes like ICS-95, ICS-60, VRAE-99, and POUND-7. These latter genotypes were recommended as standard due to their low Cd accumulation and partial resistance to *Verticillium* (Braga and Silva, 1989; End et al., 2021).

The highest N concentration in the shoot was detected in CCN-51, consistent with the findings of Reyes-Pérez et al. (2023a), who also reported that CCN-51 exhibited the highest N concentration in the shoot compared to the EET-103 genotype. Similarly, under field conditions, Carrillo et al. (2010) found that CCN-51 accumulated more leaf N compared to national genotypes such as EET-95 and EET-103.

Conclusions

Genetic variation was observed in the extraction and accumulation capacity of Cd in cocoa rootstocks. These results suggest that genotypes with limited Cd absorption could serve as an agronomic strategy for producing cocoa beans in soils contaminated with this metal. In leaf gas exchange, significant differences were found only in transpiration at 80 DAS, with the EET-103 genotype showing the highest average at 0.39 mmol m⁻² s⁻¹. Overall, at 80 DAS, a reduction in *A*, *E*, *g_s*, and WUE was observed, while *C_i* increased, which were associated to a decrease in chlorophyll content. In this study, the CCN-51 genotype exhibited greater Cd absorption and bioaccumulation in the shoot, while also showing the highest leaf N concentration. On the other hand, EET-95 showed lower Cd absorption. The POUND-12, EET-400, and EET-399 genotypes, with their low Cd extraction and accumulation capacity and resistance to *C. cacaofunesta*, are proposed as suitable candidates for use as cocoa rootstocks and for introduction into genetic improvement

programs aimed at developing Cd-excluding genotypes. The primary research constraint, however, was the use of cocoa seedlings with limited root volume and a brief exposure to Cd. This could have an impact on the results when extending these findings to mature, fruitful plants in the field. Thus, we think that more research in this area in field settings on plantations is very important.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

FF: Validation, Supervision, Writing – review & editing. MC-Z: Supervision, Methodology, Writing – original draft, Investigation. RP-G: Data curation, Writing – original draft. JR-H: Conceptualization, Investigation, Writing – original draft. RL-M: Conceptualization, Formal Analysis, Writing – review & editing. AV-N: Writing – review & editing, Conceptualization, Investigation. VR: Investigation, Methodology, Writing – review & editing. WT: Software, Methodology, Writing – original draft, Investigation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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