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Macroelements and Microelements in the Soil and Their Relationship with the Content of Steviol Glucosides in *Stevia rebaudiana* Bert from Five Regions of Colombia

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Abstract: This study was conducted to determine the effect of edaphic environmental conditions in the concentration of principal steviol glycosides and *Stevia rebaudiana* Bert yield, utilizing leaves from five Colombian regions. The structure of the experiment was a randomized complete block design with two treatments in a 5 × 2 factorial arrangement (5 locations × 2 radiation levels). In each experimental unit (UE), five healthy plants of similar physiological growth age were selected for the extraction of total glycosides (GT), stevioside (Stv), rebaudioside A (Rb-A),and leaf yield. Results were analyzed with the SAS statistical package (version 9.1). Concentrations of total glycosides and rebaudioside A showed a positive effect with the increase of nitrogen (N), phosphorus (P), magnesium (Mg), and copper (Cu). Therefore, they are important in the available phase of the soil to obtain an increase in these glycosides. Meanwhile, boron (B) presented a negative correlation under these conditions. For the production of stevioside, N, Mg, manganese (Mn) had a positive correlation, and calcium (Ca) and sodium (Na) had a negative correlation. Similarly, for leaf yields by locality, it was found that N, Ca, Mg, and B have a positive correlation with leaf production, while Mg, Mn, and iron (Fe) negatively correlate with biomass gain.The cultivation of stevia can be established in different soil conditions, precipitation and solar radiation in Colombia. Therefore, it is necessary to advance fertilization plans with these nutrients, considering the response of these metabolites to their application.

Keywords: nutrition; soil; chemical elements; cultivation; environmental conditions; metabolites

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1. Introduction

For proper growth and development, and optimal performance with high quality stevia leaf, adequate amounts of water, nutrients and solar radiation are necessary. However, yield and quality are affected by a series of external factors, over which the farmer has no control, such as precipitation and temperature, in addition to having a very important role in the soil supply of each locality [1], but optimal conditions are needed in the soil and the environment. Likewise, the environment offered, such as temperature and radiation, directly influence the metabolism and physiology of plants [2].

Additionally, for optimal growth and development of the stevia root system, medium to high fertility soils with high humus content and water storage capacity are required. Reichardt and Timm [1] reported that, at the time of harvest, the edaphoclimatic conditions presented a significant effect on dry stevia leaf yield, steviol glycoside production, and the concentration or assimilation of macronutrients found in plant tissues.

In South America, specifically in Colombia, in recent years they are looking for crops that contribute to the substitution of illicit crops, and the cultivation of stevia can be an alternative. Therefore, Stevia generates great interest in the different productive and economic sectors of the country. Consequently, due to the sweetening and therapeutic properties of its plant tissues, its sowing and establishment have increased in many regions of Colombia and the world [3]. In stevia-grown soils, chemical properties influence adequate nutrient uptake during the growing season. Furthermore, plants absorb particular nutrients from the substrate only within a certain pH range. However, there are large empty gaps in the knowledge of its agronomic behavior, mainly with regard to aspects such as nutrition and the effect of environmental variables on the development of this crop [4,5] and its relationship with the synthesis of the main sweetening molecules [6].

Nutrient deficiencies or excesses have a direct effect on the yield and quality of plants. However, each deficiency or excess manifests itself in a specific way, but all can affect the quality of the stevia leaves. In the cultivation of stevia, the relationship between the mineral nutrition of the plants, the yields, and the quality of the leaf represented in the synthesis of the main steviol glycosides has been reported by several authors [7,8]. Brandle and Telmer [9] demonstrated that an important aspect to know is the effect of nutritional elements on the synthesis of the main sweetening molecules and metabolic synthesis, and although different metabolic routes have been elucidated, they all converge at the same point, the synthesis of isopentenyl pyrophosphate (IPP) [10]. *Stevia rebaudiana* produces steviol glycosides and these compounds are synthesized through a series of enzymatic reactions that catalyze these reactions. Among them is geranyl pyrophosphate synthase, an enzyme in plastids that required Mg^{2+} or Mn^{2+} as cofactors for their maximum activity [11].

Likewise, in the edaphoclimatic conditions of Colombia, light, temperature and solar radiation are environmental factors that can affect the cultivation of stevia in the Colombian Caribbean. Michelet and Liszkay [12] demonstrated that different periods of luminosity affect many metabolic changes, such as starch accumulation, respiration, and photosynthesis during plant growth. According to Ceunen and Geuns [13] Stevia is a short-day plant, which requires around 13 h of light. Barbet et al. [14] explained that the steviol glycoside content varies between environments and generally increases between 1 and 2-year-old plants, while the steviol glycoside composition remains stable. Likewise, Woelwer [15] and Serfaty et al. [16] indicate that the productivity of the sweetener is measured by the concentration of glycosides in the dry biomass of the leaves, which vary within environmental conditions and development stages. Kumar et al. [17] and Ceunen and Geuns [13] found that there is an effect of agronomic practices and the photoperiod on the accumulation of steviol glycosides and productivity in Stevia.

Therefore, in this context, the knowledge of the responses of the edaphoclimatic supply for the production of steviol glycosides from the cultivation of stevia is an important tool to determine the optimal conditions to obtain high yields and high quality of the sheet. Consistent with this, it was proposed to determine the effect of the edaphic supply and the environmental conditions of five regions of Colombia and two levels of incident radiation, on the absorption of the different nutritional elements, the contents of stevioside, rebaudioside A and Stevia yield.

2. Materials and Methods

2.1. Location

Five experimental plots corresponding to five locations were established: (1) Montería-Córdoba (8°4′0″ N, 75°52′59″ W); (2) Campamento-Antioquia (6°58′45″ N, 75°17′45″ W); (3) Palmira–Valle del Cauca (3°32′05″ N, 76°17′44″ W); (4) Fonseca-Guajira (10°53′09″ N, 72°50′53″ W); and (5) Valledupar-Cesar (10°27′0″ N, 73°15′0″ W) (Figure 1).

Figure 1. Geographical location of the five localities where the experiment was carried out in Colombia.

In each locality, the experimental plots were divided into two radiation levels 50% and 100% of the incident radiation. Table 1 shows the radiation levels measured in each location, with IRGA Model CIRAS 2 (PP Systems) equipment.

Table 1. Average values of radiation levels, environmental variables and chemical reaction of the soils in five localities established with Morita 2 in Colombia.

Location	IR 100%	IR 50%	T	P	SB	PH
Palmira (Valle)	790	395	23.7	1065	1946	7.8
Campamento (Antioquia)	659	330	19.5	3973	1391	4.6
Montería (Córdoba)	834	417	28	1247	2180	6.4
Valledupar (Cesar)	1010	505	29	1324	2676	7.4
Fonseca (Guajira)	1097	548	28	839	2591	7.5

Incident radiation (IR) in µmoles photons $m^{-2} \cdot s^{-1}$, in two treatments using polyshade (100 and 50% of IR), average temperature (T), precipitation (P = mm) and annual solar brightness (SB = hours). pH = chemical reaction of soils.

2.2. Experimental Design

The structure of the experiment was a randomized complete block design with two treatments in a 5 × 2 factorial arrangement (five locations × two radiation levels). Each experiment per location consisted of five plots for each radiation level, called experimental units (UE) of 10 m^2 each ($\sim$62.5 plants/m^2), for a total of ten UE per location. In addition, for each radiation level, five repetitions were used, where the Morita II genotype was established.

2.3. Collection of Soil Samples

In each locality, three samples of 1 kg of soils were collected for each radiation level and between repetitions of the radiation levels. In addition, at the end of the experiment, samples of plant tissue were collected to estimate the relationship between leaf content and the amount of nutrients in the soil.

These soil samples were chemically characterized by the Soil and Water Laboratory of the University of Córdoba, Colombia: pH by the potentiometric method; organic matter (O.M) by the Walkley–Black method. P for Bray II by colorimetry; S by extraction with monobasic calcium phosphate (0.008 mol L^{-1}) and quantified by the turbidimetric method, B was extracted with HCl 0.05 mol L^{-1} and the quantification of P, S and B was performed by molecular absorption spectrophotometry in a Perkin Elmer Lambda XLS + equipment. Exchangeable bases, such as Ca, Mg, Na and K, by extraction with one normal ammonium acetate, pH 7.0. Calcium and magnesium were quantified by atomic absorption, and Na and K by atomic emission spectrophotometry. The elements Cu, Fe, Zn and Mn were determined by the dilute double acid method (Mehlich-1) and were quantified by atomic absorption in Perkin Elmer 3110 equipment [18].

2.4. Collection of Plant Material

In each experimental unit, five healthy plants were selected that were in competition with each other and of similar physiological age of growth. These plants were divided into organs, which were dried at 70 °C for 72 h to determine the yield of leaves. Finally, to evaluate the nutrient concentration, the samples collected were dried, ground and sieved through a 0.5 mm mesh. For nitrogen, 0.5 g of sample was subjected to the Kjeldahl method, with a digestion in sulfuric acid (10 mL of H_2SO_4). For the rest of the nutritional elements, 0.3 g of sample was digested wet with 10 mL of HNO_3:$HClO_4$:H_2SO_4 3:1:1 *v*/*v*) [19], to determine the macro (Ca, Mg, K, Na, P, S) and micronutrient (Fe, Mn, Cu, Zn, B) contents in the leaves. The quantification was carried out by atomic absorption in the Perkin Elmer equipment. Stevioside and rebaudioside contents were also quantified by modern HPLC techniques.

2.5. Extraction of Glycosides from Steviol, Stevioside and Rebaudioside and Analysis by HPLC

The methodology selected for the extraction was the one reported by Montoro et al. [20] with minor modifications described as follows: initially to process the stevia material from the five regions, the leaf samples were washed with distilled water and dried at 70 °C. Later they were ground and passed through a 1 mm sieve. The extracts of each material were obtained using water: ethanol (50:50) as a solvent, for a time of 60 min, with continuous stirring in an orbital shaker at 30 °C. The plant's solvent ratio was 1:10. Each extract was filtered and diluted at 1:40 before being analyzed by the chromatographic system.

Subsequently, a high-resolution chromatography was used for the detection and quantification of the glycoside compounds, stevioside and Rebaudioside A. HPLC analyses were performed using an (HPLC) Accela brand Thermo Scientific, which had a PDA detector (photodiode Array detector), with an Accela 600 quaternary pump and autosampler. The column used for the quantification of the compounds was referenced as a LiChroCART 250-4, LiChrospher 100 NH2 (5 µm), which was installed with the manuCART accessories.

The operating conditions that were defined for the calibration with the pure standards and the samples from the five regions were as follows. A mobile phase was used: Acetonitrile (ACN): Water (H_2O), 70:30, with a working flow: 500 µL/min and an injected sample volume: 5 µL. Quantified in a PDA detector 210 nm and for a running time of 20 min and the working temperature was ambient. From the HPLC grade standards, the samples were quantified against standard curves of RbA and ST (99.99% pure) Figure 2.

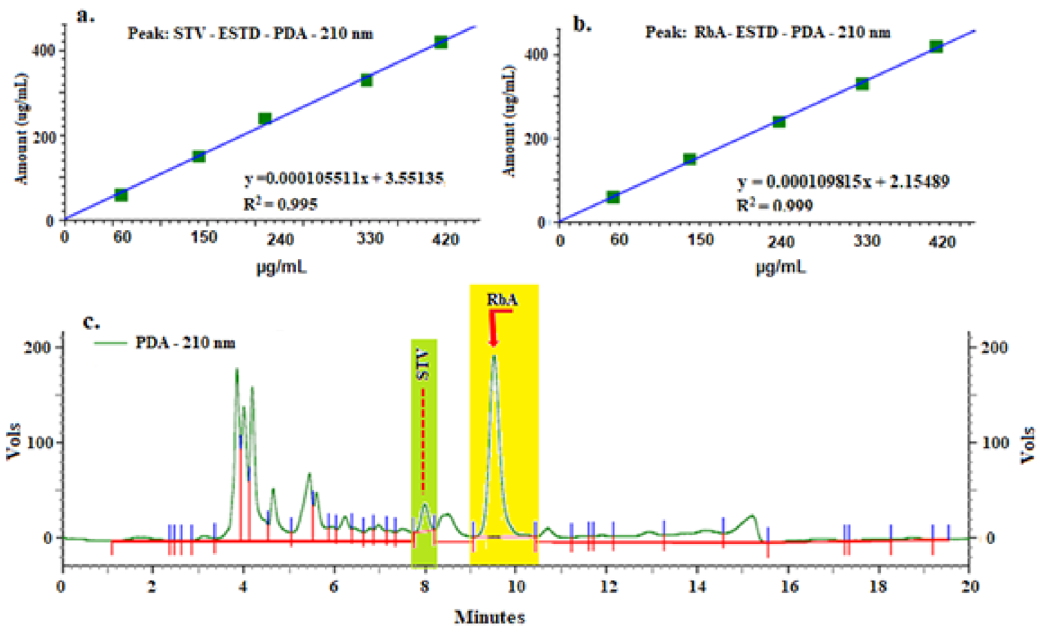

Figure 2. Chromatographic analysis of the samples in HPLC, reading of the data provided by the software and their analysis and processing. Stevioside calibration curve (**a**): Rebaudioside A calibration curve (**b**), and Chromatogram of analyzed leaf sample (**c**).

2.6. Statistical Analysis

Combined analysis of variance was performed to determine the significant differences between the nutritional contents of the soils and plants of the locations (Loc), radiation levels (RL), and the interaction of radiation level by location (Loc * RL). Where necessary, Tukey tests ($p \leq 0.05$) and multiple regression of total glycosides, rebaudioside A and stevioside vs the foliar contents of the different nutritional elements were performed, to estimate the importance of these chemical elements in the concentration of the sweetener molecules and the yields. For the analysis of the data, the statistical package SAS in version 9.1 was used.

3. Results

3.1. Chemical Characterization of Macroelements in Soils and Nutritional Content in Stevia Leaves in Five Municipalities of Colombia and Statistical Analysis between Localities

According to the results (Table 2), statistical differences ($p \leq 0.05$) were found between the chemical variables of the soil's organic matter, P, Na, potassium, Ca, and Mg for the different locations. For the OM content, it was found that the towns of Fonseca and Campamento presented the highest content with approximately 4% and the lowest content of 1.1% in Montería. The P content was higher in Campamento and Valledupar with 96 and 94.6 mg kg^{-1} and the lowest content in Montería with 12.9 mg kg^{-1}. Likewise, for the contents in cmolc kg^{-1} of interchangeable bases, Na presented contents lower than 1 and the highest K contents were found in Valledupar and Fonseca with 1.26 and 0.91. For Ca, the highest contents with 20.1 and 15.6 in Fonseca and Palmira, and Mg with 8.4 and 6.5 cmolc kg^{-1} in Montería and Palmira.

On the other hand, for the nutritional contents in leaves, statistical differences ($p \leq 0.05$) were found only for N, P, K, Ca, and Mg. According to the results, the highest 3.43% and 2.82% N contents were found in Fonseca and Campamento, and the lowest contents in Palmira with 1.96%. For K 3.66 and 3.55 g kg^{-1} were presented in Valledupar and Fonseca and the lowest 17.84 g kg^{-1} in camp. For Ca 9.8 and 8.13 g k^{-1} in Camp, finally for Mg of 4.25 and 3.4 g kg^{-1} in Palmira and Valledupar and the lowest content was in camp with 2.05 g kg^{-1}.

Table 2. Edaphic and foliar nutritional contents, and analysis of variance of macronutrients and micronutrients as a function of radiation levels in the five localities in Colombia.

Macronutrient Contents in the Soil							
Locality	O M.	P	S	Na	K	Ca	Mg
			mg kg^{-1}				
Palmira	2.0 ± 0.5b	86.1 ± 20.1b	18.4 ± 4.5bc	0.39 ± 0.20b	0.54 ± 0.05c	15.6 ± 1.0b	6.5 ± 0.5b
Valledupar	2.1 ± 0.7b	94.6 ± 20.6a	21.5 ± 9.6bc	0.45 ± 0.20b	0.91 ± 0.13b	5.6 ± 0.6d	2.2 ± 0.2d
Fonseca	4.1 ± 1.1a	68.2 ± 10.6b	31.7 ± 9.2ab	0.92 ± 0.20a	1.26 ± 0.24a	20.1 ± 2.2a	3.9 ± 0.8c
Montería	1.1 ± 0.3c	12.9 ± 6.5c	12.9 ± 5.1c	0.12 ± 0.03c	0.42 ± 0.24c	13.0 ± 1.9c	8.4 ± 1.3a
Campamento	4.0 ± 0.3a	96 ± 22a	46.3 ± 20a	0.06 ± 0.03c	0.44 ± 0.09c	3.8 ± 1.8d	2.1 ± 0.3d
Macronutrient Contents in Stevia Leaves							
Locality	N	P	S	Na	K	Ca	Mg
			g kg^{-1}				
Palmira	1.96 ± 0.1e	2.26 ± 0.2bc	0.83 ± 0.2a	0.16 ± 0.1a	18.7 ± 3.53b	8.13 ± 1.1b	4.25 ± 0.5a
Valledupar	2.50 ± 0.1c	3.66 ± 0.6a	1.21 ± 0.2a	0.10 ± 0.05a	35.7 ± 6.92a	7.64 ± 1.5bc	3.40 ± 0.5b
Fonseca	3.43 ± 0.4a	3.55 ± 0.3a	1.97 ± 2.0a	0.15 ± 0.10a	35.9 ± 7.31a	9.80 ± 1.3a	3.25 ± 0.7ab
Montería	2.26 ± 0.1d	2.88 ± 0.3b	1.40+0.3a	0.06 ± 0.01a	22.88+4.82b	6.00 ± 0.0c	3.00 ± 0.0b
Campamento	2.84 ± 0.2b	1.76 ± 0.5c	1.06 ± 0.5a	0.09 ± 0.03a	17.84 ± 9.57b	3.47 ± 0.9d	2.05 ± 0.9c
CorrCoef.	0.74 **	−0.12 ns	−0.12 ns	0.54 **	0.11 ns	0.41 *	0.23 ns
Loc	2.59 **	5.69 **	1.50 ns	0.014 ns	695.69 **	46.65 **	5.07 **
RL	0.19 **	0.25 ns	0.49 ns	0.002 ns	159.29 ns	16.05 **	3.00 **
Loc * RL	0.14 **	0.62 **	1.31 ns	0.015 *	28.77 ns	2.82 *	0.72 *

Similar letters vertically do not differ statistically, according to Tukey's test ($p \leq 0.05$), ns= not significant; * = Significant at 5%; ** = Highly significant at 5%. Corr Coef=correlation coefficient. Loc = locality: RL= radiation levels.

Table 2 shows that a significant correlation was found between the foliar and soil contents between N and OM with (0.74 **) and Na with 0.54 ** and Ca with 0.41 *, but there were no statistical differences between the contents of the soils and foliar for P, S, K, and Mg. Likewise, statistical differences were found between some locations, between radiation levels, and between location by radiation level interaction (Loc * RL). For this research, the interaction between Loc * RL was analyzed, finding that the foliar contents of N, P, Ca, Mg, and Mn presented a positive correlation, which indicates that the absorption of these nutrients was affected by radiation levels in some localities. (Table 1). Therefore, the edaphic supply of these elements has an effect on their absorption in the stevia crop, and that in some localities the absorption of nutrients can be affected by incident radiation.

3.2. Characterization of Micro Elements in Soils and Stevia Leaves

Regarding the minor elements, it was found that there were statistical differences ($p \leq 0.05$) between the contents of the microelements in the soils Cu, Fe, Zn, Mn and B between the different localities (Table 3). For the highest and lowest contents of these chemical elements, it was found that Cu in the towns of Montería and Campamento presented contents between 2.62 and 1.33 mg k^{-1} and the lowest with 0.38 mg k^{-1} for Palmira. The Fe was evaluated in Campamento and Montería with 50.43 and 26.08 mg k^{-1} and the lowest content in Palmira with 0.80 mg k^{-1}. For Zn, it was found that the towns of Campamento and Montería had the highest contents with 9.97 and 2.83 mg k^{-1} and the lowest with 1.53 mg k^{-1} in Palmira. The highest Mn contents were found in Montería and Valledupar with 54.67 and 39.2 mg k^{-1} and the lowest in Palmira with 19.82 mg k^{-1}. Finally, 0.37 and 0.3 mg k^{-1} for B were evaluated in the towns of Montería and Fonseca, and the lowest value with 0.18 mg^{-1} in Campamento.

On the other hand, only for the foliar contents in Fe, Mn, and B leaves were there statistical differences ($p \leq 0.05$) between localities, and when analyzing the results of the highest and lowest contents it is observed that, for Fe, they were presented in Valledupar and Montería with 1527.8 and 444.07 and the lowest content in Fonseca with 304.13 mg k^{-1}. For Mn with 273.8 and 269.5 mg k^{-1}, they were presented in Campamento and Palmira and the lowest content with 58.7 mg k^{-1} in Montería. For B with 23.75 and 20.2 mg k^{-1} were found in Fonseca and Valledupar, and the lowest content with 5.73 mg k^{-1} in Campamento. In Table 3, it is observed that, when performing the statistical analyses it was found that there was a significant correlation only for Mn and B between the foliar and soil contents, with a negative correlation for Mn (−0.77 **) and a positive correlation for B with 0.43 *.

Table 3. Edaphic and foliar nutritional contents and analysis of variance of macronutrients and micronutrients as a function of radiation levels in the five localities of Colombia.

Micronutrient Contents in the Soil					
	Cu	Fe	Zn	Mn	B
			mg kg^{-1}		
Palmira	0.38 ± 0.3c	0.80 ± 0.1c	1.53 ± 1.0b	19.82 ± 8.4c	0.25 ± 0.05bc
Valledupar	0.42 ± 0.3bc	10.78 ± 3.3c	2.58 ± 0.12b	39.20 ± 10.2b	0.29 ± 0.03bc
Fonseca	0.38 ± 0.4c	1.87 ± 0.4c	2.57 ± 0.8b	26.35 ± 6.4c	0.37 ± 0.11a
Montería	2.62 ± 0.5a	26.08 ± 6.2b	2.83 ± 0.4b	54.67 ± 2.3a	0.30 ± 0.16ab
Campamento	1.33 ± 0.5b	50.43 ± 23.2a	9.97 ± 2.2a	21.23 ± 5.4c	0.18 ± 0.04c
Micronutrient Contents in Stevia Leaves					
	Cu	Fe	Zn	Mn	B
			mg kg^{-1}		
Palmira	11.25 ± 3.54a	393.25 ± 5.2b	87.5 ± 23.1a	268.5 ± 35.2a	16.88 ± 2.7c
Valledupar	10.00 ± 0.00a	1527.80 ± 868a	49.9 ± 0.32a	106.1 ± 16.9bc	20.20 ± 3.1b
Fonseca	11.25 ± 3.54a	304.13 ± 133.8b	87.0 ± 34.9a	143.7 ± 39.9b	23.75 ± 2.7a
Montería	9.94 ± 0.05a	444.07 ± 235.4b	55.7 ± 6.97a	58.7 ± 5.76c	9.23 ± 0.9d
Campamento	9.93 ± 0.05a	343.34 ± 144.4b	69.4 ± 20.5a	273.8 ± 85.8a	5.73 ± 2.0e
Coef.Corr	−0.20 ns	−0.12 ns	−0.02 ns	−0.77 **	0.43 *
Loc	4.26 ns	2,589,780 **	6332.3 ns	82,844.3 **	482.89 **
RL	0.0009 ns	587,525.5 ns	4608.4 ns	5629.66 ns	11.30 ns
Loc * RL	6.251 ns	261758.6 ns	9935.5 ns	4226.31 ns	9.544 ns

Similar letters vertically do not differ statistically, according to Tukey's test ($p \leq 0.05$), ns = not significant; * = Significant at 5%; ** = Highly significant at 5%. Corr Coef=correlation coefficient. Loc = locality: RL= radiation levels.

3.3. Total Glycoside Content and Stevia Yields at Five Locations

The contribution of the different nutritional elements found in the localities affected the synthesis of steviol glycosides ($p \leq 0.05$), as observed in Table 4. Statistically significant differences were found between locations, but there were no significant statistical differences for radiation levels and the interaction of locations by radiation levels.

Table 4. Combined analysis of variance for the content of total glycosides in five locations in Colombia under two levels of radiation.

Fv	GL	Sum of Squares	Middle Square
Loc	4	15,470.69	3867.67 **
Block	4	875.83	218.95 ns
RL6	1	19.48	19.48 ns
Loc * RL	46	353.70	88.42 ns
Error	30	6328.13	210.93 ns
Total	43	23,047.85	535.99
Average		129.99	
coefficient of variation (%)		11.17	

Loc = locality: RL = radiation levels. ns = not significant; * = Significant at 5%; ** = Highly significant at 5%.

The Tukey's test ($p \leq 0.05$) (Figure 3) indicates that the localities with the highest content of total glycosides were Palmira (152.2 mg·kg^{-1}) and Montería (150.9 mg·kg^{-1}), followed by Campamento (125.9 mg·kg^{-1}), Fonseca (110.9 mg·kg^{-1}) and Valledupar (109.7 mg·kg^{-1}). Similarly, it was found that there were no significant differences for rebaudioside A, in the localities of Palmira and Montería with concentrations of 13.23% and 13.74%, but statistical differences were found between these localities and the others, presenting the lowest concentration Campamento.

On the other hand, for stevioside, statistical differences were also found with the highest concentrations in Campamento and Palmira with 3.94% and 2%, and the other localities presented similar concentrations. Finally, the yields between localities also presented statistical differences, Palmira being the locality with the highest production with 3480 kg ha^{-1}, followed by Campamento and Valledupar with 950 and 930 kg ha^{-1}, respectively.

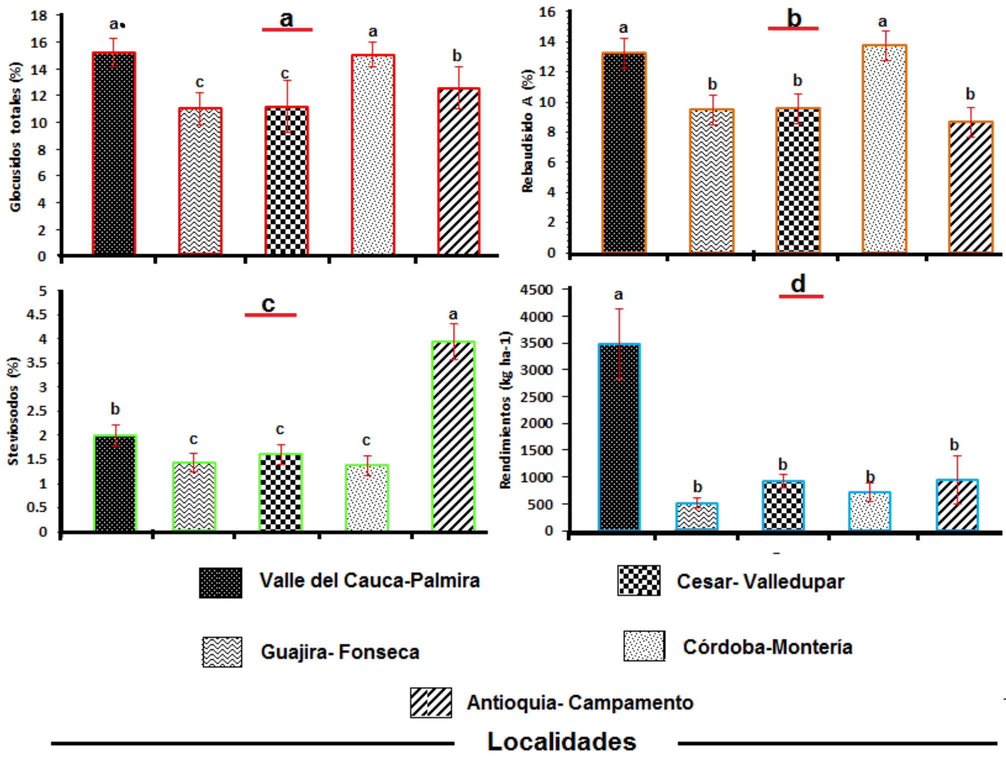

Figure 3. Effect of edaphic and environmental conditions in five locations in Colombia on the production of different metabolites and yield in stevia cultivation. Concentration of total glycosides (**a**): rebaudiosode A (**b**): steviosiodos (**c**), and yields (**d**). Similar letters vertically do not differ statistically, according to Tukey's test ($p \leq 0.05$)

3.4. Effect of Macro and Microelements on the Content of Total Glycosides and Leaf Yields in Stevia

As shown in Table 5, the results of the multiple regression with their respective level of significance indicate that the main nutritional elements that influence the concentrations of total glycosides, rebaudioside A, stevioside and yield were: macroelements N, P Ca Mg, and Na, and microelements Zn, Mn, Fe, and Cu. Therefore, the edaphological offer in the content and availability of these elements is sufficient so that, in the cultivation of stevia, metabolic reactions can be produced that originate these metabolites and increase the yield of the culture. In addition, under these conditions, contents that are not sufficient or are in high contents that produce chemical antagonisms may be present. The concentrations of total glycosides and rebaudioside A presented a positive effect with the increase of N, P, Mg, and Cu; therefore, they are necessary for achieving an increase in these glycosides. Meanwhile, B presented a negative correlation. For stevioside, N, Mg, and Mn had a positive correlation, and Ca and Na had a negative correlation with the production of this metabolite. The mathematical model that defines its effect on the concentrations of total glycosides, rebaudioside A, stevioside and yield, was the following:

$$GT = 19.86N^{**} + 25.97mg^{**} + 3.32Cu^{*} - 2.949B^{**}; R^2 = 97.1\% \quad (1)$$

$$RebA = 13.74P^{**} + 21.06Mg^{**} + 3.66Cu^{**} - 2.629B^{**}; R^2 = 96.7\% \quad (2)$$

$$Stv = 9.281N^{**} + 3.826ca^{**} + 4.07mg^{**} - 11.68Na^{**} + 0.067Mn^{*}; R^2 = 98\%. \quad (3)$$

In this study, it was found that N, Ca, and B presented a positive correlation, while Mg, Fe, and Mn showed a negative correlation with the yields of leaves by locality, and the mathematical model was:

$$Yield = 596N^{**} + 349Ca^{**} - 831Mg^{**} - 0.442Fe^{**} - 1.607Mn^{*} + 42.1B^{*}; R^2 = 94.1\%. \quad (4)$$

Table 5. Multiple regression for foliar nutrient content vs total glycoside content, rebaudioside A, stevioside and yield in the localities.

Nutrients Foliares	G T	RebA	Stv	Yield
N	19.86 **	—	9.281 **	596 **
P	-	13.74 **	-	-
S	-	-	-	-
Ca	-	-	−3.826	349 **
Mg	25.97 **	21.06 **	4.07 **	−831 **
K	-	-	-	-
Zn	-	-	-	-
Mn		-	0.06714 *	−1.607 °
B	−2.949	- 2629	-	42.1
Fe	-	-	-	−0.442 **
Na	-	-	−11.68 **	-
Cu	3.32 *	3.66 **	-	-

ns = not significant; * = Significant at 5%; ** = Highly significant at 5%, ° = Significant at 10%.

4. Discussion

4.1. Nutritional Characterization of Macro Elements in Soils and Stevia Leaves

The results indicate that the requirements in the nutritional macroelements of stevia may vary, due to factors specific to each locality, such as temperature, precipitation, pH, the soil, and climatic supply [1] that may have a differential effect on the absorption of each nutritional element and the optimal growth of the stevia crop in these regions. Consequently, in a limiting condition of OM and P (1.1%, 12.9 cmolc kg^{-1}) in the town of Montería, and Campamento, the low exchangeable content of Ca and Mg with 3.8 and 2.1 cmolc kg^{-1} can cause a decrease in the yields of stevia leaves and its secondary metabolites. According to Kuncoro et al. [21], the chemical elements can vary in different types of soil due to geographical location and climate.

Likewise, the parent materials and primary minerals that originated these soils, with chemical reaction characteristics (pH = 7) close to neutrality, can provide large amounts of essential nutrients for the cultivation of Stevia, except for Campamento that has soils with a pH less than 4.6. The rocks and their by-products reported by Fyfe et al. [22] are phosphorous sources of P, K, Ca and Mg; they also include Zn and Cu, which are essential in plant nutrition.

According to Table 1, it is observed that there are significant statistical differences ($p \leq 0.05$) in the macroelement content in stevia leaves, with low contents of 1.96% for N and phosphorus with 1.76 mg kg^{-1}. In addition, K, Ca, and Mg contents with 17.4, 3.47, and 2.05 g kg^{-1}, respectively. However, in other locations, the mineral contents in leaves were high (3.43% of N, 3.66 mg kg^{-1} of P, and 35.9, 9.8, and 4.25 g kg^{-1} in K, Ca, and Mg), contents that are in accordance with the sampling site. Therefore, the importance of the ecosystem where it develops and the agronomic management of the plant [23,24] in Stevia leaves and sweet leaf extracts found considerable amounts of minerals such as N, K, Ca, Mg, S, Na, and P [25].

Romero et al. [26], in a study carried out on native Stevia from Mexico, observed that the N content was 0.73%, which is lower than those found in this study, and in investigations carried out by Khiraoui et al. [27], they found macroelement contents in Stevia leaves (mg/100 g) that, when modified to g kg^{-1}, represented Ca, Na and Mg contents of 6.57, 1.3 and 1.88, respectively.

Results of the averages of the five localities of Colombia are similar in Ca, higher for Na, and lower in Mg. Jarma et al. [7] indicated that the concentration of macro and microelements determine the quality of Stevia.

Likewise, the cultivation of stevia needs adequate amounts of macroelements in the soils, which present a high correlation with the elements at the foliar level, and these elements are important for the physiological metabolisms of the crop. In this study, a correlation was found between the soil and foliar content of OM and N, Na: Na, and Ca: Ca; correlations that may be associated with temperature, soil moisture, and the species [28].

On the other hand, chemical elements are essential in different metabolic processes, such as N, in the formation of protein synthesis, nucleic acids, and chlorophyll [29], P in cell division, and the activation of carbohydrate metabolism [30]. Zheng et al. [31] observed the effect of Ca on pollen tube germination. Mg was the central atom of the chlorophyll a/b molecule and contributed to the photosynthetic fixation of CO_2 [32].

4.2. Nutritional Characterization of Micro Elements in Soils and Stevia Leaves

According to Table 2, the nutritional contents of microelements vary between localities, depending on factors such as temperature, precipitation, pH, the soil, and climate offer that each region presents [1]. Under these conditions, the highest contents, on average, were presented in Montería and Campamento with 1.97, 38.25, 6.4, 37.9, and 0.24 mg kg^{-1} of Cu, Fe, Zn, Mn, and B, respectively.

Meanwhile, the lowest contents of Cu, Fe, Zn, Mn, and B with 0.38, 0.8, 1.53, 19.82, and 0.25 mg kg^{-1} of these microelements, with some exceptions, were presented in the town of Palmira and Fonseca. These results are associated with parental materials that present a basic chemical reaction (pH>7) and high Ca content. In general, the extractable Zn decreases with an increase in the soil pH due to the increase in the adsorption capacity, with calcium carbonate and co-precipitation in iron oxides [33]. Therefore, soils with high levels of Ca present different problems of a chemical nature for agricultural exploitation [34].

In addition, the contents at the foliar level of Fe, Mn, and B presented significant statistical differences ($p \leq 0.05$) in stevia leaves, with high contents in averages for Cu, Fe, Zn, Mn, and B with 10.4, 602.5, 69.9, 170.16 and 15.15 mg kg^{-1}. However, under these conditions, the Fe and Mn contents are slightly excessive, which can cause phytotoxicities for this species [35]. Khiraoui et al. [27] found microelement contents in Stevia rebaudiana leaves (mg/100 g) modified to g kg^{-1} of Mn, Fe, Cu, and Zn of 64.8, 57.3, 3.5, and 17.1. These results were lower than those found in averages of the five localities of Colombia, possibly due to the incidence of the types of soils and parent materials of each region.

Romero et al. [26] and Khiraoui et al. [27] found considerable amounts of minerals in Stevia leaves and sweet leaf extracts, such as Cu, Co, Fe, Mn, Zn, Cr, Se, and Mo. Das et al. [36] observed that the biomass yield of stevia increases with the balanced application of N, P, and K fertilizers; however, micronutrients' application cannot be stopped, because their application has a favorable effect on biomass production and the quality of stevia.

4.3. Correlation between Macro and Micro FOLIAR Elements with Total Glycosides, Rebaudioside A, Stevioside, and Stevia Leaf Yield in Five Environments of Colombia

In this study, the concentration of total glycosides, rebaudioside A, stevioside, and stevia yield in the five locations showed significant statistical differences ($p \leq 0.05$). These results depend on complete nutrition, both macroelements, such as N, P, Ca, Mg and Na, and micronutrients, Zn, Mn, B, Fe, Cu, to achieve high yields and quality of the leaf, with some exceptions that presented as correlation negative. However, in this study, the soils presented optimal edaphic and nutritional conditions, but there were differences in leaf quality and yields. According to Nader et al. [37], Jarma et al. [38], Kumar et al. [17] and Ahmad et al. [39] the content of phenols, flavonoids, and diterpenes, steviol glycosides-rebaudioside A and stevioside in stevia leaves, depends on the genotype, light, temperature, precipitation, pH, and chemical conditions of the soil where it is grown. De Lima et al. [40], in a work carried out on the nutritional elements in stevia, found that their absorption depends on intrinsic factors in the metabolism of the plant material, the physiological needs of the element in the plant, the development phase, and the tissue or organ that is being used.

4.4. Correlation between Macro and Micro Nutritional Elements and Total Glycosides

Although there is little information on the true effect of macro and micronutrients on leaf quality, in this study, N, Mg, and Cu presented a positive correlation with the number of total glycosides, but B presented a negative effect. Sharma et al. [41] and Barbet et al. [14]observed that an optimal supply of N produces a higher concentration of foliar biomass and a higher concentration of steviol glycosides. Barbet et al. [14] and Tavarini et al. [42] explained that a decreased supply of N reduces the concentration of Reb-A that increases the concentration of stevioside because N deficiency suppresses the UDP-dependent glycosyltransferases responsible for the transformation of stevioside into Reb-A. However, Ceunen and Geuns [13] and Hartmann [43] state that the concentration of primary metabolites, such as steviol glycosides, exhibit great plasticity and are subject to environmental factors and agronomic management, and Nader et al. [37] found that the variation in the content of stevioside and rebaudioside A can also be associated with an increase in altitude, due to a decrease in temperature and, in turn, in the accumulation of stevioside [44]. The function of Mg as the central atom of the chlorophyll a/b molecule, and the generation of reactive oxygen species and its contribution to the photosynthetic fixation of carbon dioxide are recognized by Senbayram et al. [45] and Gerendás and Führs [46]. According to Festa and Thiele [47], Cu participates in the transport of photosynthetic electrons, mitochondrial respiration, the control of the redox state of the cell, the metabolism of the cell wall and hormonal signaling.

4.5. Correlation between Macro and Microelements and Rebaudioside A, Steviosides

The results showed that, for rebaudioside A, the macro and microelements that correlated with the increase in the concentration of this metabolite were P, Mg, and Cu, but B presented a negative effect. Likewise, a positive correlation for the increase of stevioside elements, such as N, Mg, and Mn, and Ca and Na, presented a negative effect on the gain of these metabolites. Jarma et al. [38] found that the Reb-A concentration decreases with P, S, K, or Cu deficiency. On the other hand, Barbet et al. [14] and Sun et al. [48] reported that the supply of N in stevia reduces the contents of SG because plants prefer growth than secondary metabolism related to carbon. Different reports are stated by Barbet et al. [14], who found positive correlations between the concentration of N in the leaf and rates of the assimilation of CO_2 and the negative correlations between the concentration of N in the leaf and SG of the leaf. Utumi et al. [49] showed a decrease in the concentration of SG in plants with P and N deficiencies of 7.8% and 22.8%.

In relation to the microelements, Cu contributes to the maintenance of the SG concentration in the leaves through the synthesis of isoprenoids in stevia chloroplasts, and this metabolite contributes to the production of SG in stevia chloroplasts [6]. Geeta et al. [50] found that, in plants with Cu deficiency, the total concentration of SG was low at 3%, but it was due to the relatively high yield of the leaf per plant. Humphries et al. [51] explained that Mn is important in chlorophyll synthesis and protein construction. However, Bondarev et al. [52] establishes that stevia does not demand the requirements of macro and micronutrients, because its deficiencies do not significantly affect the concentrations of steviol glycosides. On the other hand, a large number of studies have shown the negative effects of Na on photosynthesis, perspiration, the production of reactive oxygen species and, ultimately, growth and yield [53].

4.6. Correlation between Macro and Micro Elements and Performance

The results obtained in this investigation in the five localities of Colombia indicate that the yields are associated with the absorption of the macro and microelements N, Ca, and B, but the absorption of Mg, Fe, and Mn presented a negative correlation with the yield in leaves. In the town of Palmira, it was where the highest yields were found with 3480 kg ha^{-1} for each harvest that is carried out, and it is explained by the nutritional offer of the clayey soils, with vermiculite and montmorillonite clays that have a high exchange capacity cationic and storage of macro and nutritional microelements. Likewise, this region has an average temperatures of 23.7 °C, which contributes to greater retention of moisture in the soils [37].

Barbet et al. [14] found positive correlations between the N concentration of the leaf and the CO_2 assimilation rates for stevia. Likewise, germination and root elongation increase with optimal and higher Ca^{2+} contents [32]. Ahmad et al. [39] and Landi et al. [54] explain that B was favored in approximately 16 metabolic reactions in plants, mainly in the synthesis of carbohydrates and their translocation. Likewise, it is required in cell division and nitrogen fixation. Therefore, excess Mn interferes with enzymes, decreases respiration, and is related to the destruction of auxins [55], which can affect yields. On the other hand, plants subjected to high concentrations of iron adsorb and accumulate large amounts of this element in their tissues, which induces the formation of reactive oxygen species that cause severe reductions in plant growth and productivity [56].

In addition, the locality with the lowest yield was Fonseca, with 494 kg ha^{-1} for each harvest that is carried out, where there is also a high soil supply, but the average temperatures are very high (28 °C) and there is a high evapotranspiration rate [13]. Therefore, there may be a decrease in the absorption of water and nutrients such as N, P, K, Ca, Mg, and K, in addition, there is a reduction in photosynthesis that causes low yields [41].

5. Conclusions

The cultivation of stevia can be established in different soil conditions, precipitation and solar radiation in Colombia. Under these conditions, different contents of glucosides, rebaudioside A and leaf yields were obtained. In this work, it was demonstrated that there was a positive correlation between the foliar contents of N, Ca, Na, Mn and B and the nutritional contents of the soil. In relation to the chemical elements in the leaf, these fulfill specific functions in the formation of metabolites in the cultivation of stevia. Among the foliar contents, N, Mg and Cu presented a correlation with glycosides and it was explained by the mathematical model: GT = 19.86 N** + 25.97 Mg** + 3.32 Cu * − 2.949 B** with an R2 of 97.1%. In addition, the rebaudioside A presented a positive correlation with P, Mg and Cu, explained by the model RebA = 13.74 P** + 21.06 Mg** + 3.66 Cu** − 2.629 B** and the steviosides with N, Mg and Mn explained by averages of the localities: Stv = 9.281 N** − 3.826 Ca** + 4.07 Mg** − 11.68 Na** + 0.06714 Mn*. In addition, the essential elements that contribute to the increase in yields were N, Ca and B, explained by: Rend = 596 N ** + 349 Ca** − 831 Mg** − 0.442 Fe** − 1.607 Mn* + 42, 1 B*. Therefore, it is necessary to advance fertilization plans with these nutrients, considering the response of these metabolites to their application.

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