

Genetic diversity in *Coffea canephora* genotypes for leaf nutrient concentration

Diversidad genética en genotipos de *Coffea canephora* para la concentración de nutrientes en la hoja

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ABSTRACT

This study analyzed the genetic diversity in *Coffea canephora* genotypes by univariate and multivariate statistical analysis, based on concentrations of macro- and micronutrients in coffee leaves in the stages of pre-flowering and grain filling. The experiment was arranged in randomized blocks with three replications, in a 42x2 factorial design, in which factor one represented the evaluated genotypes and factor two the periods of leaf sampling, *i.e.*, pre-flowering and grain filling. The data of leaf nutrient concentrations were subjected to analysis of variance by the F test ($p < 0.01$), and genetic parameters were estimated. For the study of genetic diversity, the genotypes were grouped by the hierarchical unweighted pair-group method using arithmetic averages (UPGMA). The relative importance of a trait to predict genetic diversity was also studied. There is genetic divergence for leaf nutrient concentration in *C. canephora* genotypes. With a maximum limit of 60% of dissimilarity between genotypes, four groups were also formed by UPGMA. For the 42 evaluated genotypes, leaf S concentration was the most important trait for genetic diversity; this genotypic variability should be investigated to enhance the efficiency of nutritional diagnosis.

Keywords

Conilon coffee • sampling period • mineral nutrition • multivariate analysis

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RESUMEN

Este estudio analizó la diversidad genética en los genotipos de *Coffea canephora* mediante análisis estadístico univariado y multivariado, basado en las concentraciones de macro y micronutrientes en las hojas de café en las etapas de pre-floración y llenado de granos. El experimento se organizó en bloques aleatorios con tres repeticiones, en un diseño factorial de 42x2, donde el factor uno representaba los genotipos evaluados y el factor dos los períodos de muestreo de hojas, es decir, pre-floración y llenado de granos. Los datos de las concentraciones nutricionales de las hojas se sometieron a un análisis de varianza mediante la prueba F ($p < 0,01$), y se estimaron los parámetros genéticos. Para el estudio de la diversidad genética, los genotipos se agruparon por el método jerárquico de pares de grupos no ponderados, utilizando medios aritméticos (UPGMA). También se ha estudiado la importancia relativa de un rasgo para predecir la diversidad genética. Existe divergencia genética para la concentración de nutrientes en las hojas en los genotipos de *C. canephora*. Con un límite máximo del 60% de disimilitud entre los genotipos, el método UPGMA formó cuatro grupos. Para los 42 genotipos evaluados, la concentración de S en las hojas fue la característica más importante para la diversidad genética. La variabilidad genotípica debe investigarse para mejorar la eficiencia del diagnóstico nutricional.

Palabras clave

Café conilon • periodo de muestreo • nutrición mineral • análisis multivariante

INTRODUCTION

Two species of the genus *Coffea* are commercially produced, i.e., *C. arabica* and *C. canephora*. Brazil is the world's largest coffee producer, and in the last 10 years, *C. canephora* yields have increased over 90% (6). Among the technologies applied to raise yields, e.g., irrigation, superior genotypes, higher planting density and phytosanitary control (23), an appropriate nutritional management is also relevant (35).

The nutritional status of plants can be determined by the nutrient content in plant tissues, and the leaves are physiologically active organs that are used for the nutritional diagnosis. A correct interpretation of the leaf analysis is a fundamental tool for an adequate supply of nutrients in coffee plantations. To this end, reference values such as critical levels and sufficiency ranges are used (30, 34, 35). However, these reference values to determine the nutritional status do not take the genetic diversity for leaf nutrient concentrations in Conilon coffee genotypes into account, which is an inherent feature of self-incompatible allogamous species (19).

The high genetic variability in Conilon coffee allows the identification of plants with different characteristics within the species (17, 18, 28). The genotypes used in commercial crops differ from each other in nutrient and dry matter accumulation (24, 33), vegetative growth (32) and nutrient uptake and use efficiency (2, 26, 27). Thus, genotypic variation is one of the main factors causing differences in species nutrition (10).

Multivariate analysis is a technique that has been widely used to quantify genetic divergence and allows integrating the multiple information of a set of traits extracted from experimental units. This increases the possibilities of choosing divergent parents in breeding programs (15). For the study of genetic diversity in *C. canephora*, multivariate approaches have been used to evaluate morpho-agronomical (17, 22), morphological (7) and leaf morpho-anatomical traits (18).

Since nutrient uptake, transport and redistribution in plants are genetically controlled, genotypes can be improved and/or selected for a more efficient nutrient use (16), using multivariate techniques as analysis method. Thus, the characterization of genetic variability for leaf nutrient concentration in *C. canephora* species may contribute to more accurate diagnoses of the nutritional management of the crop and generate important information for the planning of breeding programs.

The hypothesis of the work is that the genotypes have different concentrations of nutrients in the leaves, contributing to the genetic variability of the species *C. canephora*.

Therefore, the objective of this study was to identify the genetic diversity of coffee leaf concentrations of macro- and micronutrients in the phenological stage of pre-anthesis and grain filling in *C. canephora* genotypes by univariate and multivariate statistical analysis.

MATERIAL AND METHODS

The experiment was conducted on a rural property in northern Espírito Santo (18° 39' 43" S, 40° 25' 52" W; 199 masl) where the mean annual temperature is 23 °C. According to Köppen, the predominant climate in the region is Aw (tropical with a dry season) (1). The soil at the site is a Latossolo Vermelho-Amarelo, distrófico, with clayey texture and a wavy relief (38). The chemical and physical characteristics are described in table 1.

Table 1. Chemical and granulometric analysis of soil in the experimental area. Nova Venécia, ES - Brazil.

Tabla 1. Análisis químico y granulométrico del suelo en el área experimental. Nova Venécia, ES - Brasil.

Chemical attributes	Depth (cm)					
	0-10	10-20	20-30	30-40	40-50	50-60
K (mg dm ⁻³)	110	95	74	57	52	46
S (mg dm ⁻³)	15	11	29	15	15	17
Ca (cmol dm ⁻³)	3.8	3.4	1.9	1	0.7	0.6
Mg (cmol dm ⁻³)	1	0.9	0.4	0.3	0.1	0.1
Al (cmol dm ⁻³)	0	0	0.3	0.7	0.8	0.8
H+Al	1.6	1.8	2.4	2.9	3.1	3.1
pH-H ₂ O	6.6	6.5	5.3	4.8	4.8	4.8
Organic matter (dag dm ⁻³)	2.1	1.7	1.1	0.8	0.7	0.5
Fe (mg dm ⁻³)	140	138	126	94	88	87
Zn (mg dm ⁻³)	10.2	4.5	2.9	1.1	0.6	0.5
Cu (mg dm ⁻³)	3.4	4.3	3	1.9	1.2	1
Mn (mg dm ⁻³)	207	174	104	46	44	40
B (mg dm ⁻³)	0.81	0.83	0.58	0.55	0.56	0.61
Na (mg dm ⁻³)	11	37	8	6	5	4
Granulometry (g kg ⁻¹)						
Sand	434	352	188	368	366	376
Silt	86	168	212	32	74	124
Clay	480	480	600	600	560	500

In 2014, Conilon coffee was planted, consisting of 42 *C. canephora* genotypes, grown under full sun, in rows spaced 3 m and plants spaced 1 m apart, *i.e.*, at a plant density of 3333 plants per hectare. The cultural treatments were applied according to the technical guidelines for the crop, basically with herbicide weed control and manual cutting, preventive phytosanitary management, liming, fertilization and drip irrigation.

The treatments received 500, 100, and 400 kg ha⁻¹ year⁻¹ of N, P₂O₅, and K₂O, respectively, applied depending on plant requirements and phenological stages. Soil micronutrients were corrected by applying 2 kg ha⁻¹ year⁻¹ Zn, 1.0 kg ha⁻¹ year⁻¹ B, 2.0 kg ha⁻¹ year⁻¹ Cu, and 10 kg ha⁻¹ year⁻¹ Mn.

The experiment was arranged in randomized blocks with three replications, in a 42x2 factorial design, in which factor one represented the evaluated genotypes (table 2, page 25) and factor two the sampling periods (pre-flowering and grain filling). Each experimental plot consisted of seven plants, considering the five central plants for evaluation.

Table 2. Identification of 42 *Coffea canephora* genotypes. Nova Venécia, ES - Brazil.**Tabla 2.** Identificación de 42 genotipos de *Coffea canephora*. Nova Venécia, ES - Brasil.

Code	Name	Code	Name	Code	Name
1	Verdim R	15	Bamburral	29	Tardio C
2	B01	16	Pirata	30	A1
3	Bicudo	17	Peneirão	31	Cheique
4	Alecrim	18	Z39	32	P2
5	700	19	Z35	33	Emcapa 02
6	CH1	20	Z40	34	Emcapa 153
7	Imbigudinho	21	Z29	35	P1
8	AD1	22	Z38	36	LB1
9	Graudão HP	23	Z18	37	122
10	Valcir P	24	Z37	38	Verdim D
11	Beira Rio 8	25	Z21	39	Emcapa 143
12	Tardio V	26	Z36	40	Ouro negro 1
13	AP	27	Ouro Negro	41	Ouro negro 2
14	L80	28	18	42	Clementino

Genotype 33 belongs to cv. Emcapa 8111 and genotypes 34 and 39 to cv. Emcapa 8131 (4). Genotypes 1, 11, 15, 16 and 30 belong to cv. Tributun (18, 37) and 30 and 35 to cv. Andina (28, 36).

In June, coffee leaf samples were collected in the pre-flowering period and in December during grain filling. In both periods, leaf samples were collected on either side of each tree, from the middle third of the plant, taking the third or fourth pair of leaves from the apex of the plagiotropic branches. The leaves were placed in paper bags and dried in a forced air circulation oven at 65 °C to constant weight.

The collected material was sent to a plant tissue analysis laboratory to determine the leaf concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) and boron (B), according to the methodology described by Silva (2009).

The values for leaf nutrient concentration were subjected to analysis of variance by the F test ($p < 0.01$) for each trait separately to detect genetic variation between the genotypes. For the leaf concentration of each nutrient, the coefficient of experimental variation (C_{Ve}), coefficient of genetic variation (C_{Vg}) and coefficient of genotypic determination (H^2) were estimated. The Scott Knott algorithm ($p \leq 0.05$) was used to group the genotypes according to the mean leaf nutrient concentrations.

For the study of genetic diversity, Mahalanobis' generalized distance matrix (D^2) was established as similarity measure and the genotypes were grouped by the Unweighted Pair Group Method using Arithmetic Means (UPGMA). The study of the relative importance of leaf nutrient concentrations to predict genetic diversity was also applied, as proposed by Singh (1981). All statistical analyses were performed using Genes software (8).

RESULTS AND DISCUSSION

According to the analysis of variance, the differences in leaf concentration of all nutrients between the evaluated periods and genotypes were significant at 1% and 5% probability by the F test, except for Zn, which was not significant for either source of variation (table 3, page 26). Significant interactions between evaluated periods and genotypes were only observed for Ca, S and Cu. The significant results indicate differentiated responses of the evaluated genotypes, suggesting the existence of variability in leaf nutrient concentrations, which makes studies related to genetic diversity possible.

Table 3. Summary of variance analysis, estimates of experimental coefficient of variation (CVe), coefficient of genetic variation (CVg) and coefficient of genotypic determination (H^2) for leaf concentration of macro- and micronutrients in 42 *Coffea canephora* genotypes during pre-flowering and grain filling. Nova Venécia, ES - Brazil.

Tabla 3. Resumen del análisis de varianza, estimaciones del coeficiente de variación experimental (CVe), coeficiente de variación genética (CVg) y coeficiente de determinación genotípica (H^2) para la concentración foliar de macro y micronutrientes en 42 genotipos de *Coffea canephora* durante la floración previa y relleno de grano. Nova Venécia, ES - Brasil.

Nutrients	Mean square				Mean	CVe (%)	CVg (%)	H^2 (%)
	Stage (St)	Genotypes (G)	St x G	Error				
N	724.71**	5.47*	2.47 ^{ns}	3.23	26.63	6.75	2.29	40.81
P	2.11**	0.02**	0.01 ^{ns}	0.01	1.08	8.39	4.71	65.41
K	1352.42**	7.26**	3.50 ^{ns}	2.81	11.63	14.42	7.40	61.23
Ca	1067.45**	32.14**	16.72**	8.33	18.34	15.73	10.85	74.07
Mg	290.87**	5.73**	1.42 ^{ns}	1.32	5.86	19.58	14.61	76.96
S	10.50**	0.70**	0.16**	0.08	2.05	14.13	15.72	88.12
Fe	35145.14**	638.16**	402.20 ^{ns}	282.11	90.65	18.52	8.49	55.79
Zn	6.34 ^{ns}	4.93 ^{ns}	4.32 ^{ns}	3.53	7.79	24.11	6.19	28.39
Cu	80.01**	36.13**	17.35**	8.06	16.21	17.51	13.33	77.67
Mn	4053847.00**	76890.91**	22610.86 ^{ns}	31852.94	596.43	29.92	14.52	58.57
B	5040.19**	489.81**	110.32 ^{ns}	92.93	63.25	15.24	12.85	81.02

^{ns}, ** and *, not significant, significant at 1 and 5% probability, respectively, by the F test.

^{ns}, ** y *, no significativo, significativo a 1 y 5% de probabilidad, respectivamente, por la prueba F.

For most leaf concentrations, the experimental coefficient of variation (CVe) was < 20%, which is an acceptable value for experiments with perennial crops such as coffee (11). The lowest leaf concentration was that of N and the highest of Mn, which suggests the lowest environmental influence on N and the highest on Mn (table 3). Lower CVe values for leaf concentration of N and higher values for Mn were also found by Partelli *et al.* (2007, 2016, 2018) and Gomes *et al.* (2016), who evaluated the leaf concentration of these nutrients during pre-flowering and grain filling.

Leaf concentrations from 2.29% for N to 15.72% for S were observed for the coefficient of genetic variation (CVg) (table 3). This is an important parameter that allows conclusions about the range of variability contained in the population for different traits, allowing a comparison of the levels of genetic variability in different genotypes (11). Since the parameter is directly linked to genetic variability, it can provide breeders with an idea of the relative magnitude of changes accessible by selection (14).

Considering the genotype effect as fixed, the genotypic coefficient of determination (H^2), *i.e.*, the limit of heritability, was estimated at 28.39% for Zn (minimum), and 88.12% for S (maximum). Values of H^2 close to 100% indicate a high genetic control for the traits in question, along with a low environmental influence (table 3). Together with S, the leaf concentrations of Cu and Mg were the highest for H^2 . Evaluating macronutrient concentrations in plant tissues of a *C. canephora* genotype, Starling *et al.* (2019) also observed that the S and Mg concentrations stood out among the macronutrients with highest H^2 values.

During grain filling, the leaf concentrations of the macronutrients N, P and K of the genotypes were 13.59%, 18.18% and 49.67% higher, respectively, than in the pre-flowering period (table 4, page 27). These results agree with those of Partelli *et al.* (2016; 2018), in that the leaf concentrations of N, P and K are higher during grain filling. In this period, the growth rate of *C. canephora* trees is higher (31), and these nutrients play a fundamental role in plant metabolism and are essential for the functioning of the photosynthetic apparatus. Nitrogen is a constituent of many plant cell components such as chlorophyll, amino acids and nucleic acids, P is a component of respiration and photosynthesis intermediates, as well as nucleotides used in plant energy metabolism such as ATP, and K plays an important role in regulating the osmotic potential of plant cells, with direct participation in stomatal opening (42).

Conversely to the primary macronutrients (N, P and K) the leaf concentrations of Mg, Fe, Mn and B were higher during pre-flowering, namely 44.88%, 29.95%, 54.01% and 15.22% higher, respectively, than during grain filling (table 4). Higher concentrations of these nutrients during pre-flowering were also reported by Partelli *et al.* (2016; 2018). All of these nutrients are important for plant development and their concentrations should be maintained at appropriate levels in plant tissues, according to the metabolic demand. Magnesium is a constituent of the chlorophyll molecule and required by a series of enzymes involved in phosphate transfer; Fe plays an important role as a component of enzymes involved in electron transfer; B is involved in cell elongation and nucleic acid metabolism, and Mn is required for some enzyme activities, such as decarboxylases and dehydrogenases involved in the Krebs cycle (42).

Table 4. Mean leaf concentrations of N, P, K, Mg, Fe, Mn and B in 42 *Coffea canephora* genotypes during pre-flowering and grain filling. Nova Venécia, ES - Brazil.

Tabla 4. Concentraciones medias de hojas de N, P, K, Mg, Fe, Mn y B en 42 genotipos de *Coffea canephora* durante la floración previa y el llenado de grano. Nova Venécia, ES - Brasil.

Period	Macronutrients (g.kg ⁻¹)				Micronutrients (mg.kg ⁻¹)		
	N	P	K	Mg	Fe	Mn	B
Pre-flowering	24.93 b	0.99 b	9.32 b	6.94 a	102.46 a	723.26 a	67.72 a
Grain filling	28.32 a	1.17 a	13.95 a	4.79 b	78.84 b	469.60 b	58.77 b

Distinct letters in a column differ from each other by the F test at 5% probability.

Las letras distintas en una columna difieren entre sí por la prueba F con una probabilidad del 5%.

Based on the mean grouping by the Scott-Knott test, the genotypes were divided into two dissimilar groups for leaf concentrations of N, P, K, Fe and Mn. For Mg and B concentrations of the genotypes, the variability was highest and three and four dissimilar groups, respectively, were formed (table 5, page 28). The same genotypes identified in the group with highest means for a given nutrient also appear in the group with the lowest means for another nutrient. However, genotype 3 stood out for appearing more frequently in the group with highest means and was grouped in the cluster of highest means for six nutrients (N, K, Mg, Fe, Mn, B). On the other hand, genotypes 5, 8, 14, 17 and 30 were not found in the group with highest means for leaf concentrations of any evaluated nutrient.

Significant differences between leaf nutrient concentrations of *C. canephora* genotypes were also reported by Gomes *et al.* (2016) and Martins *et al.* (2019b). These differences between genotypes may be related to nutrient uptake affinity, compartmentalization in roots or other plant organs, mobility in xylem and phloem vessels and changes in the rhizosphere during growth (25). Another important factor is that the biomass accumulation rates of *C. canephora* genotypes differ from each other (32), *i.e.*, nutrient dilution effects may occur in genotypes with higher and effects of nutrient concentration in genotypes with lower biomass accumulation rates. These genetic variations cause the differences in leaf nutrient contents, indicating a higher or lower efficiency of nutrient uptake, translocation or use by the plant between cultivars or lines (12), and consequently, the possibility of improving and/or selecting more efficient cultivars for nutrient use (13).

The significance of interactions for Ca, S and Cu concentrations indicated a differential response of genotypes to the two sampling periods. For pre-flowering, the genotypes were clustered into three groups for leaf concentrations of Ca, S and Cu. For grain filling, two groups were formed for Ca and Cu and four for S (table 6, page 29).

For nutrient Ca, the leaf concentration of none of the evaluated genotypes was higher during grain filling than pre-flowering, but was mostly statistically equal in both periods or lower during grain filling, indicating a tendency to higher Ca concentrations during pre-flowering (table 6, page 29). Higher Ca concentrations during pre-flowering were also reported by Partelli *et al.* (2016; 2018). Genotypes 1, 6, 10, 11 and 16 stood out for leaf Ca concentration for being clustered in the group with the highest means for both periods. This nutrient fulfills two distinct functions in plants - a structural and a signaling, *i.e.*, it serves as a secondary messenger that triggers plant responses to environmental stimuli (9).

Table 5. Mean leaf concentration of N, P, K, Mg, Fe, Mn and B in 42 *Coffea canephora* genotypes. Nova Venécia, ES - Brazil.**Tabla 5.** Concentración media de hojas de N, P, K, Mg, Fe, Mn y B en 42 genotipos de *Coffea canephora*. Nova Venécia, ES - Brasil.

Means followed by the same letter in a column do not differ from each other by the Scott-Knott test at 5% probability. Las medias seguidas de la misma letra en una columna no difieren entre sí en la prueba de Scott-Knott con una probabilidad del 5%.

Genotype	Macronutrients (g.kg ⁻¹)				Micronutrients (mg.kg ⁻¹)		
	N	P	K	Mg	Fe	Mn	B
1	28.23 a	1.06 b	10.52 b	6.29 b	86.33 b	675.16 a	82.16 a
2	28.71 a	1.05 b	11.87 a	5.53 c	84.33 b	832.33 a	70.33 b
3	28.71 a	1.05 b	12.29 a	7.26 a	111.16 a	647.50 a	88.83 a
4	25.28 b	1.08 b	12.39 a	5.25 c	84.50 b	519.83 b	50.50 d
5	25.41 b	1.03 b	11.35 b	5.77 c	87.50 b	550.16 b	61.00 c
6	26.13 b	0.97 b	9.89 b	8.53 a	92.66 b	680.50 a	83.16 a
7	27.53 a	1.15 a	10.21 b	7.55 a	83.50 b	757.83 a	65.50 c
8	26.85 b	1.01 b	10.21 b	6.58 b	89.16 b	553.66 b	58.50 c
9	26.25 b	1.16 a	11.56 a	5.82 c	88.16 b	748.50 a	63.66 c
10	26.21 b	0.99 b	11.56 a	6.44 b	105.83 a	535.33 b	59.33 c
11	25.18 b	1.02 b	11.77 a	6.00 b	101.16 a	628.83 a	70.16 b
12	26.42 b	1.14 a	12.81 a	5.63 c	88.16 b	719.16 a	53.83 d
13	28.66 a	1.11 a	12.08 a	6.33 b	85.00 b	572.66 b	65.00 c
14	24.94 b	1.05 b	10.00 b	6.82 b	85.00 b	530.00 b	61.66 c
15	26.32 b	1.03 b	11.56 a	6.41 b	87.16 b	630.33 a	66.50 c
16	26.62 b	1.05 b	10.94 b	7.72 a	97.50 a	675.66 a	78.16 a
17	25.66 b	1.04 b	10.73 b	5.64 c	84.83 b	485.50 b	54.00 d
18	26.42 b	1.09 a	12.08 a	6.11 b	88.83 b	767.16 a	64.50 c
19	26.20 b	1.03 b	13.02 a	5.13 c	103.66 a	478.33 b	54.33 d
20	26.33 b	1.03 b	12.29 a	4.34 c	78.83 b	542.66 b	50.50 d
21	26.55 b	1.17 a	12.40 a	6.06 b	76.50 b	411.33 b	63.16 c
22	26.76 b	1.02 b	12.60 a	7.31 a	89.16 b	585.50 b	63.00 c
23	26.84 b	1.12 a	13.33 a	5.34 c	88.50 b	711.33 a	71.33 b
24	26.47 b	1.17 a	11.25 b	5.45 c	88.00 b	491.83 b	63.83 c
25	28.21 a	1.16 a	11.56 a	5.39 c	122.16 a	333.66 b	52.83 d
26	27.77 a	1.12 a	14.48 a	4.69 c	89.66 b	471.00 b	60.33 c
27	27.09 a	1.12 a	11.77 a	5.41 c	85.83 b	718.00 a	67.50 b
28	27.61 a	1.08 b	14.06 a	4.47 c	85.16 b	536.66 b	53.16 d
29	25.88 b	1.11 a	11.88 a	4.69 c	109.33 a	571.50 b	53.00 d
30	25.88 b	0.99 b	10.00 b	5.25 c	86.33 b	545.83 b	68.50 b
31	27.63 a	1.21 a	10.83 b	4.69 c	112.16 a	353.83 b	65.66 c
32	26.16 b	0.97 b	11.15 b	5.24 c	94.83 b	609.16 a	59.00 c
33	27.83 a	1.12 a	12.29 a	5.38 c	92.16 b	611.33 a	58.50 c
34	26.37 b	1.15 a	12.19 a	5.13 c	78.50 b	623.66 a	50.16 d
35	26.51 b	1.05 b	12.81 a	4.48 c	88.16 b	540.16 b	55.66 d
36	27.46 a	1.16 a	10.52 b	6.84 b	94.00 b	829.33 a	64.16 c
37	26.86 b	1.11 a	11.35 b	6.17 b	96.16 b	626.00 a	65.83 c
38	26.56 b	1.01 b	10.42 b	7.11 a	86.33 b	688.00 a	69.00 b
39	25.34 b	1.05 b	11.04 b	6.27 b	74.00 b	611.16 a	73.16 b
40	27.40 a	1.18 a	11.87 a	5.46 c	76.83 b	574.00 b	63.50 c
41	26.64 b	1.06 b	12.19 a	4.33 c	81.33 b	589.33 b	52.33 d
42	24.82 b	1.03 b	9.58 b	5.98 b	99.16 a	486.50 b	61.16 c

Table 6. Partitioning of interaction for Ca, S and Cu leaf concentrations in 42 *Coffea canephora* genotypes during pre-flowering and grain filling. Nova Venécia, ES - Brazil.**Tabla 6.** Particionamiento de la interacción para las concentraciones de hojas de Ca, S y Cu en 42 genotipos de *Coffea canephora* durante la floración previa y el llenado de granos. Nova Venécia, ES - Brasil.

Means followed by equal letters, lowercase letters and uppercase letters do not differ from each other by the F and Scott-Knott tests, respectively, at 5% probability.

Las medias seguidas de letras iguales, letras minúsculas y letras mayúsculas no difieren entre sí por las pruebas F y Scott-Knott, respectivamente, con un 5% de probabilidad.

Genotypes	--- Ca (g.kg ⁻¹) ---		--- S (g.kg ⁻¹) ---		--- Cu (mg.kg ⁻¹) ---	
	Pre-flowering	Grain filling	Pre-flowering	Grain filling	Pre-flowering	Grain filling
1	26.31 Aa	18.40 Ab	1.82 Ca	1.65 Da	12.66 Ca	14.66 Ba
2	17.39 Ca	17.30 Aa	1.82 Ca	2.10 Ca	9.33 Cb	14.00 Ba
3	22.06 Ba	18.90 Aa	1.71 Ca	2.17 Ca	10.33 Cb	15.33 Ba
4	17.84 Ca	13.37 Ba	1.98 Ca	2.29 Ca	16.00 Ba	16.66 Ba
5	17.57 Ca	14.78 Ba	1.55 Ca	1.96 Ca	15.66 Ba	16.00 Ba
6	27.29 Aa	18.24 Ab	1.89 Cb	2.56 Ba	15.66 Ba	16.66 Ba
7	18.01 Ca	18.95 Aa	1.78 Ca	2.20 Ca	15.00 Ca	19.00 Aa
8	17.65 Ca	20.21 Aa	1.52 Cb	2.06 Ca	9.00 Cb	17.00 Ba
9	22.11 Ba	20.84 Aa	2.13 Bb	2.88 Ba	16.66 Ba	16.66 Ba
10	24.74 Aa	19.28 Ab	1.75 Cb	2.56 Ba	15.00 Cb	25.00 Aa
11	24.80 Aa	21.35 Aa	1.88 Ca	2.20 Ca	12.00 Ca	15.00 Ba
12	22.39 Ba	14.95 Bb	1.62 Ca	2.02 Ca	17.00 Ba	18.00 Aa
13	21.26 Ba	15.93 Bb	1.88 Ca	2.20 Ca	19.33 Aa	20.66 Aa
14	20.72 Ba	14.00 Bb	1.86 Ca	1.59 Da	14.00 Cb	20.00 Aa
15	22.08 Ba	18.05 Aa	1.78 Cb	2.56 Ba	16.33 Ba	18.00 Aa
16	25.21 Aa	19.63 Ab	2.17 Ba	2.35 Ca	15.66 Ba	15.00 Ba
17	22.65 Ba	16.72 Bb	1.92 Ca	2.05 Ca	15.33 Ba	16.00 Ba
18	17.51 Ca	17.32 Aa	1.75 Ca	1.92 Da	14.33 Cb	19.00 Aa
19	19.41 Ca	16.99 Aa	1.63 Ca	2.00 Ca	17.00 Ba	16.33 Ba
20	18.80 Ca	13.93 Bb	1.62 Ca	1.57 Da	13.00 Ca	13.00 Ba
21	19.83 Ca	15.58 Ba	1.71 Ca	2.03 Ca	21.00 Aa	15.33 Bb
22	16.92 Ca	18.67 Aa	2.09 Bb	3.52 Aa	19.00 Aa	16.33 Ba
23	16.41 Ca	14.72 Ba	1.86 Ca	2.12 Ca	20.33 Aa	18.66 Aa
24	20.80 Ba	13.33 Bb	1.55 Ca	1.68 Da	15.66 Ba	16.33 Ba
25	16.04 Ca	12.22 Ba	1.59 Ca	1.74 Da	11.66 Cb	18.33 Aa
26	18.37 Ca	20.57 Aa	2.45 Bb	3.06 Ba	14.00 Ca	15.00 Ba
27	21.93 Ba	12.06 Bb	1.62 Cb	2.10 Ca	16.66 Ba	16.66 Ba
28	15.57 Ca	15.05 Ba	1.72 Cb	2.23 Ca	18.33 Aa	15.66 Ba
29	17.91 Ca	12.29 Bb	1.76 Cb	2.30 Ca	13.00 Ca	13.33 Ba
30	28.06 Aa	15.99 Bb	1.65 Ca	2.09 Ca	10.00 Ca	13.33 Ba
31	20.03 Ca	15.74 Ba	1.85 Ca	1.74 Da	13.33 Ca	15.33 Ba
32	18.86 Ca	14.32 Ba	1.89 Ca	1.85 Da	13.33 Ca	15.00 Ba
33	17.91 Ca	18.90 Aa	2.19 Bb	2.67 Ba	17.33 Ba	21.33 Aa
34	16.06 Ca	14.68 Ba	1.72 Ca	2.16 Ca	20.33 Aa	18.33 Aa
35	19.67 Ca	14.62 Bb	1.72 Cb	2.23 Ca	17.66 Ba	15.33 Ba
36	22.30 Ba	15.53 Bb	1.69 Ca	2.09 Ca	19.33 Aa	21.33 Aa
37	21.51 Ba	15.37 Bb	3.03 Ab	3.81 Aa	21.33 Aa	16.66 Bb
38	21.00 Ba	16.16 Bb	2.02 Cb	2.56 Ba	19.33 Aa	17.66 Aa
39	22.57 Ba	15.98 Bb	2.03 Ca	2.23 Ca	14.00 Ca	12.00 Ba
40	21.45 Ba	16.07 Bb	1.90 Ca	2.36 Ca	22.66 Aa	18.00 Ab
41	16.09 Ca	13.33 Ba	1.82 Cb	2.39 Ca	18.66 Aa	16.66 Ba
42	21.65 Ba	13.52 Bb	1.58 Cb	2.79 Ba	11.00 Cb	16.00 Ba
Mean	20.40	16.28	1.84	2.25	15.65	16.78

For leaf S concentration, the genotypes responded inversely to that of Ca, since the S concentrations of 16 genotypes were highest during grain filling, while those of the others were considered statistically the same in both periods, indicating a tendency to higher S concentrations during grain filling (table 6, page 29). Genotype 37 stood out for forming a separate group of high S concentration during pre-flowering, superior to the other groups. For the grain filling period, genotype 37 and 22 formed a group with the highest means. The S content in plant tissues of these lines is extremely important, as this nutrient is a constituent of coenzymes, vitamins and certain amino acids that are essential for the metabolism, while a deficiency, similarly as in the case of nitrogen, can lead to plant growth reduction (5).

Similar to S, a trend of higher Cu concentration during grain filling can be observed, except for genotypes 21, 37 and 40, for which the mean concentrations were higher during pre-flowering (table 6, page 29). The importance of adequate Cu concentrations in plants is related to the functions of the element, which is essential for mitochondrial respiration, carbon and nitrogen metabolism, for protection of oxidative stress and necessary for cell wall synthesis (3). It also participates in photosynthetic reactions. since more than half of the Cu in plants is found in chloroplasts (21). The genotypes 13, 23, 34, 36, 38 and 40 were found to have the highest leaf Cu concentrations in both evaluated periods.

The grouping of genotypes by the UPGMA hierarchical clustering, using Mahalanobis' generalized distance (D^2) as a measure of genetic dissimilarity for the macro- and micro-nutrient leaf concentrations in the periods pre-flowering and grain filling, allowed the formation of a dendrogram. By establishing a maximum limit of 60% dissimilarity between genotypes, the formation of four groups was observed (figure 1).

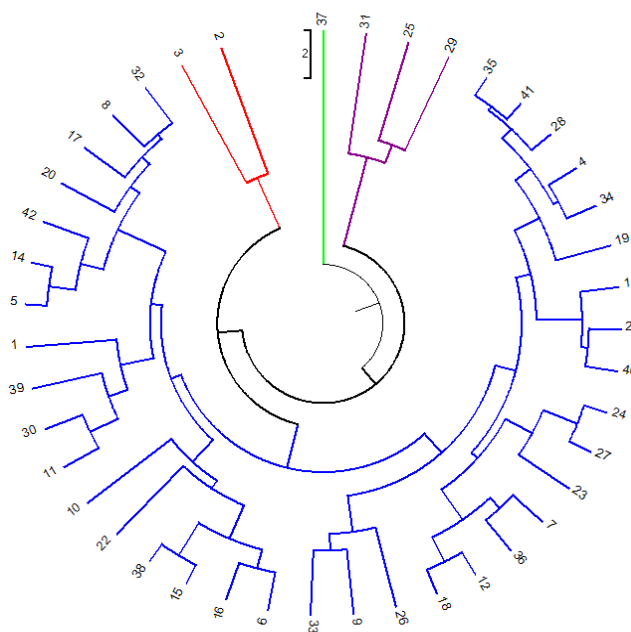


Figure 1. Representative dendrogram of genetic dissimilarity among 42 *C. canephora* genotypes, obtained by UPGMA clustering, using Mahalanobis' generalized distance (D^2) for leaf concentrations of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, copper, manganese and boron during pre-flowering and grain filling. Cophenetic correlation = 0.62.

Figura 1. Dendrograma representativo de la diferencia genética entre 42 genotipos de *C. canephora*, obtenidos por agrupamiento UPGMA, utilizando la distancia generalizada de Mahalanobis (D^2) para las concentraciones foliares de nitrógeno, fósforo, potasio, calcio, magnesio, azufre, hierro, cobre, manganeso y boro durante pre-floración y relleno de grano. Correlación cophenetic = 0,62.

The first group established by the UPGMA method consisted of only genotype 37. The second group comprised three genotypes, 31, 25 and 29. Genotypes 2 and 3 formed the third group, and the fourth group contained 36 genotypes, representing 85.71% of all evaluated genotypes. The formation of the groups, considered divergent, indicates the variability between *C. canephora* genotypes for leaf nutrient concentrations. By the hierarchical UPGMA clustering, Gomes *et al.* (2016) and Martins *et al.* (2019b) also observed the formation of divergent groups of *C. canephora* genotypes for nutritional characteristics. In breeding programs, the study of genetic diversity by multivariate techniques is useful for planning and to define work strategies (22).

According to the grouping by the UPGMA method, the mean of the evaluated characteristics was calculated for each group, thus allowing a comparison of the leaf concentrations that differentiate the groups (table 7). In the first group, with only genotype 37, the highest S and Cu and lowest K concentrations were found. The second group had the highest P and Fe and lowest Mg, Mn, B, Ca and S leaf concentrations. For the third group, highest N, K, Mg, Mn, B and Ca concentrations and lowest P and Cu concentrations were recorded. The fourth group differs from the others by not presenting highest leaf concentrations of any of the evaluated nutrients, but the lowest N and Fe concentrations.

Table 7. Means of macro- and micronutrient leaf concentrations in *Coffea canephora* for groups formed by UPGMA clustering based on Mahalanobis' generalized distance (D^2). Nova Venécia, ES - Brazil.

Tabla 7. Medias de las concentraciones de hojas de macro y micronutrientes en *Coffea canephora* para grupos formados por agrupamiento UPGMA basado en la distancia generalizada de Mahalanobis (D^2). Nova Venécia, ES - Brasil.

Group	Macronutrients						Micronutrients			
	----- (g.kg ⁻¹) -----						----- (mg.kg ⁻¹) -----			
	N	P	K	Ca	Mg	S	Fe	Cu	Mn	B
G1	26.86	1.11	11.35	18.44	6.17	3.42	96.16	19.00	626.00	65.83
G2	27.24	1.16	11.42	15.71	4.92	1.83	114.55	14.16	419.66	57.16
G3	28.71	1.05	12.08	18.91	6.40	1.95	97.75	12.25	739.92	79.58
G4	26.52	1.07	11.64	18.53	5.90	2.04	88.12	16.53	602.37	62.77

To determine the relative contribution of leaf concentration of evaluated nutrients, we used the method of Singh (1981), resulting in values from 4.69 to 22.52% (table 8). Leaf concentrations that contributed most to genetic divergence among the 42 genotypes were nutrients S (22.52%), Cu (11.54%), B (11.46%) and Mg (11.10%), which together accounted for 55.62% of the variability between the genotypes.

Table 8. Relative contribution of leaf concentration of macro- and micronutrients to genetic diversity in 42 *Coffea canephora* genotypes, according to Singh's method (1981), Mahalanobis' generalized distance (D^2). Nova Venécia, ES - Brazil.

Tabla 8. Contribución relativa de la concentración foliar de macro y micronutrientes a la diversidad genética en 42 genotipos de *Coffea canephora*, según el método de Singh (1981), distancia generalizada de Mahalanobis (D^2). Nova Venécia, ES - Brasil.

Nutrients	S.j	S.j (%)	Cumulative S.j (%)
S	2539.352978	22.52	22.52
Cu	1300.724814	11.54	34.06
B	1291.781259	11.46	45.52
Mg	1250.1168	11.10	56.62
P	1122.41325	9.96	66.58
Ca	1102.525267	9.78	76.36
Fe	747.869333	6.63	82.99
Mn	730.187555	6.48	89.47
K	660.416076	5.86	95.33
N	528.287387	4.69	100

S. j: value estimated by the statistic of Singh (1981).

S. j: valor estimado por la estadística de Singh (1981).

Leaf N concentration was the trait that contributed least to the genetic diversity among genotypes (table 8, page 31). Similarly, Starling *et al.* (2019) reported that the N concentration in plant tissues of *C. canephora* genotypes had the lowest relative contribution to genetic diversity by the method of Sing (1981). The study of the relative importance of traits for genetic divergence is highly relevant, since it estimates values based on which those of minor importance for genotype discrimination can be eliminated (20).

CONCLUSIONS

Genetic divergence is available among *C. canephora* genotypes for leaf nutrient concentration during the phenological stages pre-anthesis and grain filling.

Genotype 3 (Bicudo) stands out with the highest leaf concentrations of six evaluated nutrients (N, K, Mg, Fe, Mn and B).

Leaf S concentration contributed most to the genetic diversity among the 42 evaluated genotypes, followed by Cu, B and Mg concentrations.

To improve the efficiency of nutritional diagnosis, it is suggested that apart from the sampling periods of pre-flowering and grain filling, the genotypic variability for leaf nutrient concentration should also be taken into consideration.

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