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GENETIC VARIABILITY ON NUTRIENT CONTENTS IN *Coffea canephora* GENOTYPES CULTIVATED AT 850 METERS OF ALTITUDE IN TWO CROP SEASONS

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Abstract: The variation in the climatic conditions throughout the year can influence the foliar nutrient contents in *Coffea canephora*, impacting the fertilization management. We evaluated the influence of the climatic seasonality on the foliar nutrient contents of 28 *C. canephora* genotypes cultivated at 850 meters of altitude, in cold winter. The work was carried out in Morrinhos, State of Goiás, Brazil. A randomized complete block design in a 2 x 28 factorial arrangement was used, with two crop seasons, winter and summer, and 28 *C. canephora* genotypes, with four replications, each replicate composed by five plants, and a spacing of 3.5 m x 1.0 m. The third and fourth pairs of leaves, of productive branches located in the middle third of the plant, were collected in six-year-old crops. The leaves were dried, and the mineral contents were analyzed, they were, then, subjected to multivariate analysis of principal components, dissimilarity and clustering. The results reveal the existence of different nutritional contents among leaves collected in the winter and summer. There is a tendency of higher macro and micronutrient contents in leaves collected in the winter than in the summer. The nutritional diagnosis should consider the group of genotypes and the crop season.
Keywords: Conilon; Environment; Macronutrients; Micronutrients

Introduction

More than 150 million bags of coffee were harvested in 2016, worldwide, from which 95 million were produced by *Coffea arabica* L. and 56 million by *C. canephora* Pierre ex A. Froehner. Currently, the largest producing countries are Brazil, Vietnam, Colombia, Indonesia, Ethiopia, Honduras and India (ICO, 2018). The coffee income is around USD 173.000 million for the entire coffee chain of value (ICO, 2018), presenting an immense social and economic role in many tropical developing countries, with an estimation that the livelihoods of 25 million smallholder farmers depend on this crop (Van der Vossen, 2016).

Coffea canephora develops well at annual average temperatures of 22 ºC to 26 ºC (Ramalho et al., 2014). Tolerance at temperatures up to 37 ºC is observed through the maintenance or reinforcement of photoprotection and antioxidative mechanisms (Martins et al., 2016; Rodrigues at el., 2016). Although, heat may impact physical attributes, showing decreases in bean mass and yield of coffee (Ramalho et al., 2018a). For *C. canephora* cv. Conilon, growth is retarded when the minimum average temperature is below 17 ºC and above 31 ºC under field conditions (Partelli et al., 2010; Covre et al., 2016). In general, when the temperature falls below 13 ºC, the coffee tree suffers several metabolic and membrane alterations in the composition and structure of the photosynthetic pigment complexes and present negative impact on the photosynthetic machinery functioning (Praxedes et al., 2006; Partelli et al., 2011; Batista-Santos et al., 2011; Scotti-Campos et al., 2014). However, some tolerance has been observed at low positive temperatures that vary according to *Coffea* species and genotypes, depending on the reinforcement of antioxidative mechanisms and on the dynamics and alteration of the lipid matrix of the chloroplast membranes (Fortunato et al. 2010; Partelli et al., 2011, Ramalho et al., 2014).

Under conditions that promote stomatal closure (eg, water deficit, low temperature), the transpiration flow will be reduced and, hence, the translocation of nutrients from the roots to the leaves will also be reduced (Covre et al., 2018). On the other hand, in plants, the efficiency and priority of certain nutrients absorption by the roots are genetically defined characteristics (Larcher, 2000). In the coffee tree, the mineral nutrients absorption by the roots and their accumulation in the plant vary with location, climate and season of the year, age, organs and tissues (Prezotti and Bragança, 2013). Several studies indicate differences in foliar nutrient content among genotypes of *C. canephora* under the same management conditions (Martins et al., 2015; Gomes et al., 2016). This fact can be explained by the wide intra and interspecific variability among genotypes of *C. canephora*, especially regarding characteristics such as growth, maturation cycle, nutrient accumulation and stress tolerance (Marraccini et al., 2012). This allows the exploration of the diversity for the identification of genetic material more adapted to the several soil and climate conditions existing in the producing regions (Martins et al., 2015).

Considering the lack of studies concerning the foliar nutrient content in genotypes of *C. canephora* cultivated in climate of high altitude, we aimed to evaluate the influence of climatic seasonality on foliar contents of different genotypes of *C. canephora* cultivated at 850 meters of altitude, with possibility to infer the nutritional diagnosis, under these cultivation conditions.

Material and methods

The experiment was carried out in Morrinhos, State of Goiás, Brazil, at an altitude of 850 m (Latitude: 49'30'' S Longitude: 49° 12'01'' W). The region is characterized by water deficit from April to October, flat topography, with an average annual temperature of 20 °C (minimum air temperature varies from 10 to 20 °C in the winter months, and the average minimum annual temperature is of 18 °C), presenting some days with temperature below 8 ºC. The experimental crop included 28 genotypes of *C. canephora* Pierre ex A. Froehner (Table 1).

The experiment was established under a randomized block design in a 2 x 28 factorial arrangement, considering two leaf crop seasons (winter: August 9, late summer: March 12) and 28 genotypes (Table 1), with four replications, each composed by five plants of each genotype.

The spacing used in the experimental crop was 3.5 m x 1 m. The cultural treatments corresponding to the fertilization were carried out according to the technical orientations for the coffee crop. Pruning was made to control the number of branches per area, maintaining the standard of 12,000-15,000 stems per hectare. We carried out a manual weeding (tracing the fertilization site), a mechanized weeding and a chemical weeding (per year). No micronutrients, insecticides or fungicides were applied during the study year.

The third and fourth pairs of leaves were collected from productive branches located in the middle third of the plant. After harvest, the leaves were dried in a forced circulation oven for 48 hours at 60-70 ºC. Then, leaves were grounded for the nutrient content analysis. For the determination of N content, the plant material was submitted to sulfur digestion, in which the nitrogen was determined by the Nessler method. The other nutrients P, K, Ca, Mg, S, B, Fe, Zn, Mn and Cu were quantified by ICP-OES, after digestion with concentrated HNO_3 and H_2O_2 in an open digestion system. ICP conditions: plasma gas 8.0 L min^{-1}, auxiliary gas 0.70 L min^{-1} and carrier gas 0.55 L min^{-1}, according to Peters' procedures (2005).

Soil samples were collected at 0-20 and 20-40 layers and analyzed regarding the chemical characteristics by the Embrapa (2009), methodology (Table 2).

Data of the foliar nutrient content of the 27 genotypes evaluated in the study (one of the genotypes died due to the low temperature) were analyzed by the multivariate method through clustering, what generated a dendrogram elaborated from the Euclidean distance matrix and a clustering carried out by the complete linkage clustering (farthest neighbor linkage) (Barroso and Artes, 2003). For the groups formation, a cut-off point was calculated using the constant of 1.25 considering what was suggested by Milligane and Cooper (1985), using the formula: (M + 1.25* sd), where: M = average of the junction points values; sd = standard error of the junction points values. A multivariate ordering analysis was performed involving principal component analysis.

The data were submitted to analysis of variance using the F test, with a 5% level of probability. To compare the average contents of nutrients in coffee leaves, the values obtained for the interaction environment x groups were analyzed using the Tukey test, adopting a 5% probability level. All statistical analyzes were performed using the R Core Team program (2016).

Results and discussion

The cut-off point for the groups separation in the dendogram provided the formation of nine groups of *C. canephora* genotypes based on the evaluated characteristics (Figure 1).

The first, second, third, fourth and eighth groups were composed of a single genotype, 2, 14, 18, 19 and 16, respectively. The fifth group consisted of genotypes 1, 4, 6 and 13, the sixth by genotypes 25 , 9, 5, 8, 28, 20 and 27, the seventh by genotypes 11, 17, 3, 7, 10, 21 and 24 and the ninth by genotypes 12, 22, 23 and 26. These nine clusters are considered divergent among each other regarding the foliar nutrient contents from the *C. canephora* genotypes cultivated at an altitude of 850 meters, where high diversity was identified in terms of foliar nutrient contents. The study of genetic diversity using multivariate techniques is important for planning and defining work strategies in breeding programs (Guedes et al., 2013; Machado et al., 2017). In fact, the use of plant material with genetic variability is determinant for successive breeding programs, providing greater gains in selection (Cruz et al., 2004).

The analysis of variance (Table 3) shows that there was no significant interaction between the factors groups of genotypes and crop seasons for the levels of N, Mg and Fe (Table 3). Nevertheless, significant differences were found for these nutrients content when the factors genotype group and crop season were observed separately. The existence of differences between groups of genotypes is lined up with the results obtained through the analysis of divergence (Figure 1). In addition, levels of P, K, Ca, S, B, Cu, Mn and Zn demonstrate significant interaction between the groups of genotypes and crop seasons in the analysis of variance (Table 3).

Regarding the two seasons under analysis, the nutrients N, Mg and Fe presented higher leaf content in the winter (Table 4). These nutrients have important roles in the photosynthetic machinery, since they determine the level of protein (N), of the enzymes from the Calvi-Benson cycle, in particular of ribulose 1,5-bisphosphate carboxylase oxygenase (RuBisCO), the most abundant plant protein, while Mg is an integral part of the chlorophyll molecule (Taiz et al., 2015). Besides, *ca*. 80% of the foliar Fe occurs in chloroplasts, where it plays an important role in the photosynthesis and biosynthesis of proteins and chlorophyll (Marschner, 1995).

The coffee plant Conilon, when submitted to cultivation at a temperature lower than 17 ºC, undergoes leaf dehydration, with stomatal and mesophyll limitations, followed by metabolic alterations, causing damages to the components of the photosynthetic process with differentiated intensities among the genotypes, besides reduced vegetative growth (Partelli et al., 2010; Ramalho et al., 2018b). Thus, it is possible that genotypes of *C. canephora* evaluated in this study suffered stress from temperatures below 17 ºC, which occurred during the winter period. This would explain the differences between the contents of these nutrients at this crop season.

A significant interaction between groups of genotypes and seasons of the year (Table 3) was observed for the remaining levels of macro (P, K, Ca, S) and micro (B, Cu, Mn and Zn) nutrients. For P foliar contents, significant difference between the seasons of the year was only observed in the third clustering (genotype 18 or NV8), with the lowest value reached in the summer (Table 5), which may be related to the post-flowering phase and filling of grains in which the plants were found. This was also observed in the leaves of Conilon Robusta Tropical in the post-flowering phase, relative to P, N and K (Prezotti and Bragança 2013).

The foliar contents of K in groups 1, 3 and 4 were also lower in the late summer, period of growth and ripening of fruits. These are the main drain of the plant in this phase, promoting the translocation of minerals and photoassimilates from the leaves to the fruits (Partelli et al. 2014). Also, foliar contents of S, Ca, Cu, Mn and Zn present a similar pattern in some groups of coffee genotypes (Table 5), due to fruit ripening, but also due to the higher vegetative growth that some of these genotypes present at that season of the year (Partelli et al., 2014; Covre et al., 2016; Gomes et al., 2016). On the other hand, greater values of minerals such as Cu, Zn, Fe, Mn in the coolest part of the year, might be related with the strengthening of the antioxidative defense mechanisms, necessary under cold conditions (Ramalho et al., 2013).

B was the only foliar nutrient, which content was higher in the summer in 5 of the 9 groups (4, 6, 7 and 9) (Table 5). B is considerably immobile in plants, and its movement into the roots occurs by mass flow until an equilibrium between its concentration in the roots and in the soil is achieved. For that reason, the higher water availability, characteristic of late summer in Brazil, may have resulted in higher B accumulation in the plants (Covre et al., 2018). This micronutrient participates in cell growth, in the biosynthesis of cellular components, in the metabolism of phenols, nucleic acids, carbohydrates and Indole-3-acetic acid (IAA), besides conferring stability and structure to the cell wall (Marschner, 1995).

Regarding the relative contribution of the several nutrients to the discrimination of the formed groups, the analysis of principal components for leaves harvested in the winter showed that the macronutrient Ca is the one that describes, with more intensity of response, the nutrient contents in group 2 (Figure 2A). For the 7th genotype group, Mg was the macronutrient that best described the behavior of this group.

The nutrients N, P and S describe group 8 more strongly, where P represents a greater influence on the responses of the nutrient contents to group 8 when compared to nutrients N and S. The nutrient K discriminates, in a greater degree, the response of the groups of genotypes 9 and 4. For the groups of genotypes 1, 5, 6 and 3, there was no macronutrient in this study that explains, with great intensity, the response of these groups regarding the nutrient contents (Figure 2A). K and Mg were the most important nutrients to discriminate the groups of genotypes of *C. canephora* according to the nutrient contents in leaves harvested in the winter.

In a similar analysis for the micronutrients (Figure 2B), Fe explains with great intensity the group of genotypes 1 and 2. In comparison with Fe, the micronutrient Zn describes group 2 in a greater degree. The response according to the nutrient content of groups of genotypes 7 and 8 are strongly explained by the Cu micronutrient, and this nutrient explains in a greater degree the behavior for the nutrient content of group 7 when compared to group 8. Regarding groups 9, 5, 4, 3 and 6, no micronutrients were found to describe the response of these groups to the nutrient content, with great intensity, suggesting that the contribution of micronutrients to these groups of genotypes occurred more evenly.

Regarding the macronutrient foliar content in the summer (Figure 3A), N explains the nutrient content response of group 5. Magnesium describes, in a greater degree, group 7, as in the winter. Calcium describes groups 2 and 3 with different intensities (Figure 3A) and is similar to that observed in the winter for group 2. Because it is a nutrient with low mobility in the plant, it is expected little variation between leaves crop seasons within the same genotype groups. Finally, S explains with certain degree of discrimination, groups of genotypes 3 and 4, and P explains with great intensity, the behavior for groups 8 and 6, with more emphasis on the latter. The response to the ninth genotype group was largely explained by K. Only the first genotype group does not seem to be described by any of the evaluated macronutrients.

Regarding the foliar micronutrient content in the summer (Figure 3B), Zn, B, Mn and Cu describe in a high degree the response of group 3. Manganese explains, although with small intensity, the response of group 4 for the micronutrient contents in the leaves in the summer. Groups 2 and 7 are described by Fe, while groups of genotypes 1, 5, 6, 8 and 9 were not discriminated with great intensity by any of the micronutrients (Figure 3B).

In general, there is a pattern of nutrient content differentiated when the interaction between the variables macro and micronutrients and groups of genotypes is observed in a comparison between the two sampling seasons at altitude (Figures 2 and 3). The response pattern that more strongly discriminates the results for analysis of principal components of this study were presented by macronutrients in leaves harvested in the summer (Figure 3A). This response can be explained by the favorable climatic conditions in which the plants grew when the leaves were collected (late summer).

Finally, the results of the study indicate that groups of genotypes of *C. canephora* cultivated at high altitudes suffered significant changes in the nutrient content of leaves collected in the winter and late summer. This difference indicates the presence of promoting or inhibiting factors (edaphoclimatic and other factors) occurring during the year in these climatic conditions that along with the genetic variability of the coffee trees, caused variations in nutrient contents in the leaves collected in winter and summer, at high altitude. Thus, the nutritional management of these plants should consider season and genotypes. The results observed in this study can be considered similar to that identified by Giles et al. (2018), in which, by studying promising genetic coffee conilon materials, they concluded that among the evaluated genotypes there is genetic diversity indicating potential for actions aimed at the improvement of coffee plants.

Table 1. List of the 28 genotypes of *C. canephora* used in the experiment, in the Institute Federal Goiano, in Morrinhos - GO.

A	AC	A	AC
1V	1	NV2	14
2V	2	14 from EMCAPA 8121	15
3V	3	Clone 18	16
4V	4	NV1	17
5V	5	NV8	18
6V	6	P1	19
7V	7	3 from EMCAPA 8121	20
8V	8	P2	21
9V	9	4 from Bahia	22
10V	10	NV3	23
11V	11	Verdim TA	24
12V	12	A1	25
13V	13	Robustão Seeds	26, 27 and 28

*(A): Accessions; (AC): Accessions code, corresponding to 28 genotypes of *C. Canephora* analyzed in the experiment. V1 to V2 - genotypes that compose the Vitória cultivar. A1 - composes the *Tributun* cultivar. 3 and 14 from Emcapa - compose the Emcapa 8121cultivar.

Table 2. Chemical and granulometric characterization of soils collected in the experimental area of the study, Morrinhos - GO.

Samples	pH	P	K	Na	Ca	Mg	Al	H+Al	SB	t	T	V	m	OM
	H_2O	mg dm^{-3}			cmolc dm^{-3}							%	%	g Kg^{-1}
0-20	5.45	1.43	92	5	2.44	0.39	0.05	5.28	5.09	3.14	8.37	36.92	1.59	32.23
20-40	5.41	0.61	51	3	1.74	0.34	0.05	4.78	2.22	2.27	7.00	31.69	2.20	30.23

Samples	Total sand	Silt	Clay	Classification
	%	%	%	
0-20	34	6	60	Argillaceous texture
20-40	33	6	61	Very argillaceous texture

*Available phosphorus (P), exchangeable potassium (K), sodium (Na), calcium (Ca^{2+}) and magnesium (Mg^{2+}), exchangeable aluminum (Al^{3+}), Sum of exchangeable bases (SB), cation effective capacity at pH7 (t) and exchangeable capacity (T), base saturation (V), organic matter (OM), and aluminum saturation index (m).

Table 3. Analysis of variance for the characteristic foliar nutrient contents, harvested in different seasons (winter and summer), from different *C. canephora* genotypes cultivated at high altitude, Morrinhos - GO.

		Mean square and significance											
		(g/Kg)						(mg/Kg)					
VS	DF	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	
Block	3	1.09 ^{ns}	0.00139 ^{ns}	3.89 ^{ns}	1.559 ^{ns}	0.07412 ^{ns}	0.00235 ^{ns}	96.8 ^{ns}	17.178 ^{ns}	1305 ^{ns}	147.4 ^{ns}	0.79 ^{ns}	
Season	1	361.63 **	0.49667 **	16.255 ^{ns}	147.09 **	1.6805 **	1.53125 **	10287.3 **	4.268 ^{ns}	813822 **	1558.1 **	140 **	
Genotype Group	8	14.09 **	0.11594 **	90.226 **	34.435 **	1.1214 **	0.18448 **	3906.1 **	24.554 ^{ns}	24962 **	11410.5 **	3.09 *	
Season* Group	8	2.59 ^{ns}	0.0667 *	25.215 **	5.39 **	0.1383 ^{ns}	0.08666 **	1665.4 **	158.444 **	6737 ^{ns}	845.7 **	3.08 *	
Residue	51	2.08	0.02793	8.157	1.852	0.07993	0.02747	84.5	21.75	4036	347.9	1.182	
CV (%)		7.28	14.06	11.82	9.45	15.11	8.48	14.02	15.02	27.10	17.44	13.93	

*VS – Variation Source; DF – Degrees of Freedom; CV – Coefficient of Variation; $^{(ns)}$ non-significant; $^{(*)}$ significant at the level of 5% of probability; $^{(**)}$ significant at the level of 1% of probability, both using the F test.

Table 4. N, Mg and Fe contents from *C. canephora* leaves harvested in the winter and summer in coffee trees cultivated at high altitude, located in Morrinhos - GO.

	Nutrients		
Crop seasons	N (g kg^{-1})	Mg (g kg^{-1})	Fe (mg kg^{-1})
Winter	22.0 a	2.02 a	340.7 a
Summer	17.5 b	1.71 b	128.1 b

*$^{(1)}$ In the columns the averages with different letters significantly differ among each other, considering the level of 5 % of probability, evaluated by the F test.

Table 5. Interaction of *C. canephora* foliar nutrient contents cultivated at high altitude and the crop season.

Groups	Season	Nutrients							
		(g kg^{-1})				(mg kg^{-1})			
		P	K	Ca	S	B	Cu	Mn	Zn
1	Winter	1.20 a	23.3 a	14.1 a	1.93 a	45.2 a	34.7 a	123.6 a	9.20 a
	Summer	1.01 a	17.9 b	12.8 a	1.49 b	49.8 a	23.8 b	110.8 a	5.15 b
2	Winter	1.25 a	18.6 a	20.0 a	2.15 a	69.6 b	27.7 a	139.0 a	9.90 a
	Summer	1.00 a	18.6 a	15.5 b	1.95 a	99.8 a	34.3 a	95.0 b	6.80 b
3	Winter	1.30 a	25.2 a	18.0 a	1.97 a	69.0 b	21.8 b	182.3 a	9.82 a
	Summer	0.73 b	19.3b	16.1 a	2.00 a	62.5 b	42.7 a	160.6 a	7.80 b
4	Winter	1.17 a	31.5a	15.5 a	2.35 a	53.4 b	32.1 a	165.5 a	10.20 a
	Summer	1.20 a	27.3 b	14.5 a	1.90 b	70.6 a	33.0 a	137.5b	6.63 b
5	Winter	1.19 a	25.6 a	13.4 a	1.91 a	43.6 a	32.1 a	76.5 a	9.35 a
	Summer	1.12 a	24.6 a	10.1 b	1.55 b	45.8 a	27.4 a	61.7 a	5.56 b
6	Winter	1.18 a	26.1 a	14.5 a	1.90 a	46.9 b	27.7 a	91.8 a	7.72 a
	Summer	1.18 a	23.6 a	12.9 a	1.80 a	69.2 a	28.0 a	92.1 a	6.84 a
7	Winter	1.28 a	23.6 a	17.2 a	2.18 a	49.6 b	32.3 a	109.5 a	9.66 a
	Summer	1.05 a	23.7 a	13.9 b	1.81 b	81.7 a	32.1 a	114.4 a	6.58 b
8	Winter	1.52 a	29.0 a	15.6 a	2.37 a	58.8 a	35.3 a	73.7 a	9.05 a
	Summer	1.32 a	26.9 a	9.65 b	1.75 b	54.9 a	27.9 b	49.6 a	5.32 b
9	Winter	1.33 a	27.2 a	14.1 a	2.12 a	46.4 b	33.4 a	70.2 a	7.83 a
	Summer	1.31 a	27.0 a	11.3 b	2.01 a	63.4 a	33.2 a	70.6 a	6.98 a

* (1) In the columns the averages with different letters, in the same genotype group, significantly differ among each other, considering the level of 5 % of probability, evaluated by the Tukey test.

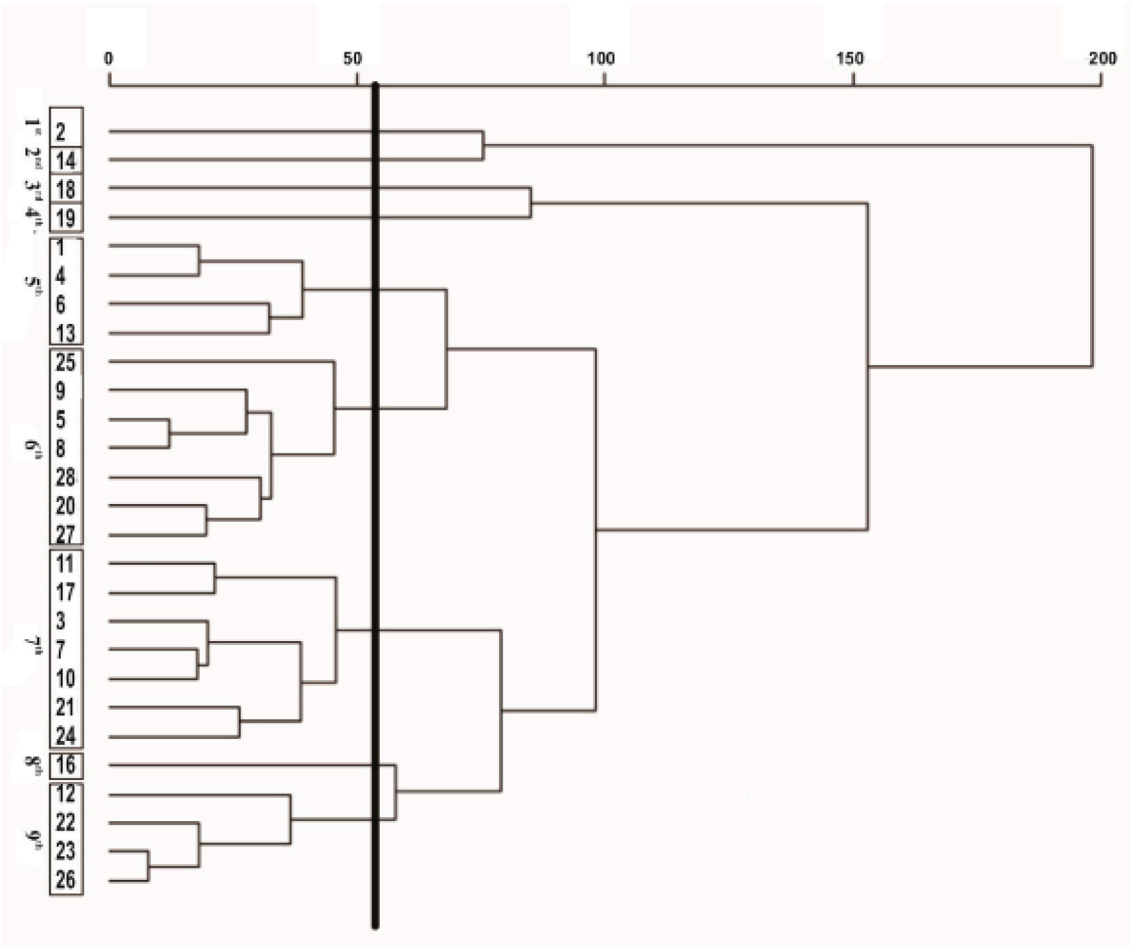

Figure 1. Dendrograms created with the complete linkage, from the genetic dissimilarity matrix using the Euclidean distance, the variable average foliar nutrient content in leaves of 27 genotypes of *C. canephora*, collected in two seasons (winter and summer), cultivated in the south of the cerrado region of Goiás. Morrinhos - GO.

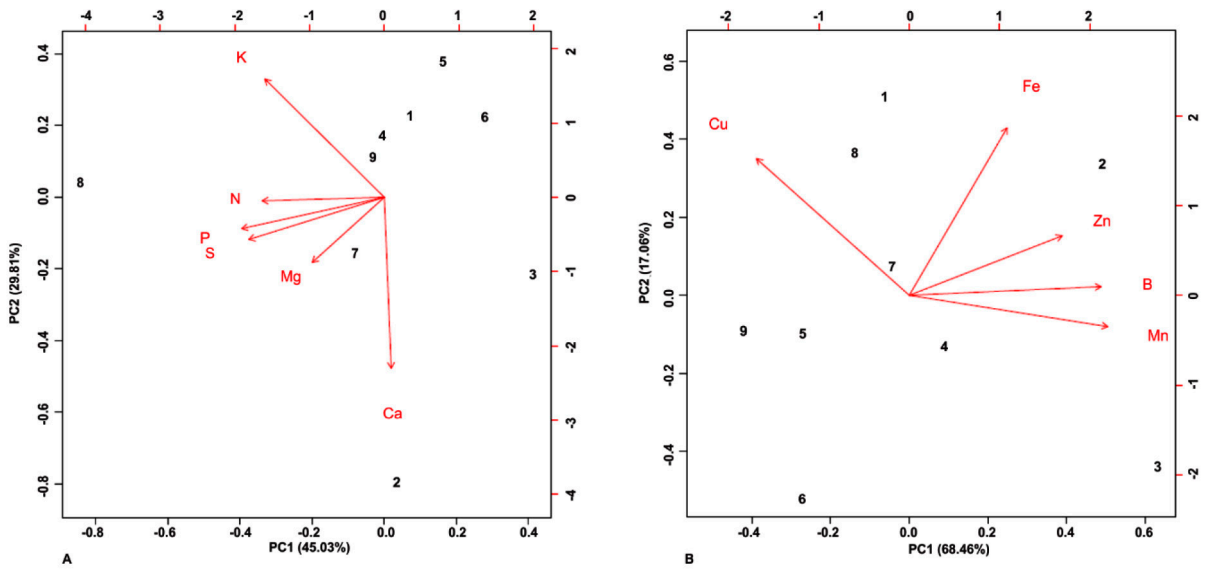

Figure 2. Graph of scores of the PC1 x PC2 referring to the macronutrients (A) and micronutrients content (B) in leaves of coffee trees collected in the winter, representative of nine groups of genotypes of *C. canephora*, cultivated at high altitude in Morrinhos - GO.

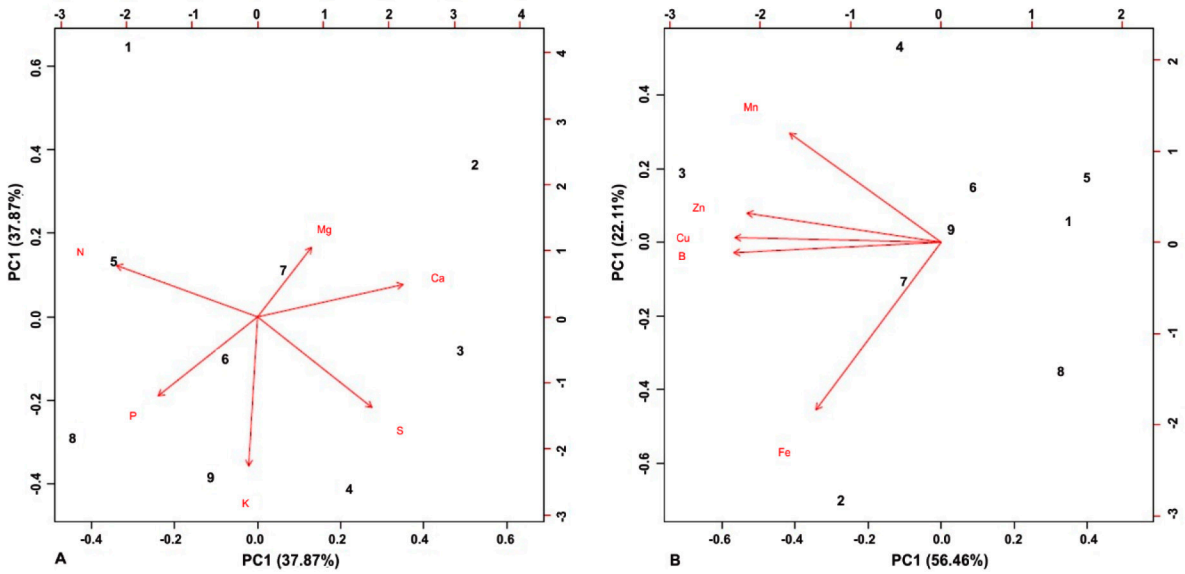

Figure 3. Graph of scores of the PC1 x PC2 referring to the macronutrients (A) and micronutrients content (B) in leaves of coffee trees collected in the summer, representative of nine groups of genotypes of *C. canephora*, cultivated at high altitude in Morrinhos - GO.

Conclusions

In conclusion, we observed difference in foliar macro and micronutrient contents between the two seasons (winter and summer) of the studied year, somewhat differently among the 28 studied genotypes of *C. canephora* cultivated at an altitude of 850 m. The nutrients N, Mg and Fe were found with higher content in the leaves harvested in the winter in comparison to the leaves harvested in the summer. Significant interactions are present between nutrient content of the leaves harvested in the winter and summer, and the analysis of principal components highlighted the nutrients that discriminated, to a greater or lower degree, the content of nutrients present in the leaves of the different coffee groups collected at 850 m altitude, in the winter and summer. The nutritional diagnosis should consider the group of genotypes and the crop season.

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