COFFEE SCIENCE
ISSN: 1984-3909

eISSN: 1984-3909
Coffee Science, e182164, 2023

Doi: https://doi.org/10.25186/.v18i.2164

Caffeine and chlorogenic acid content of *Coffea canephora* cultivars in different environments

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Received in August 21, 2023 and approved in December 11, 2023

ABSTRACT

Coffee plants of the *Coffea canephora* species are currently grown in tropical regions throughout the world, and both higher yield efficiency and higher beverage quality are important considerations. The aim of this study is to characterize the content of caffeine and chlorogenic acids of *C. canephora* cultivars in different environments. According to the maturation cycle of each clone, samples of cherry coffee were collected from ten cultivars evaluated in the environments of Porto Velho, RO; Ouro Preto do Oeste, RO; and Manaus, AM in Brazil. These environments with contrasting characteristics represent most of the coffee fields established in the Af and Aw climate types in *Latossolos Vermelhos* and *Latossolos Amarelos* typical of the Western Amazon. The results were analyzed considering the factorial design to quantify the effects of genotypes, of environments, and of the genotype × environment interaction (GE) on the caffeine and chlorogenic acid content. Despite the significant genetic-environment (GE) effects, the caffeine and chlorogenic acid content primarily exhibited genetic control. In comparison to caffeine content, chlorogenic acid content showed a stronger environmental influence, resulting in more variations in genotype performance across different environments. The significant positive association between the caffeine content and chlorogenic acid content favors the selection of plants that simultaneously have higher or lower content of both traits. The dispersion of the first two principal components, linked with reference points, enabled the identification of genotype performance across all environments in a single analysis. The selection of clone BRS3210 results in a gain from selection of 14.99% in caffeine content, while cultivation of clone BRS3193 yields a gain from selection of 10.81% in chlorogenic acid content. The selection of clone BRS2299 results in a reduction of 21.85% in caffeine content and of 9.15% in chlorogenic acid content.

Key words: Conilon; robusta; western Amazon; genotype × environment interaction.

1 INTRODUCTION

Grown in tropical regions throughout the world, the *Coffea canephora* coffee plant is characterized by its high yield potential and good adaptation to tropical climate regions (Ferrão et al., 2023; Akpertey et al., 2022; Partelli et al., 2022). In the past, when it was considered a product of inferior quality, this coffee plant was already grown with the lowest possible cost of production, which contributed to low quality coffee, with characteristics that were not valued or that could not be recognized by consumers (Filete et al., 2022).

The high yield potential and the increasing coffee value have contributed to the adoption of technologies through effective management, harvesting, and post-harvest practices (Rocha et al., 2021; Ferrão et al., 2021). As of 2010, the protocol for evaluation of beverage quality (UCDA, 2010) has allowed growers and consumers to recognize the characteristics of this beverage, which is differentiated by its lower sweetness, greater body, and higher caffeine and chlorogenic acid content (Viencz et al., 2023).

Caffeine is an alkaloid naturally synthesized by this coffee plant in its leaves, fruit, and beans; it contributes to notes of bitterness in the beverage and is known for its stimulant effect on the central nervous system. Caffeine is known to have various effects on metabolism, including increasing alertness and cognitive performance, elevating blood pressure, and acting as a diuretic (Perrois et al., 2015).

C. canephora species have higher caffeine content compared to the *Coffea arabica*, which has caffeine content ranging from 1.00 to 1.20% (Teixeira et al., 2012). Ky et al. (2001) studied the caffeine content of 32 accessions of *C. arabica* and 40 accessions of *C. canephora* from different regions of the African continent and observed mean content of 1.22% for *C. arabica* and 2.54% for *C. canephora*. Caffeine content higher than 3.50% was observed by Viencz et al. (2023) in evaluation of 57 *C. canephora* accessions of the Robusta botanical variety.

While caffeine production arises from purine compounds, the metabolism of chlorogenic acids (CGA) is associated with the synthesis of polyphenols, which are characterized by their antioxidant action. Over the past few years, CGA consumption has been associated with a variety of health advantages, such as a decreased risk of cardiovascular disease, type 2 diabetes, and Alzheimer's disease, as well as antibacterial and anti-inflammatory effects (Farah et al., 2008). Although the metabolic pathways of these compounds are well known, the expression of these traits in different environments is much less known (Filete et al., 2022).

The change in plant response from one environment to another can be investigated considering the effects of the genotype × environment interaction (GE). Significant effects of the GE interaction are the result of the non-additive relation of the genotype and environment effects that cause change in genotype performance across locations (Cruz et al., 2021). Altitude, temperature, and soil fertility are factors that affect the differential performance of the plants (Mori et al., 2018).

In order to expand the options available for coffee cultivation and enhance genetic diversity in the Western Amazon region, Embrapa has undertaken efforts to develop new cultivars (Rocha et al., 2021). These cultivars have high yield potential and agronomic traits that characterize the Conilon and Robusta botanical varieties, as documented by Teixeira et al. (2020).

In this context, this study aimed to assess how different *C. canephora* cultivars interact with different environments, considering the caffeine and chlorogenic acid content.

2 MATERIAL AND METHODS

2.1 Field trials

Three clonal competition trials were set up in the following locations in Brazil: Manaus, AM (2°39'09" S and 60°03'15" W), Porto Velho, RO (8°48'05.5" S and 63°51'02.7" W), and Ouro Preto do Oeste, RO (10°43'55.3" S and 62°15'23.2" W) (Lourenco et al., 2022). These environments are at altitudes of 97, 88, and 245 meters above sea level, respectively. Table 1 presents the soil chemical properties in the 0–10 and 20–40 depth layers.

2.2 Experimental design

To quantify the effects of genotypes, environments, and the genotype × environment interaction, a completely randomized experimental design was used in a factorial arrangement, with three replications (Cruz, 2013). This study evaluated a set of 10 cultivars specifically selected for cultivation in diverse regions of the Western Amazon (Table 2). These cultivars, distinguished by the prefix BRS, are organized into three distinct compatibility groups and display different maturation cycles, namely, early, medium, and late, as documented by Rocha et al. (2021) and Moraes et al. (2018).

The genotypes were harvested when approximately 70% of the fruit reached the red-cherry stage, considering the maturation cycles classified as early (270-290 days to maturation), medium (approximately 300 days to maturation), and late (>315 days to maturation). Samples of washed cherry coffee were then separately collected for each genotype and they underwent a natural drying process on raised drying beds for 12 to 15 days until the moisture content approached 12%. Moisture levels were assessed using a Gehaka (G600) grain moisture meter.

We interpreted stability and adaptability using the centroid method, which takes into account the maximum and minimum performance across all environments in a single analysis. Vector data served as references for the "ideal" minimum (min), medium (med), and maximum (max) performance of the genotypes in both favorable (f) and unfavorable (uf) environments. Subsequently, the genotypes were categorized based on the Euclidean distance between each genotype and the known reference of response (centroids), as described by Rocha et al. (2005) Equation (1):

$$D_{ik} = \sqrt{\sum_{j=1}^{n}\left(x_{ij} - c_{jk}\right)^2} \quad (1)$$

where D_{ik} is the Euclidian distance from the ith genotype to the kth centroid (k=1,2,...n), x_{ij} is the performance of the ith genotype in the jth environment, and c_{jk} is the performance of the kth centroid in the jth environment. Based on these distances (D_{ik}), the genotypes were classified as follows: I = high overall adaptability: max(f) and max(uf), II = adaptability specific to favorable environments: max(f) and min(uf), III = adaptability specific to unfavorable environments: min(f) and max(uf), IV = low adaptability:min(f) and min(uf), V = medium overall adaptability: med(f) and med(uf), VI = adaptability specific to favorable environments: max(f) and med(uf), and VII = adaptability specific to unfavorable environments: med(f) and

Table 1: Chemical properties at the 0–10 and 20–40 depth layers of soil in clonal competition trials conducted in three contrasting environments of the Western Amazon. Soil analyses were conducted in the year 2019, at the time of the experiment installation.

Environment	Depth Layer	pH	P	K	Ca	Mg	Al+H	Al	OM	V
	(cm)		mg dm^{-3}	cmolc dm^{-3}					g kg^{-1}	%
Manaus, AM (E1)	0–20	4.9	18.9	1.39	1.9	0.6	2.7	0.13	42	48
	20–40	4.6	40.6	1.55	1.2	0.3	2.5	0.2	36	38
Porto Velho, RO (E2)	0–20	5.4	2	0.09	1.48	1.02	13.53	0.87	51	16
	20–40	4.9	2	0.05	0.39	0.37	13.37	1.65	41	6
Ouro Preto do Oeste, RO (E3)	0–20	5.2	15	0.23	2.42	0.66	4.95	0.1	18	40
	20–40	5.4	8	0.32	2.71	0.88	5.94	0	17	40

pH in water (1:2.5); OM: organic material, by wet digestion; P and K by the Mehlich I method; exchangeable Ca, Mg, and Al; OM using 1 mol KC.

Table 2: List of cultivars evaluated in clonal competition trials in three environments of the Western Amazon: Manaus, AM (E1); Porto Velho, RO (E2); and Ouro Preto do Oeste, RO (E3).

Cultivar	Maturation cycle	Compatibility group	Genealogy
BRS 1216	Medium	I	EMCAPA03 × IAC1675
BRS 2299	Medium	II	Open pollination
BRS 2314	Late	II	EMCAPA03 × IAC640
BRS 2336	Late	II	Open pollination
BRS 2357	Late	II	Open pollination
BRS 3137	Early	III	Open pollination
BRS 3193	Early	III	Open pollination
BRS 3210	Medium	III	EMCAPA03 × IAC2258
BRS 3213	Medium	III	EMCAPA03 × IAC2258
BRS 3220	Medium	III	EMCAPA03 × IAC1675

Cultivars preceded by the prefix BRS were registered in MAPA/RNC (2019) (Teixeira et al., 2020). IAC: Instituto Agronômico de Campinas, EMCAPA: Empresa Capixaba de Pesquisa Agrícola.

max(uf). We also considered the Euclidean distance between the genotypes and the ideal reference plants, which represent superior performance across all environments (Lin; Binns, 1988).

Genotypic values served as the basis for quantifying the selection gain (SG) of genotypes with varying caffeine content. Estimates of SG took into account the selection differential and the genotypic determination coefficient within each and across all environments (Cruz et al., 2021) Equation (2):

$$SG = SD \,.\, H^2 \quad (2)$$

where SG refers to the selection gain, *SD* refers to the selection differential, H^2 refers to the genotypic coefficient of determination. The GENES software (Cruz, 2013) was used in statistical analyses.

2.3 Evaluations of caffeine and chlorogenic acid content

Chromatographic analyses were conducted in liquid phase chromatography in the Alliance e2695 model (Waters) with photodiode array detector model 2998 (Waters) with quantification in 290 nm. Separation occurred in reversed phase using two columns in series, Hypersil BDS C_{18} (100 × 4.6 mm; 2.4 µm) and Hypersil BDS C_{18} (50 × 4.6 mm; 2.4 µm) (Thermo). Elution was carried out in gradient mode at 1 ml / min of A – 0.15% phosphoric acid (Sigma) in water and B – acetonitrile (Tedia). The gradient had an initial condition of 95% A and 5% B. The concentrations of the mobile phases over time were 92% A and 8% B in 8 minutes, 88% A and 12% B in 12 minutes, 80% A and 20% B in 18 minutes, 70% A and 30% B in 25 minutes. and 40% A and 60% B in 25.1 minutes. They remained up to 25.9 minutes and returned to the initial condition of 95% A and 5% B at 26 minutes, with equilibrium until 28 minutes. The chromatographic columns were kept at 35 °C and the injection volume was 3 µL. External analytical curves were prepared of the following substances: neochlorogenic acid (Sigma) ranging from 0.0078 to 0.5000 mg/mL, chlorogenic acid (Sigma) ranging from 0.1312 to 4.2000 mg/mL, caffeine (Sigma) ranging from 0.0737 to 2.3000 mg/mL, caffeic acid (Sigma) ranging from 0.0062 to 0.2000 mg/mL, 4-O-caffeoylquinic (Sigma) ranging from 0.02968 to 0.9500 mg/mL, and 4-O-feruloylquinic (Sigma) ranging from 0.0875 to 1.4000 mg/mL. The acids 4,5-Di-O-caffeoylquinic (Sigma), 3,6-Di-caffeoylquinic (Sigma), and 3,4-Di-O-caffeoylquinic (Sigma).

The samples of green coffee beans were ground in a micro-hammermill (model TE 600 – Tecnal). After grinding, approximately 0.2 g of the samples were extracted with 4 mL of methanol:water solution (50:50, v/v, pH 2), according to Nascimento et al. (2017). The extract was shaken for an hour at ambient temperature and then centrifuged at 6,000 rpm for 10 minutes. The supernatant was collected and a new extraction was made from the residue with 4 mL of acetone:water solution (70:30, v/v), repeating the previous steps of shaking and centrifuging. The supernatant of this second extraction was also collected and mixed with the first supernatant obtained. The mixture of the extracts was then placed in a vortex for 30 seconds and transferred to the vial of the chromatography injector (Nascimento et al., 2017).

3 RESULTS

The effects of genotypes, of environments, and of the GE interaction were significant both for caffeine and for evaluations of chlorogenic acids. The significance of the effect of the GE interaction indicates that the cultivars evaluated had differentiated performance in the environments (Table 3). The estimates of the experimental coefficient of variation can be

considered low both for caffeine and for chlorogenic acids, indicating good experimental accuracy.

Table 3: Summary of analysis of variance of the caffeine and chlorogenic acid content of ten cultivars evaluated in three environments of the Western Amazon: Manaus, AM (E1); Porto Velho, RO (E2); and Ouro Preto do Oeste, RO (E3).

SV	DF	Caffeine	Chlorogenic acids
		F	F
Genotypes	9	13.32**	6.45**
Environments	2	193.86**	598.43**
G × E	18	40.95**	15.11**
Residual	60		
Total	89		
Overall mean		2.51	8.92
Mean E1		2.42b	7.97a
Mean E2		2.47b	9.36b
Mean E3		2.64a	9.43b
$CV_{e(\%)}$		1.78	2.06
$CV_{g(\%)}$		13.35	6.24
H^2		92.49	84.50
CV_g/Cv_e		7.50	3.03

**: significant at 1% probability, SV: source of variation, DF: degrees of freedom, SS: sum of squares, MS: mean square, F: value of the F statistic of the variance analysis. Means of environments followed by the same lowercase letters do not differ according to the Scott-Knott test at 5% probability, CV_e: coefficient of experimental variation, CV_g: coefficient of genetic variation, H^2: coefficient of genotypic determination.

In relation to caffeine content, the environment of Ouro Preto do Oeste, RO, grouped separately from the environments of Manaus, AM, and of Porto Velho, RO (Table 3). For chlorogenic acid content, the environments grouped in a different manner, since the environment of Manaus, AM, grouped separately from the environments of Ouro Preto do Oeste, RO, and Porto Velho, RO. The genotypes had both higher caffeine content and chlorogenic acid content in the favorable environment of Ouro Preto do Oeste, RO (Table 3).

The relation between the coefficient of genetic variation and the coefficient of experimental variation greater than one indicates potential for obtaining gains from selection for higher or lower content. Although the estimates for caffeine content and chlorogenic acid content were greater than one, this relation was higher for caffeine (CV_g/Cv_e = 7.50) than for chlorogenic acids (CV_g/Cv_e = 3.03) (Table 3).

The coefficient of genotypic determination (H^2) refers to the relation between the estimates of genotypic variance and phenotypic variance when the effects are fixed, and it is interpreted to quantify the predominance of variance of a genetic nature in expression of these traits. Although the genotypes exhibited changes in their performance from one environment to another, the estimates of H^2 greater than 80 indicate greater importance of the effect of genotypes for expression of these traits. The magnitude of this estimate for caffeine content was also superior than the magnitude for expression of chlorogenic acids (Table 3).

The order of the genotypes according to their proximity to an ideal plant of maximum performance (Pi) shows that the clones BRS3210, BRS2314, and BRS3193 had the highest caffeine content in all the environments, while the clones BRS 3137, BRS3220, and BRS2299 had the lowest content (Table 4). This same ordering for chlorogenic acids shows that the clones BRS 3193, BRS2314, and BRS 3210 exhibited higher content; and the clones BRS 3137, BRS 1216, and BRS 2299 lower content in all the environments. The Scott Knott test for grouping means placed the genotypes in six different groups according to caffeine content and five groups according to chlorogenic acid content (Table 4).

The scatterplot of the mean performance of the clones in the environments, interpreted as their genotypic value, indicates a positive association between the evaluations of caffeine and of chlorogenic acids (Figure 1). This indicates a trend of genotypes of higher caffeine content to also have higher chlorogenic acid content (r = 0.79**) (Figure 1).

In occurrence of the GE interaction, the performance of the genotypes should be considered separately in each environment. The scattering of the first two principal components with maximum and minimum reference points in favorable and unfavorable environments, represent the responses of the genotypes in all the environments (Rocha et al., 2005). The genotypes BRS3210 and BRS2314 performed in a way similar to the ideotype that represents the ideal plant of maximum caffeine content in all the environments. The cultivars BRS3213, BRS2336, and BRS3193, in turn, had better performance in the favorable environments of Ouro Preto do Oeste and Porto Velho, RO. The cultivars BRS1216, BRS3137, BRS2357, and BRS3220 had performance similar to the mean of all the environments, while the cultivar BRS2299 had performance similar to the ideotype that represents the plant of lowest caffeine content in all the environments (Figure 2A).

Performance in relation to chlorogenic acid content was also interpreted considering the performance of the plants in all the environments in relation to the ideal references of known response (Figure 2B). The cultivars BRS3193 and BRS2314 had performance similar to the ideotype that represents the ideal plant of maximum content of chlorogenic acids in all the environments. The cultivars BRS3213 and BRS2357, in turn, had better performance in the favorable environments of Ouro Preto do Oeste and Porto Velho, RO. The cultivars BRS2336, BRS3220, BRS3210, and BRS1216 had performance similar to the mean value of all the

environments, while cultivars BRS2299 and BRS 3137 had performance similar to the ideotype that represents the plant with lower chlorogenic acid content. The original variation of the data explained by the principal components was greater than 70% for both caffeine content and chlorogenic acid content (Figure 2).

Table 4: Mean caffeine and chlorogenic acid content of ten cultivars evaluated in three environments of the Western Amazon: Manaus, AM (E1); Porto Velho, RO (E2); and Ouro Preto do Oeste, RO (E3). The means were grouped using the Scott Knott test with a 5% significance level.

Caffeine content (%)					
Cultivar	E1	E2	E3	Mean	Pi
BRS 1216	2.31Ce	2.58Bc	2.69Ad	2.53	6
BRS 2299	1.77Bg	1.99Ae	1.94Ag	1.90	10
BRS 2314	2.95Aa	2.32Ba	2.95Ab	2.74	2
BRS 2336	2.68Ac	2.57Bc	2.74Ad	2.66	5
BRS 2357	2.25Ae	2.31Ad	2.30Af	2.29	7
BRS 3137	2.25Be	1.86Cf	2.57Ae	2.23	8
BRS 3193	2.79Ab	2.74Ab	2.77Ac	2.77	3
BRS 3210	2.80Bb	2.85Ba	3.05Aa	2.90	1
BRS 3213	2.52Cd	2.91Aa	2.82Bc	2.75	4
BRS 3220	1.90Cf	2.04Be	2.59Ae	2.18	9
Chlorogenic acid content (%)					
Cultivar	E1	E2	E3	Mean	Pi
BRS 1216	7.04Ce	9.29Ad	8.83Bd	8.39	9
BRS 2299	7.06Be	8.56Ae	8.48Ae	8.03	10
BRS 2314	9.15Bb	9.40Bc	10.26Aa	9.61	2
BRS 2336	7.53Bd	9.62Ac	9.41Ac	8.85	6
BRS 2357	7.63Bd	9.85Ab	9.59Ac	9.02	5
BRS 3137	7.62Bd	8.79Ae	8.88Ad	8.43	8
BRS 3193	9.42Ba	10.16Aa	10.33Aa	9.97	1
BRS 3210	9.06Bb	9.20Bd	9.50Ac	9.25	3
BRS 3213	7.99Cc	9.65Bc	9.96Ab	9.20	4
BRS 3220	7.23Be	9.06Ad	9.08Ad	8.46	7

Mean values followed by the same uppercase letters in the horizontal direction do not differ statistically from each other. Mean values followed by the same lowercase letters in the vertical direction do not differ statistically from each other by Scott-Knott test ($P < 0,05$). Pi: Lin & Binns, 1988.

From the estimates of genotypic variance, the gains from selection were interpreted for higher and lower content of caffeine and of chlorogenic acids. Cultivation of clone BRS3210 selected for higher caffeine content is associated with a gain from selection of 14.99% in relation to the mean value of the crop, with a new mean value of cultivation of 2.90% in all the environments. This clone had the highest caffeine content of 3.05% in the favorable environment of Ouro Preto do Oeste (Table 5). Cultivation of clone BRS 3193, in turn, resulted in a gain of 10.81% in the chlorogenic acid content, associated with a new mean value of 9.97. Cultivation of clone BRS2299, selected for lower content of caffeine and chlorogenic acids, is associated with reductions in the mean value of 21.85% and of 9.15%, respectively (Table 5).

4 DISCUSSION

Although quantification of caffeine and chlorogenic acid content was the object of other studies (Tsai; Jioe, 2021), few studies have considered the expression of these traits in different environments. The GE interaction can be understood as the change in the performance of plants from one environment to another, which can be classified as being of a simple nature, when the change in plant performance does not result in changes in classification, or of a complex nature, when the change in performance results in changes in classification of the genotypes (Cruz et al., 2021). Although both traits showed significant GE effects, the chlorogenic acid content showed greater changes in the classification of the genotypes across the environments (Tables 3 and 4). This greater change can be observed in the greater contrasts among mean values of different environments and in the greater scattering observed in the first two principal components (Table 3, Figure 2).

In the Western Amazon, coffee growing can be genuinely sustainable and combined with preservation of the forest. The region in which coffee is grown in the Western Amazon is in climate types Af and Aw, characterized as typical tropical climates, hot and humid, with low annual thermal amplitude and expressive daily thermal amplitude from May to September (Alvares et al., 2013). The Porto Velho, RO, and Manaus, AM, environments are also characterized by acid soils of low natural fertility, whereas the environment of Ouro Preto do Oeste, RO, is characterized by soils of higher fertility and Aw climate type. It is characteristic of the coffee fields in the south of this region, with the geographical designation of "*Matas de Rondônia*" (Rondônia Forests) (Instituto Nacional de Propriedade Industrial - INPI, 2022).

The higher base saturation of the environment of Ouro Preto do Oeste, RO (V=40%) and Manaus, AM (V=48%), which favors plant growth in comparison with the environment of Porto Velho, RO (V=16%), also favored the higher caffeine and chlorogenic acid content values observed in this environment. Although it is less than the contrast of 32% observed between locations of low and high altitudes (Sridevi; Giridhar, 2014), contrasts of 8.33% were observed for caffeine content and of 15.48% for chlorogenic acid content among the environments evaluated (Table 3).

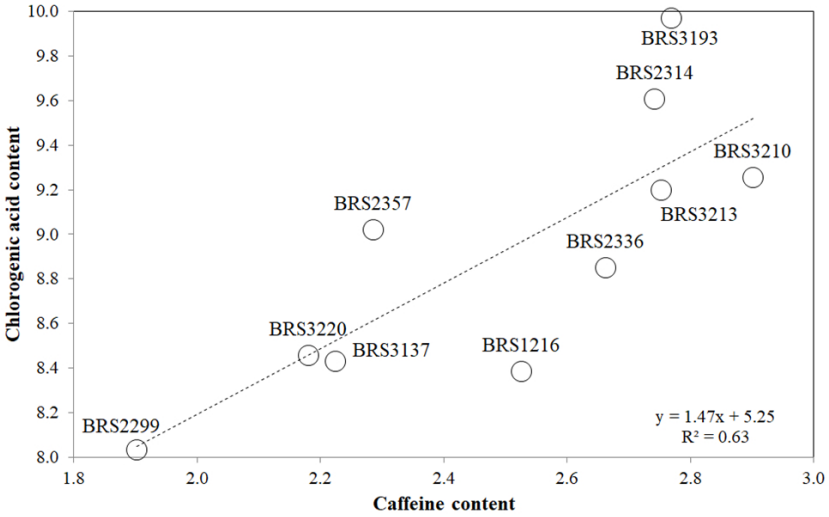

Figure 1: Scattering of the genotypic values of caffeine and chlorogenic acid content of ten *Coffea canephora* cultivars grown in different environments of the Western Amazon (Manaus, AM; Porto Velho, RO; and Ouro Preto do Oeste, RO).

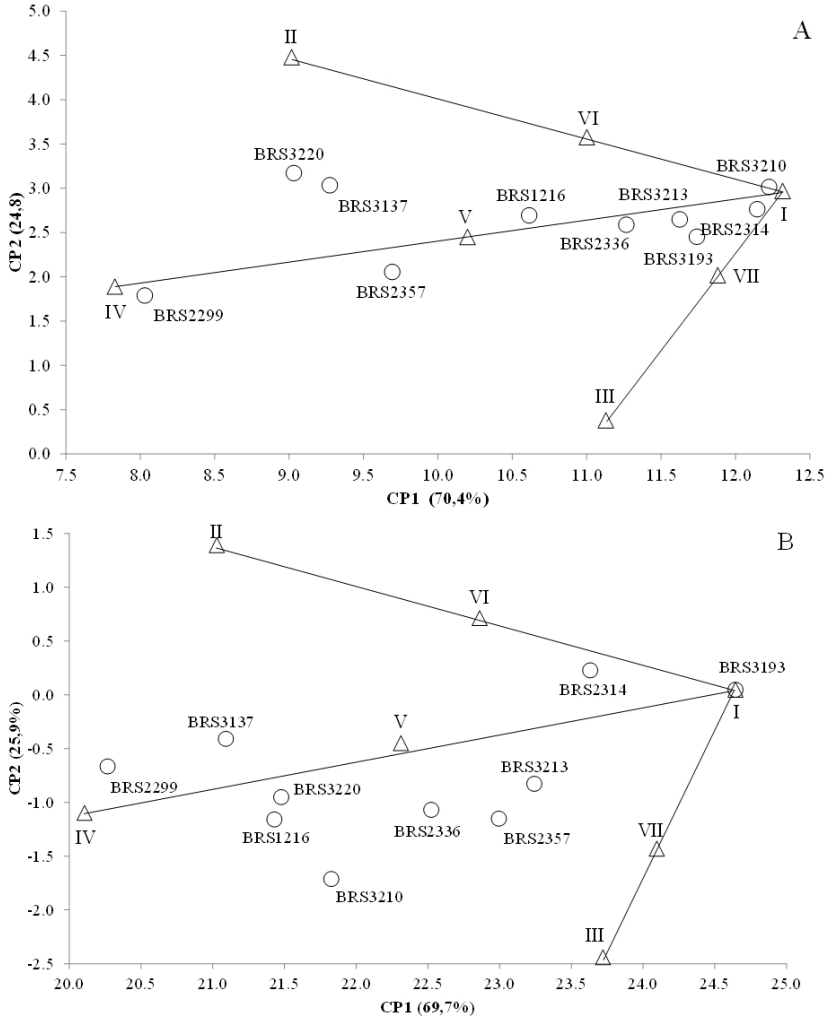

Figure 2: Scatterplot of the first two principal components of caffeine content (A) and of chlorogenic acid content (B) of ten *Coffea canephora* cultivars evaluated in three environments of the Western Amazon. The Roman numerals indicate points of reference of wide and specific adaptability to the environments. I: wide adaptability, II and VI: specific adaptability to favorable environments, III and VII: specific adaptability to unfavorable environments, V: mean performance in all the environments, IV: low overall adaptability.

Table 5: Estimates of gain from selection (GS) of the genotypes with higher caffeine content and genotypes with lower caffeine content in three environments of the Western Amazon: Manaus, AM (E1); Porto Velho, RO (E2); and Ouro Preto do Oeste, RO (E3).

Selection for higher caffeine content (BRS3210)			
Environment	GS	GS%	New mean
E1	0.35	14.29	2.80
E2	0.40	16.45	2.85
E3	0.38	14.31	3.05
All environments	0.37	14.99	2.90
Selection for lower caffeine content (BRS2299)			
Environment	GS	GS%	New mean
E1	-0.60	-24.81	1.77
E2	-0.39	-16.15	1.99
E3	-0.64	-24.34	1.94
All environments	-0.54	-21.85	1.90
Selection for higher chlorogenic acid content (BRS3193)			
Environment	GS	GS%	New mean
E1	1.33	16.70	9.42
E2	0.74	7.86	10.16
E3	0.83	8.76	10.33
All environments	0.96	10.81	9.97
Selection for lower chlorogenic acid content (BRS2299)			
Environment	GS	GS%	New mean
E1	-0.84	-10.53	7.06
E2	-0.74	-7.87	8.56
E3	-0.87	-9.25	8.48
All environments	-0.82	-9.15	8.03

GS: gain from selection in the original unit, GS%: gain from selection in percentage.

As they do not represent a sample coming from a population, the genotypes evaluated in this study were interpreted as fixed effects, and the conclusions referred only to these cultivars studied. The genotypes grown in the Western Amazon are characterized by their hybrid nature, resulting from pollination between plants of the Conilon and Robusta botanical varieties (Espindula et al., 2022).

The lower estimates of the coefficient of genotypic determination indicate that the chlorogenic acid content was more affected by environmental factors than caffeine content was (Table 2). The greater genetic determination of the caffeine content may be associated with the smaller number of genes and lower complexity of the metabolic pathways of this metabolite in comparison with chlorogenic acids (Perrois et al., 2015). High estimates of the coefficient of genotypic determination (79 to 95%) for caffeine content were also observed by Teixeira et al. (2012) in evaluation of 75 *C. arabica* genotypes.

Estimates of the coefficient of experimental variation ranging from 1.78 to 2.06% indicate that the experiment was well conducted, and they can be considered low (Cruz et al., 2021). Similar estimates were also observed by Lemos et al. (2020), who observed estimates of the coefficient of variation of 2.54% for caffeine and 3.09% for chlorogenic acids.

The performance of the clones in the environments was interpreted considering their proximity to ideal plants that express maximum content of caffeine and of chlorogenic acids in all the environments (Pi). The clones BRS3210, BRS 3193, and BRS 2314 had high content of both caffeine and of chlorogenic acids in the environments evaluated, while the clones BRS3137 and BRS 2299 had lower content for both traits.

Considered individually, the magnitude and the direction of the correlations may make selection based on a single trait result in undesired changes in other yield components. These changes are called correlated responses, and their direction should be considered in plant selection (Cruz et al., 2021). The positive and significant association between caffeine content and chlorogenic acid content observed in this study favors the selection of plants that simultaneously have higher or lower content for these traits (r = 0.79*) (Figure 2). Positive association between caffeine and chlorogenic acid content was also observed by Viencz et al. (2023), who observed correlation estimates of 0.51 in evaluation of 57 accessions of the species *C. canephora*.

Although some studies consider the effect of different environments on expression of these traits, results that simultaneously considered the effects of genotypes and of environments were not found. (Sridevi et al., 2014) evaluated the caffeine content in coffee plants of the *C. canephora* species grown at altitudes from 3000 to 3700 meters and observed a reduction of 32% in caffeine content in the plants grown at higher altitudes. From a larger number of genotypes evaluated in a single environment, Viencz et al. (2023) observed a variation from 1.63–3.57% for caffeine and from 3.93–6.37% for chlorogenic acids.

The scattering of the first two principal components associated with reference points was used to interpret the performance of all genotypes in all the environments in a single analysis (Rocha et al., 2005). In this context, adaptability can be understood as the ability of the genotypes to respond to improved environmental conditions, and stability as the predictability of their response in different environments (Nascimento et al., 2009).

In addition to the genotypes that exhibited specific adaptation, other genotypes were observed that had higher and lower content in all the environments evaluated (Figure 3). While clones BRS 3210 and BRS 3193 had the highest caffeine and chlorogenic acid content in all the environments, clone BRS2299 had the lowest content for both traits. These

estimates indicate the possibility of obtaining gains from selection, and plants can be selected for growing considering higher or lower caffeine and chlorogenic acid content simultaneously.

Individual characterization of these cultivars allows them to be grown with flexibility in the composition of crop fields, according to the grower's preference. In addition to higher caffeine and chlorogenic acid content, the clone BRS3210 also stands out for its uniform maturation, while the clone BRS2314 has been grown for its greater potential for producing a quality beverage (Rocha et al., 2021).

The selection of superior materials, plant nutrition, thinning, irrigation, and pest control are activities that are part of the routine of coffee growers of the region of geographical indication called "Matas de Rondônia" (INPI, 2022). Along with yield increase, caffeine and chlorogenic acid content may be important traits that reveal expression of the genetic nature of each genotype in different growing environments.

5 CONCLUSIONS

The caffeine and chlorogenic acid content show predominantly genetic control, in spite of the significant effects of the genotype × environment interaction. Compared to the caffeine content, the chlorogenic acid content show greater environmental effects and greater changes in the performance of the genotypes evaluated in different environments. The positive association between caffeine content and chlorogenic acid content favors the selection of plants that simultaneously exhibit higher or lower content of caffeine and of chlorogenic acids. Cultivation of the clone BRS 3210 results in an increase of 14.99% in caffeine content and of 10.81% in chlorogenic acid content. In contrast, cultivation of the clone BRS2299 results in a reduction of 21.85% in caffeine content and reduction of 9.15% in chlorogenic acid content.

6 ACKNOWLEDGEMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) for the scholarship granted, and the Secretaria Estadual de Desenvolvimento Econômico, government of the state of Rondônia and Consórcio Pesquisa Café for funding.

RBR, EAA, and ALT authored the manuscript, carried out field experiments, and conducted statistical analyses. HCT performed field experiments. HRB, RA, SP, and MCPAS conducted laboratory analyses, reviewed the manuscript, and approved the final version of the work.

7 AUTHORS' CONTRIBUTION

RBR, EAA, and ALT authored the manuscript, carried out field experiments, and conducted statistical analyses. HCT performed field experiments. HRB, RA, SP, and MCPAS conducted laboratory analyses, reviewed the manuscript, and approved the final version of the work.

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