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*Scientific Article*

# Analysis of bioactive compounds, organic acids, and genetic parameters of ten amazonian robusta cultivars

# Análise de compostos bioativos, ácidos orgânicos e parâmetros genéticos de dez cultivares de robustas amazônicos

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**ABSTRACT** - *Coffea canephora* beans are used for various industrial purposes, among which the use as soluble coffees stands out for producing beverages in blends with *Coffea arabica*. Due to the increase in demand, EMBRAPA launched ten monoclonal *C. canephora* cultivars, named Amazonian Robustas, adapted to the growing conditions of the Brazilian Amazon. However, the chemical composition of the beans of these cultivars is still little known. The present study aimed to estimate genetic parameters for the evaluated characteristics and determine the levels of bioactive compounds and organic acids in ten *C. canephora* cultivars. The experiment was set in Manaus, Amazonas, consisting of plants from the cultivars BRS 1216, BRS 2299, BRS 2314, BRS 2336, BRS 2357, BRS 3137, BRS 3193, BRS3210, BRS 3213, and BRS 3220. The cultivars were characterized according to the profile of bioactive compounds and organic acids. Analysis of variance, mean test, and genetic parameters (genetic, environmental, and phenotypic variance and heritability) were conducted. The heritability of characters was considered from intermediate 63.76% (trigonelline) to high 88.44% (caffeine). Of the compounds studied, trigonelline contents ranged from 0.54 to 0.78 g.100g$^{-1}$, chlorogenic acids from 3.77 to 5.31 g.100g$^{-1}$, caffeine from 2.31 to 4.13 g.100g$^{-1}$, and citric acid from 0.76 to 1.28 g.100g$^{-1}$. It was observed that there is genetic variability among the cultivars for the compounds studied, and the cultivars can be used in breeding programs for the development of new cultivars.

**Keywords**: *Coffea canephora.* Caffeine. Trigonelline. Chlorogenic acids. Plant genetics.

**RESUMO** - Grãos da espécie *Coffea canephora* são utilizados para diversos fins industriais, dentre os quais se destacam o uso como cafés solúveis e para produção de bebidas em blends com a espécie *Coffea arabica*. Devido ao aumento da demanda, a EMBRAPA lançou dez cultivares monoclonais de cafeeiros *C. canephora*, com nome Robustas Amazônicos, adaptados às condições de cultivo da Amazônia brasileira, entretanto, a composição química dos grãos destes clones ainda é pouco conhecida. O objetivo do estudo foi estimar parâmetros genéticos para as características avaliadas e determinar os teores de compostos bioativos e de ácidos orgânicos nas dez cultivares. A unidade experimental foi instalada em Manaus, Amazonas, constituída de plantas das cultivares BRS 1216, BRS 2299, BRS 2314, BRS 2336, BRS 2357, BRS 3137, BRS 3193, BRS3210, BRS 3213 e BRS 3220. As cultivares foram caracterizadas quanto ao perfil de bioativos e ácidos orgânicos. Foi realizada análise de variância, teste de médias e estimados parâmetros genéticos como variância genética, ambiental, fenotípica e herdabilidade. A herdabilidade dos caracteres foi considerada de mediana, 63,76%, para trigonelina, a alta, 88,44%, para cafeína. Dos compostos estudados, os teores de trigonelina variaram de 0,54 a 0,78 g.100g$^{-1}$; os de ácidos clorogênicos de 3,77 a 5,31 g.100g$^{-1}$; o de cafeína de 2,31 a 4,13 g.100g$^{-1}$ e o de ácido cítrico de 0,76 a 1,28 g.100g$^{-1}$. Observou-se que existe variabilidade genética entre as variedades para os compostos estudados e as cultivares podem ser utilizadas no melhoramento genético para desenvolvimento de novas cultivares.

**Palavras-chave**: *Coffea canephora*. Cafeína. Trigonelina. Ácidos clorogênicos. Genética de plantas.

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## INTRODUCTION

Brazil is the largest coffee producer in the world, and the most produced species are *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* Pierre ex A. Froehner (Conilon or Robusta coffee). According to Gomes and Partelli (2021), Brazilian production exceeded 67 million 60-kilogram bags in 2019-2000, comprising around 70% Arabica and 30% Conilon.

Robusta coffee, due to the sensory characteristics of neutrality in terms of sweetness, acidity, the marked aroma of roasted cereals, and a more pronounced body than Arabica coffee (PEREIRA et al., 2019), is mainly used to comprise blends in the soluble coffee industry (RIBEIRO et al., 2014). Thus, the main concerns regarding its production are just characteristics linked to environment adaptability, yield, and resistance to pests and diseases, among other factors for crop development (GOMES; PARTELLI, 2021).

However, the world's growing demand for specialty coffees has made Robusta coffee a target of several studies seeking to improve beverage quality. These studies include genetic improvement, management, harvest, and post-harvest processing methods, including fermentation, drying, storage, and roasting.

The state of Espírito Santo in Brazil has shown great aptitude in producing high-quality Robusta coffee. They have been called special Robustas (CONAB, 2017).

The Amazon region, characterized by producing unique flavor coffees, has also emerged in the search for special Robustas. This advance in coffee production is directly linked to technologies, mainly the adoption of clonal coffee production, making it possible to know beverage quality from each genetic material planted.

According to Viencz et al. (2023), the significance of studying *Coffea canephora* for the coffee industry is linked to the analysis of its chemical composition and bioactive compounds. This information is valuable for the industry as it can be utilized in developing coffee products with specific sensory profiles and potential health benefits. Moreover, the study helps underscore *Coffea canephora* as a quality coffee choice, potentially positively impacting the industry by diversifying the range of coffees in the market and potentially improving prices for producers.

Among the genetic and phenotypic parameters that can help direct the selection of the most promising coffee trees, genetic and phenotypic variances, heritability, and expected genetic progress stand out. Estimating the genetic parameters allows us to know the genetic structure and the inference of the genetic variability present in the population, providing subsidies to predict the genetic gains and the possible success of the breeding program. As for heritability, it must be as accurate as possible due to its importance in predicting the genetic improvement of a trait (FERRÃO et al., 2008).

According to the literature, in addition to different physical and sensory characteristics, Robustas differ chemically from Arabica coffees. The chemical composition of raw coffee is related to factors such as species, genotype, crop location, management, processing, and storage, and the interaction of these factors directly interferes with the beverage's characteristics. Concerning bioactive compounds such as trigonelline, chlorogenic acids, and caffeine, robustas have lower trigonelline and higher chlorogenic acid and caffeine contents than arabica coffee (FROST-MEYER; LOGOMARSINO, 2012; ZAIN; SHORI; BABA, 2017).

These compounds are essential in coffees since they actively participate in flavor formation reactions during roasting. During roasting, trigonelline contributes to coffee aroma by forming degradation products, mainly nicotinic acid. The group of chlorogenic acids, the main phenolics found in coffee, is degraded during roasting, originating pigments and aromatic volatiles. Caffeine is a relatively stable alkaloid in the thermal process and contributes to beverage bitterness (SOUZA et al., 2010).

Another group of compounds that have been closely related to coffee quality is organic acids (FARAH; LIMA, 2019). The primary organic acids in coffee are acetic, citric, lactic, malic, quinic, tartaric, and oxalic. Each acid stands out for its characteristic flavors, such as the lemon flavor of citric acid or the undesirable fermentation conferred by acetic acid (LINGLE, 2011).

Given the above, this research aimed to characterize ten Amazonian Robusta coffee cultivars for the genetic parameters (genetic, environmental, and phenotypic variance and heritability) of their characteristics regarding the content of bioactive compounds (trigonelline, chlorogenic acids, and caffeine) and organic acids.

## MATERIAL AND METHODS

The field experiment was conducted in Manaus (between the coordinates 2º38'20 "S - 2º39'10"S and 60º40'W - 60º30'W), Amazonas, Brazil. The experiment was conducted using clonal seedlings produced in the experimental field of Embrapa Rondônia, Ouro Preto do Oeste, Rondônia. Ten cultivars developed by Embrapa were evaluated, all of which were recommended for cultivation. The crop was implanted in January 2019 (Table 1).

**Table 1**. Genotypes, origen and genealogy with the registration date of 2019 in the National Cultivar Registry of the Ministry of Agriculture, Livestock, and Food Supply RNC/MAPA.

| Genotypes[1] | Origin | Genealogy |
|---|---|---|
| BRS 1216 | Controlled hybridization | Emcapa 03 x Robusta 1675 |
| BRS 2299 | Active Germplasm Bank | Open pollination |
| BRS 2314 | Controlled hybridization | Emcapa 03 x Robusta 640 |
| BRS 2336 | Active Germplasm Bank | Open pollination |
| BRS 2357 | Active Germplasm Bank | Open pollination |
| BRS 3137 | Active Germplasm Bank | Open pollination |
| BRS 3193 | Active Germplasm Bank | Open pollination |
| BRS 3210 | Controlled hybridization | Emcapa 03xRobusta 2258 |
| BRS 3213 | Controlled hybridization | Emcapa 03xRobusta 2258 |
| BRS 3220 | Controlled hybridization | Emcapa 03xRobusta 1675 |

[1]The genotypes were registered individually as a monoclonal cultivar.
Source: Espindula et al. (2019).

Before planting, the soil chemical analysis was conducted, and the results were: pH ($H_2O$): 5.04; organic matter 288.93 $g.kg^{-1}$; phosphorus 177 $mg.dm^{-3}$; potassium 399 $mg.dm^{-3}$; sodium 69 $mg.dm^{-3}$; calcium 1.50 $cmol_c.dm^{-3}$, magnesium 1.0 $cmol_c.dm^{-3}$; potential acidity 5.50 $cmol_c.dm^{-3}$; cation-exchange capacity 9.89 $cmol_c.dm^{-3}$; base saturation 45.0%. Based on the chemical analysis of the soil, liming was conducted two months before planting to increase base saturation to 70%. Tanned chicken manure (1.0 $kg.m^{-2}$) was used as fertilization in the seedling transplanting. Topdressing fertilizations were conducted 10 and 20 days after transplanting seedlings, with 0.1% urea in foliar fertilization in the irrigation water. The test was conducted according to the technical recommendations for the crop (MARCOLAN et al., 2009).

The experiment was set in a randomized block design with ten treatments (cultivars) and three replications. Each replication consisted of eight plants. The first harvest was conducted between May and June 2021, respecting the maturation cycle of each cultivar.

Coffee samples of each cultivar were randomly obtained after harvesting the mixture of beans from the eight plants that comprise each replication for each cultivar. After harvesting, 3 kg of fruits of each replication of each genotype were sent for drying and processing at the Experimental Farm – FAEXP, of the Federal University of Amazonas – UFAM.

After drying and processing, the samples were sent to the UFLA Agricultural Product Processing Laboratory, where they were prepared for analysis, as described below.

First, the samples were separated by size and defects (broken beans, floaters, outer shell, inner shell, and other similar things) using sieves. For the chemical analyses, the raw coffee samples were ground in an IKA 11A mill in the presence of nitrogen and placed in an ultra-freezer for subsequent extraction.

Non-volatile compounds such as caffeine, trigonelline, and chlorogenic acid were determined by high-performance liquid chromatography (HPLC), following the methodology adapted from Vitorino et al. (2001). A calibration curve, with 10 points and concentration between 0.002 and 0.8 mg. $mL^{-1}$, was plotted for identification and quantitative analysis using caffeine, trigonelline, and 5-caffeoylquinic acid (5-CQA) as standards.

Organic acids were determined by HPLC based on the methodology described by Jham et al. (2002). Standards of citric, malic, tartaric, succinic, lactic, quinic, and acetic acids were used to identify the chromatogram peaks, compare the retention times, and calculate their concentration in the samples.

Data were submitted for analysis of variance ($p<0.05$), and the means were grouped by the Scott-Knott Clustering Algorithm ($p<0.05$) using the SISVAR statistical software (SISVAR, version 5.3). The following genetic parameters were estimated: genetic, environmental, and phenotypic variance and heritability (CRUZ; CARNEIRO; REGAZZI, 2014). Aiming to understand the relationship between treatments (ten cultivars with three replicates each) and chemical composition (caffeine, trigonelline, chlorogenic acid, and organic acids), principal component analysis (PCA) was performed using the statistical software CHEMOFACE (CHEMOFACE, version 1.64). An M x N matrix was plotted with the content of identified chemical compounds and sensory attributes for the evaluated samples.

## RESULTS AND DISCUSSION

The trigonelline content did not differ significantly among the cultivars, with concentrations ranging from 0.54g to 0.78g per 100g. Similarly, no variations were noted in chlorogenic acid levels among the cultivars, with values ranging between 3.77g and 5.31g per 100g (Table 2). However, distinctions were observed in the caffeine and citric acid levels among the cultivars, suggesting likely genetic diversity within the cultivars studied.

Caffeine levels ranged from 2.31g to 4.13g per 100g. The highest concentration was observed for cultivar BRS 2299, with 4.13g. Cultivars BRS 1216, BRS 2314, BRS 3137, BRS 3193, BRS 3210, and BRS 3213 showed intermediate values, and cultivars BRS 2336, BRS 2357, and BRS 3220 showed the lowest values (Table 2).

Regarding citric acid contents, the values ranged from 1.28g to 0.76g per 100g. Cultivars BRS 1216, BRS 3137, and BRS 3193 showed higher levels of citric acid, while the other cultivars did not differ concerning this characteristic (Table 2).

The results found in this research agree with those presented in other studies that indicate trigonelline contents between 0.6 and 1.7% in conilon coffee (SOUZA et al., 2010). Caffeine contents were also similar to those described in the literature, between 1.7 and 3.5$g.100g^{-1}$ (HEČIMOVIĆ et al., 2011). The only cultivar that presented a higher content than that found in the literature was BRS 2299. However, for total chlorogenic acids, the levels are below those described for Conilon and closer to Arabica coffee, which vary between 4 and 8$g.100g^{-1}$. As described, the chlorogenic acid levels for Conilon coffee range between 7 and 10$g.100g^{-1}$ (MONTEIRO; TRUGO, 2005).

Studies mention that trigonelline contributes to the formation of desirable aromas during coffee roasting, but it is still not possible to assess how the content of this compound acts on the other sensory attributes (MACRAE, 1985). Controversial reports on this compound are found, such as that of Farah et al. (2006), who associate higher trigonelline contents with higher sensory quality, or Fassio et al. (2016), who point out that the bioactive trigonelline, chlorogenic acid, and caffeine did not show a good relationship with sensory attributes.

Chlorogenic acid and its derivatives contribute to the final acidity but are associated with beverage astringency and bitterness, in addition to participating in the formation of pigments (VARIYAR et al., 2003; SCHENKER; ROTHGEB, 2017), being related to a lower sensory quality (FARAH et al., 2006; FASSIO et al., 2016). It is one of the reasons why Conilon coffee is always referred to as more bitter than Arabica. In the present study, the levels of chlorogenic acids were very close to those of Arabica coffees, which may indicate a potentially higher quality for these *C. canephora* cultivars.

**Table 2**. Content of bioactive compounds and citric acid per 100 g of dry coffee sample from Robusta cultivars.

| Cultivars | Trigonelline | Chlorogenic Acid | Caffeine | Citric Acid |
|---|---|---|---|---|
| | | ($g.100g^{-1}$) | | |
| BRS 1216 | 0.66±0.13a | 4.56±0.16a | 3.19±0.38b | 1.28±0.05a |
| BRS 2299 | 0.76±0.03a | 5.11±0.70a | 4.13±0.73a | 0.81±0.10b |
| BRS 2314 | 0.54±0.06a | 5.31±0.31a | 3.38±0.68b | 0.77±0.20b |
| BRS 2336 | 0.67±0.08a | 3.77±0.33a | 2.31±0.46c | 0.76±0.10b |
| BRS 2357 | 0.63±0.06a | 4.48±0.10a | 2.95±0.25c | 0.93±0.13b |
| BRS 3137 | 0.78±0.06a | 5.39±0.23a | 3.22±0.27b | 1.20±0.07a |
| BRS 3193 | 0.74±0.06a | 5.02±0.53a | 3.26±0.63b | 1.14±0.06a |
| BRS 3210 | 0.70±0.11a | 4.92±0.74a | 3.52±0.21b | 0.90±0.23b |
| BRS 3213 | 0.73±0.04a | 5.44±0.36a | 3.26±0.25b | 0.78±0.06b |
| BRS 3220 | 0.73±0.10a | 4.68±0.80a | 2.56±0.09c | 0.97±0.24b |
| Mean | 0.69 | 4.86 | 3.17 | 0.95 |

Mean values in the same column followed by different letters belong to different groups ($p < 0.05$) by the Scott-Knott Clustering Algorithm.

Caffeine is associated with bitterness, which, as is known, can make several people reject the beverage (MONTEIRO; TRUGO 2005). The caffeine content and, consequently, the coffee quality are genetically influenced (KY et al., 2001; AGUIAR et al., 2005) and suffer environmental and management influences. Caffeine has high heritability (MONTAGNON et al., 1998) and suffers less external influence, while trigonelline and chlorogenic acids have intermediate heritability and are more influenced by the environment and management. Thus, given the correlation of these compounds with quality, this study reinforces the influence of the relationship between genotype, environment, and management on the chemical and sensory quality of Amazonian Robusta coffees.

Regarding organic acids, the only acid detected and quantified by the methodology used was citric acid, and the contents of this organic compound vary between 0.76 and $1.28 g.100g^{-1}$. For Arabica coffee, the relationship of sensory quality with citric acid is positive (KOSHIRO et al., 2015; RODRIGUES et al., 2007; ROGERS et al., 1999). However, studies on organic acids in Conilon coffees are scarce in the literature.

BRS 2314 had the lowest citric acid content, as well as the lowest trigonelline content and the highest levels of caffeine and chlorogenic acids. According to Garambone and Rosa (2008), who verified the possible benefits of chlorogenic acid in *in vitro* tests, chlorogenic acid was indicated as a potent antioxidant in all experiments of their study, showing the need for further research with cultivars that present higher levels of this compound for consumption that benefits consumer health.

The contents of trigonelline, chlorogenic acid, caffeine, and citric acid were submitted to principal component analysis (PCA), and a biplot was generated (Figure 1).

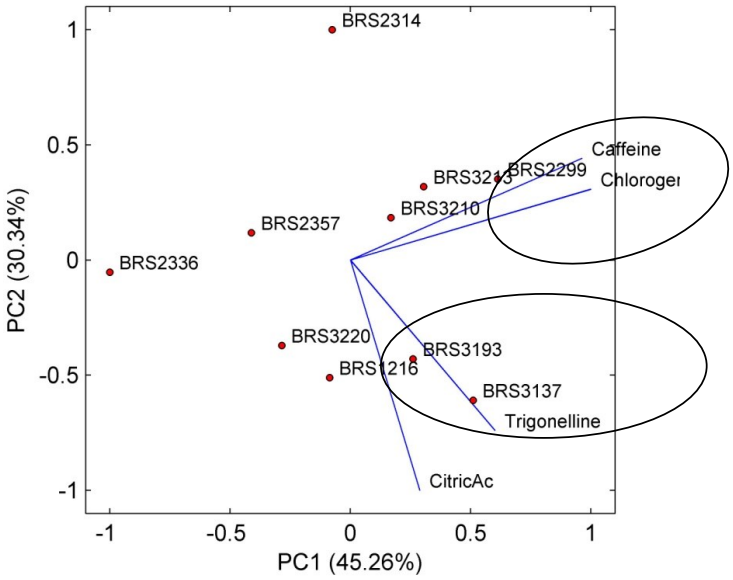


**Figure 1**. Principal component analysis (PCA) of trigonelline, chlorogenic acids, caffeine, and citric acid present in raw coffee beans of the ten cultivars analyzed.

In the biplot graph, using the first two principal components, the vectors represent the variables and the red dots, the samples, and their codes. The first component (PC1) accumulated 77.55% of the variance while the second (PC2), 15.07%. The larger the vector, the greater the influence of the variable on the grouping, and the smaller the angle between the vectors, the more significant the correlation between the variables. Therefore, it is possible to observe the relationship between trigonelline and citric acid graphically; that is, in the samples in which higher trigonelline contents were identified, there is also a higher citric acid content; the same relationship is observed for caffeine and chlorogenic acids; samples with higher levels of chlorogenic acids also had higher caffeine contents. In addition, it was possible to see the variables responsible for separating the samples. Caffeine and chlorogenic acids show an inverse relationship with citric acid and trigonelline.

The samples were reasonably distributed, but it is possible to identify only one group with higher levels of chlorogenic acid and caffeine (BRS3213, BRS2299, and BRS3210) and one group with higher levels of citric acid and trigonelline (BRS3220, BRS1216, BRS3193, and BRS3137). Two cultivars were opposite to the vectors (BRS2336, BRS2357), which shows that they have lower levels of the measured compounds and an isolated cultivar with high levels of caffeine and chlorogenic acid and low levels of other components (BRS2314). The distribution of cultivars in the graph demonstrates considerable variability in chemical composition. This variability is of great importance to understand the individualities of each cultivar and direct their use.

The statistically significant difference observed for cultivars in the analysis of trigonelline, chlorogenic acid, caffeine, and citric acid shows the presence of phenotypic variability for the evaluated genotypes (Table 3). The coefficients of experimental variation (CVe) were below 20%, indicating good experimental precision in the experiment (DUBBERSTEIN et al., 2020).

The heritability coefficient - the relationship between genotypic and phenotypic variance - ranged from 63.76% for Trigonelline to 88.44% for Caffeine. These values are considered intermediate (50% < $h^2$ < 80%) and high ($h^2$ > 80%) (FALCONER, 1987). The CVg/CVe ratio values ranged from 0.76 to 1.6 for all characteristics studied (Table 3). CVg/CVe values close to or greater than 1.0 and high heritability indicate favorable situations for selecting superior genotypes in breeding programs (FALUBA et al., 2010; CRUZ; CARNEIRO; REGAZZI, 2014).

**Table 3**. Mean squares and genetic and environmental parameters of the contents of trigonelline, chlorogenic acids, caffeine, and citric acid of *C. canephora* cultivars evaluated in Manaus, AM in 2021, Brazil.

| Source of Variation | DF | Mean squares | | | |
|---|---|---|---|---|---|
| | | Trigonelline | Chlorogenic Acids | Caffeine | Citric acid |
| Blocks | 2 | 0.013 | 0.138 | 1.225 | 0.427 |
| Cultivars | 9 | 0.015* | 0.784* | 0.754** | 0.070** |
| Residue | 18 | 0.005 | 0.250 | 0.087 | 0.017 |
| Mean | | 0.693 | 4.869 | 3.179 | 0.954 |
| Minimum | | 0.485 | 3.565 | 1.912 | 0.414 |
| Maximum | | 0.841 | 5.903 | 4.967 | 1.332 |
| CVe (%)[1] | | 10.670 | 10.260 | 9.290 | 14.420 |
| | | Genetic parameters | | | |
| Phenotypic Variance | | 0.005 | 0.261 | 0.251 | 0.023 |
| Environmental Variance | | 0.002 | 0.083 | 0.029 | 0.006 |
| Genotypic Variance | | 0.003 | 0.178 | 0.222 | 0.0178 |
| Heritability (%) | | 63.762 | 68.520 | 88.440 | 76.060 |
| CVg (%)[2] | | 8.171 | 8.670 | 14.830 | 14.840 |
| CVg/CVe[3] | | 0.770 | 0.840 | 1.600 | 1.020 |

**; * $p < 0.01$, $p < 0.05$, respectively, by the F-test [1]Coefficient of experimental variation; [2]Coefficient of genetic variation; [3] Ratio between the coefficient of experimental variation and coefficient of genetic variation. DF: degree of freedom.

## CONCLUSIONS

There is genetic variability among the ten *Coffea canephora* cultivars concerning bioactive compounds and organic acids in the beans.

The genotypes analyzed can be used in breeding programs to increase the contents of bioactive compounds and organic acids to develop new cultivars.

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