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Genetic parameters and chemical characterization of conilon coffee accessions under irrigation in the Cerrado

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ABSTRACT. The objective of this study was to identify the genetic, phenotypic and environmental parameters related to chemical compounds associated with beverage quality in a conilon coffee collection, grown in an irrigated system, in the Cerrado. The experiment was carried out at Embrapa Cerrados using green grains harvested in two years from 84 genotypes of the cultivar Robusta Tropical. Caffeine content, protein, ether extract, total soluble solids, pH and titratable acidity were evaluated. The data were subjected to analysis of variance. Significant differences were observed at 1% probability among accessions for all evaluated chemical characteristics in both harvest years. The high heritability, genetic coefficients of variation and selective accuracy reveal the potential for achieving genetic gains via the selection of genotypes adapted to the irrigated system in the Cerrado combined with high beverage quality.

Keywords: *Coffea canephora* Pierre ex Froehner; chemical compounds; genetic diversity.

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Introduction

The agricultural production system in the Cerrado has brought together technological innovations derived from the growing need for crop diversification. Species previously considered unsuitable are now adapted to the region thanks to research aimed at introducing and adapting genotypes.

In this context, conilon coffee has shown promise as an alternative crop that is adapted to the Cerrado. Coffee is one of the most consumed drinks worldwide, contributing significantly to the economy of producing countries as it is one of the most traded commodities (United States Department of Agriculture [USDA], 2023; Pereira et al., 2021).

The introduction of cultivars and progenies of *Coffea canephora* of different origins into the edaphoclimatic environment of the Cerrado is an opportunity to increase our knowledge of genetic variability and mechanisms of tolerance to abiotic stresses and their effects on grain quality, and to gather the first information about this species in this new production environment.

Little is known about the parameters affecting *Coffea canephora* production in the Cerrado region. Studies that will provide the tools for the selection (genetic parameters) of the most promising accessions for the irrigated production system are imperative, taking into account the genotype x environment interaction for the years evaluated.

The chemical composition of the coffee beans is of great importance in the classification and characterization of conilon coffee in breeding programs, requiring knowlege of the individual variation within each accession to enable evaluation of the genetic variability (Akpertey, Anim-Kwapong, Adu-Gyamfi, & Ofori, 2022).

Complementing the characterization of accessions, knowledge of genetic parameters is very important in the evaluation of the variability and proportion in which the desirable characters are inherited, and should make the selection and evaluation process more efficient (Vencovsky & Barriga, 1992).

The objective of the present work was to characterize the chemical composition of the coffee grains and to identify genetic parameters of accessions of conilon coffee under irrigation in the Cerrado of the *Planalto Central do Brasil*, in two consecutive years, and thus to identify promising genotypes.

Material and methods

The work was conducted at the Food Science Technology Laboratory of Embrapa Cerrados. It consisted of the evaluation of green coffee beans from 84 genotypes of conilon coffee from a collection, originating from open natural crosses of the Emcaper 8151 – Robusta Tropical variety, in an experimental field of the *Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural do Espírito Santo – Incaper*.

The collection was planted in April 2009, with a spacing of 3.5 m between rows and 1.0 m between plants, in the experimental field of Embrapa Cerrados, Planaltina, Distrito Federal, Brazil, at 15°35'57'' S, 47°42'30'' W and 1,030 m., in Typic Haplustox soil, under an irrigation system with a central pivot.

The irrigation regime was based on the Cerrado Irrigation Monitoring Program proposed by Embrapa Cerrados, and water stress management was used to standardize flowering (Guerra et al., 2005).

To prepare the soil prior to planting, liming and plastering were performed with 2 Mg ha^{-1} of dolomitic limestone and 2 Mg ha^{-1} of agricultural gypsum, respectively. Based on the results of a soil analysis, 58 g of P_2O_5 was added to each pit during planting and maintenance fertilization was conducted annually with 450 kg ha^{-1} N, 450 kg ha^{-1} K_2O, and 300 kg ha^{-1} P_2O_5.

For the purposes of this study, the harvest was carried out manually during June and July 2014 and 2015, collecting from each plant only those fruits in the cherry phase, which were immediately processed naturally and separately, in a conventional yard, until they reached 11% humidity. After peeling, the green coffee beans were ground and passed through a 20 mesh sieve and the samples were stored in closed glass containers covered with aluminum foil to protect them against light until analysis. The caffeine content was determined based on the method suggested by the Instituto Adolfo Lutz [IAL] (2008), the protein content by the Kjeldhal method, and the total soluble solids (TSS) and ether extract (EE), the pH and total titratable acidity according to Association of Official Analytical Chemists [AOAC] (2010). These analyses were performed in triplicate in a completely randomized design.

Individual and joint analysis of variance was performed for each character according to the statistical model: Yijk = μ + Gi + Aj + GAij + εijk, where: Yij= relative observed value of the characteristic of the i-th genotype and j-th year in the k-th repetition; μ = general average; Gi = effect of the i-th genotype (i = 1, 2, ..., i); Aj = effect of the j-th year (j = 1, 2, ...,j); GAij = effect of the interaction between i-th genotype and j-th year; εijk = random error (uncontrolled factors), εij ~ NID (0, σ²) (Table 1).

Table 1. Scheme of joint analysis of variance of a completely randomized design with first order interaction, with the mean square expected values and F test for the sources of variation, considering fixed effects of genotypes, years and genotype x year interaction.

FV	GL	QM	E (QM)	F
Genotype (G)	g – 1	QMg	$\sigma^2 + a\ r\ \sigma^2_g$	QMg/QMe
Year (A)	a – 1	QMa	$\sigma^2 + g\ r\ \sigma^2_a$	QMa/QMe
G x A	(g – 1) (a – 1)	QMga	$\sigma^2 + r\ \sigma^2_{ga}$	QMga/QMe
Error	ga (r – 1)	QMe	σ^2	

The phenotypic variance at the mean level ($\sigma^2 f$), the genotypic quadratic component (Øg), the heritability at the mean level (h^2), and the coefficients of experimental variation (CV_e), genetic variation (CV_g), and relative correlation were estimated (CV_r) and selective accuracy ($\hat{r}_{\hat{g}g}$) for each characteristic analyzed:

Phenotypic variance between means of genotypes: $\sigma^2_f = \frac{QMg}{ra}$

Genotypic quadratic component: $\sigma^2_g = \frac{QMg - QMe}{ra}$

Heritability at the mean level (coefficient of genotypic determination): $h^2(\%) = \frac{\sigma^2_g}{\sigma^2_f}$

Environmental variation coefficient: $CV_e(\%) = \frac{100\sqrt{QMe}}{m_c}$, where m_c = character average.

Coefficient of genetic variation: $CV_g(\%) = \frac{100\sqrt{\sigma^2_g}}{m_c}$, where m_c = character average.

Relative coefficient of variation: $CV_r = \frac{CV_g}{CV_e}$

Selective accuracy: $\hat{r}_{\hat{g}g} = \sqrt{1 - 1/F}$

The means were grouped using the Scott-Knott test at 1% probability and the phenotypic, genotypic and year correlations were measured from the estimates of phenotypic, genotypic and year variances and covariance between the characters two by two.

All statistical analyses were performed using the software GENES.

Results and discussion

The joint analysis of variance showed the existence of significant differences at 1% by the F test between the genotypes of conilon coffee for all evaluated characteristics (Table 2). This demonstrated the presence of the expected genetic variability in the study population, as it is a diploid allogamous species with gametophytic self-incompatibility (Partelli et al., 2019; Silva et al., 2017). Santin, Coelho, Sayd, Peixoto, and Amabile (2019) had already verified genetic variability in this same population in relation to yield and maturation cycle, which highlights the possibility for genetic gains by selection.

Table 2. Analysis of variance, F values and genetic parameters of protein, caffeine, crude ether extract (EE), total soluble solids (TSS), pH and total titratable acidity, evaluated in raw beans of 84 genotypes of conilon coffee harvested in the years 2014 and 2015. Planaltina, Distrito Federal, Brazil, 2016.

FV	G.L.	F Values					
		Caffeine	Protein	EE	TSS	pH	Acidity
Genotype	83	24.08**	916.56**	48.99**	6.69**	3.81**	13.80**
Year	1	485.90**	164552.98**	15039.75**	9.26**	269.62**	3.96*
G x A	83	18.24**	315.97**	33.14**	6.29**	2.60**	12.29**
Residue	332						
σ^2_g		0.016	0.192	0.231	4.256	0.152	369.422
σ^2_f		0.017	0.192	0.236	5.004	0.207	398.276
σ^2_e		0.004	0.001	0.029	4.488	0.033	173.121
h^2 (%)		95.85	99.89	97.96	85.05	73.73	92.76
CV_e (%)		3.08	0.44	3.73	6.91	3.15	9.50
CV_g (%)		6.05	5.39	10.55	6.72	2.16	13.88
CV_r (%)		1.96	12.35	2.83	0.97	0.68	1.46
$\hat{r}_{gg}$		0.979	0.999	0.990	0.922	0.859	0.963

*Significant at 5% probability by the F test. **Significant at 1% probability by the F test.

The effect of year was significant at 1% for caffeine, protein, crude ether extract (EE), total soluble solids (TSS) and pH and at 5% for total titratable acidity. The effect of the genotype x year interaction was significant at 1% for all characters, revealing that the order of classification of the genotypes was influenced by the year factor.

The significant effect of the genotype x environment interaction for all chemical characteristics indicates that the effects of treatments and years did not explain the total variation contained in the characters, as a result of the different behaviors in the years evaluated, probably caused by different climatic conditions during grain filling. According to Fagan, Souza, Pereira, and Machado (2011), the photosynthates accumulated by the plant during the expansion of the fruits are decisive in the final quality of the product, and any stress at this stage, such as disease attack, water deficit or high temperatures, can harm the accumulation of these compounds. In the current study, the average maximum temperatures in January differed significantly between the years 2014 (29°C) and 2015 (30.7°C) (Figure 1), varying by almost 2.0°C, which may be the reason for this interaction. Borém et al. (2019) found that among the evaluated meteorological variables, temperature was the variable that most influenced the sensorial quality of the evaluated coffees.

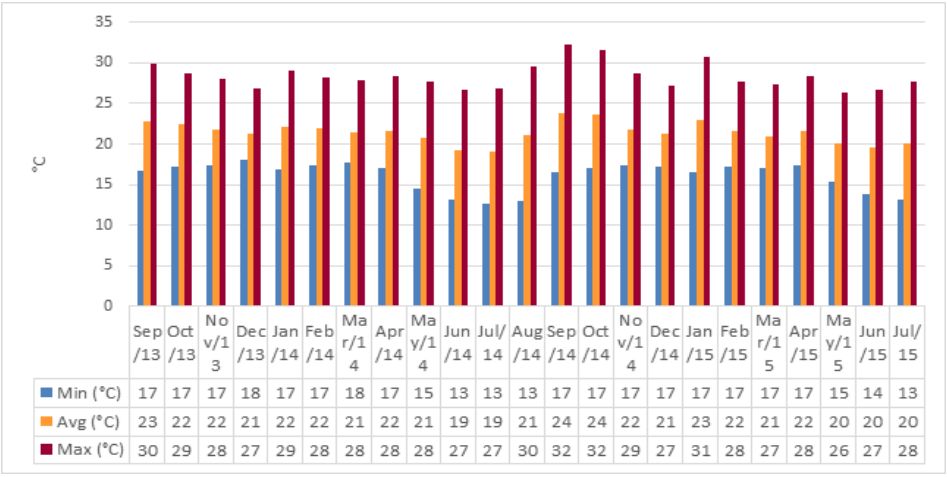

Figure 1. Maximum air temperature (Max), average air temperature (Avg) and minimum air temperature (Min) monthly for January to July 2014 and 2015, collected from the Embrapa Cerrados weather station. Planaltina, Distrito Federal, Brazil, 2016.

The use of Snedecor's F test, which aims to compare estimates of variance, allows the level of genotypic variation and the number of repetitions in the evaluation of the tests to be considered simultaneously (Resende & Duarte, 2007). The F values found in the analysis of variance were between 2.6 and 164,552.98, which reflects a high to very high level of accuracy of the experiment, according to Resende and Duarte (2007). These authors stated that in plant breeding, F values greater than 2.0 indicate greater accuracy in the selection process. The coefficients of environmental variation (CV_e) showed low values for all characteristics (Table 2), ranging from 0.44% for protein content to 9.50% for total titratable acidity, indicating high experimental precision.

It was found that the estimated genotypic variance, with the exception of the TSS characteristic, accounted for more than 90% of the phenotypic variance, indicating highly accessible genetic variability, adequate environmental control, experimental efficacy and genotypic accuracy. This can be attributed to the appropriate harvesting and post-harvesting practices used to carry out the work, the choice of the experimental design such as the number of repetitions, as well as laboratory assertions. This corroborates the results obtained by Brige et al. (2019), who found that pH was the characteristic that contributed least to the genetic variability in a population of conilon coffee. On the other hand, the selective accuracy values ($\hat{r}_{\hat{g}g}$) were classified as high for pH (0.859) and very high for caffeine (0.979), protein (0.999), crude ether extract (EE) (0.990), total soluble solids (TSS) (0.922) and total titratable acidity (0.963) according to the categories proposed by Resende and Duarte (2007) (Table 2). Genetic variability and experimental accuracy are fundamental to helping direct the genetic improvement program and ensuring adequate selection and genetic gains. The variability observed in the context of conilon coffee cultivation in the Cerrado shows the potential for genetic gains via the selection of genotypes adapted to edaphoclimatic conditions combined with beverage quality.

The proportion of genetic variability in populations and in different characteristics can be inferred through the genetic variation coefficient (CV_g). CV_g was superior to CV_e for caffeine (6.05%), protein (5.39%), EE (10.55%) and total titratable acidity (13.88%), giving these characters the greatest potential for improvement. However, the CV_g of TSS (6.72%) and pH (2.16%) proved to be inferior to the respective CV_e, suggesting less favorable conditions for the selection of these characteristics (Table 2). The coefficients of relative variation (CV_r) resulting from the CV_g/CV_e ratio were greater than 1.0 for caffeine (1.96), protein (12.35), EE (2.83), and total titratable acidity (1,46), suggesting the possibility of success in phenotypic selection based on these characteristics, since the genetic variance surpassed the environmental variance. However, they were lower than 1 for SST (0.97) and pH (0.68), indicating less favorable conditions for the selection of these characteristics.

The coefficient of genotypic determination or heritability is directly related to experimental accuracy and genotypic variance, indicating how much a character can be transferred to its descendants and how much the phenotypic value represents the genotypic value of the character analyzed. With the exception of TSS (85.05%) and pH (73.73%), high heritability values were found, in general, based on the mean for each character (Table 2), indicating a predictive correspondence between the phenotypic and genotypic value as discussed by Vencovsky and Barriga (1992). This is supported by the high contribution of the genotypic variance in relation to the environmental variance.

The determination coefficients (h^2) for caffeine and ether extract were 95.85 and 97.96%, respectively (Table 2). Montagnon, Guyot, Cilas, and Leroy (1998) evaluated the genetic parameters of biochemical components in progenies obtained by crossing individuals from the Congolese and Guinean groups and obtained lower values, in the order of 80.0% for caffeine and 74.0% for the ether extract, respectively, but considered these high values in the strict sense. Priolli et al. (2008) found heritability values for caffeine in the order of 91.0% in interspecific progenies of *Coffea arabica* and *C. canephora*. Protein and total titratable acidity had heritability values of 99.89 and 92.76%, respectively (Table 2). These high values of heritability and adequate environmental controls at harvest and post-harvest, as well as in laboratory analyses, favored the expression of the genotypic variability of the studied characteristics. Such high heritability values, genetic variation coefficients and selective accuracy for all traits (with the exception of pH), reveal the potential for achieving genetic gains through selection.

Regarding the estimates of the phenotypic, genotypic and environmental correlation coefficients (Table3), the signs of the phenotypic and genotypic coefficients were the same, due to the absence of errors in sampling and evaluation. The genotypes have different chemical characteristics, which were influenced by the year effect and the genotype x year interaction.

Table 3. Estimates of the phenotypic, genotypic and environmental correlation coefficients between caffeine, protein, ether extract (EE), total soluble solids (TSS), pH and total titratable acidity in raw beans of 84 genotypes of irrigated conilon coffee in the Cerrado, harvested in 2014 and 2015. Planaltina, Distrito Federal, Brazil, 2016.

		Protein	EE	TSS	pH	Acidity
Caffeine	r_f	0.177	-0.0779	-0.0079	-0.0093	-0.2342
	r_g	0.1829	-0.0734	-0.0121	-0.0072	-0.2519
	r_a	-0.2994	-0.231	0.0378	-0.0311	0.0598
Protein	r_f		0.2034	0.2742	0.1866	-0.2158
	r_g		0.2071	0.2977	0.2163	-0.2234
	r_a		-0.3177	-0.0144	0.0601	-0.0902
EE	r_f			0.2661	0.1612	0.0034
	r_g			0.2917	0.1919	0.0022
	r_a			-0.0023	-0.0249	0.0326
TSS	r_f				0.3148	0.1308
	r_g				0.4021	0.1387
	r_a				-0.0186	0.0734
pH	r_f					-0.5514
	r_g					-0.5466
	r_a					-0.7207

The lowest levels of caffeine were found in the genotypes CPAC 15 (1.78%), CPAC 36 (1.83%), CPAC 70 (1.81%), CPAC 85 (1.73%), CPAC 134 (1.86%), CPAC 145 (1.7%), CPAC 148 (1.76%), CPAC 178 (1.73%), and CPAC 201 (1.85%), with no significant differences among these genotypes (Table 4). These are relatively low values in relation to those mentioned by Spiller (2019) for raw coffee. The highest levels of caffeine, for the same year, were found in the genotypes CPAC 38 (2.37%), CPAC 162 (2.33%), CPAC 193 (2.29%), and CPAC 224 (2.39%), again with no significant differences among these genotypes. In 2015, the genotypes with the lowest caffeine content were CPAC 7 (1.81%), CPAC 16 (1.83%), CPAC 39 (1.77%), CPAC 105 (1.72%), CPAC 148 (1.86%), CPAC 218 (1.84%), and CPAC 231 (1.75%), with no significant differences among these genotypes. CPAC 36 (2.74%) had a significantly higher caffeine content than all other genotypes. CPAC 148 was in the lowest group in both years, indicating that this may be a promising genotype for selection for low levels of caffeine, while CPAC 36 had divergent results for each year evaluated, disfavoring its selection.

Regarding protein content, the genotypes CPAC 171 and CPAC 219 had the highest values in 2014, at 8.34 and 8.39%, respectively, being statistically similar to each other. In 2015, the highest values were found in the genotypes CPAC 171 (9.86%), CPAC 178 (9.93%), and CPAC 219 (9.9%) (Table 4). CPAC 171 and CPAC 219 had the highest protein content in both years. These values were below those reported for robusta coffee (Dong, Hu, Chu, Zhao, & Tan, 2004; Quast & Aquino, 2004), which may explain the inferiority of conilon coffee in relation to its aroma when compared to arabica coffee (Montagnon et al., 1998).

Regarding the total soluble solids (TSS) content, the genotypes with the highest averages in 2014 (which did not differ significantly) were CPAC 5 (34.17%), CPAC 8 (33.33%), CPAC 16 (35.0%), CPAC 23 (33.33%), CPAC 26 (34.17%), CPAC 47 (33.33%), CPAC 59 (33.33%), CPAC 60 (33.33%), CPAC 70 (33.33%), CPAC 102 (34.17 %), CPAC 162 (37.5%), CPAC 165 (33.33%), CPAC 171 (34.17%), CPAC 178 (35.0%), CPAC 182 (37.5%), CPAC 212 (34.17%), and CPAC 215 (35.83%). In 2015, the genotypes with the highest averages (which did not differ statistically) were CPAC 5 (37.83%), CPAC 6 (35.83%), CPAC 38 (35.83%), CPAC 134 (35.0%), CPAC 135 (35.0%), CPAC 147 (35.83%), CPAC 171 (35.83%), CPAC 193 (36.67%), CPAC 197 (35.83%), CPAC 215 (36.67%), CPAC 229 (39.17%), and CPAC 235 (35.0%) (Table 4). Values of this magnitude in conilon coffee were also observed by Macedo et al. (2020).

In 2015, the genotypes that had the lowest total titratable acidity, in mL of NaOH/100 g of dry matter (which did not differ statistically) were CPAC 9 (113.87), CPAC 10 (101.68), CPAC 59 (83.45), CPAC 60 (107.34), CPAC 90 (112.06), CPAC 100 (94.33), CPAC 123 (94.20), CPAC 132 (92.08), CPAC 167 (107.56), CPAC 171 (98.38), CPAC 178 (87.85), CPAC 197 (104.02), CPAC 214 (107.46), CPAC 215 (94.46), and CPAC 230 (99.07). The genotypes with the lowest values for this characteristic were CPAC 127 (213.78) and CPAC 193 (199.91) (Table 4). The total titratable acidity of coffee beans is inversely related to the quality of the coffee as a beverage (Borém, Salva, & Silva, 2008).

Table 4. Genotypes with majors means in the Scott-Knott test at 1% significance for caffeine, protein, ether extract (EE), pH and total soluble solids (TSS) and with minors means for total titratable acidity, within of a population of 84 genotypes of irrigated conilon coffee in the Cerrado, in two years. Planaltina, Distrito Federal, Brazil, 2016.

Caffeine (%)				Protein (%)			
GEN	2014	GEN	2015	GEN	2014	GEN	2015
224	2.39 a	36	2.74 a	219	8.39 a	178	9.93 a
38	2.37 a			171	8.34 a	219	9.90 a
162	2.33 a					171	9.86 a
193	2.29 a						

EE (%)				pH[1]			
GEN	2014	GEN	2015	GEN	2014	GEN	2015
176	5.10 a	102	7.32 a	167	6.06 a	162	6.36 a
229	5.05 a						
93	4.95 a						
171	4.84 a						

TSS (%)				Total tritable acidity[2]			
GEN	2014	GEN	2015	GEN	2014	GEN	2015
182	37.50 a	229	39.17 a	129	78.19 e	59	83.45 e
162	37.50 a	5	37.83 a	235	90.06 e	178	87.85 e
215	35.83 a	215	36.67 a	8	92.19 e	132	92.08 e
16	35.00 a	193	36.67 a	151	98.25 e	123	94.20 e
178	35.00 a	171	35.83 a	224	98.73 e	100	94.33 e
102	34.17 a	147	35.83 a	38	98.86 e	215	94.46 e
171	34.17 a	197	35.83 a	37	98.93 e	171	98.38 e
26	34.17 a	6	35.83 a	230	99.99 e	230	99.07 e
5	34.17 a	38	35.83 a	218	101.24 e	10	101.68 e
212	34.17 a	135	35.00 a	59	102.26 e	197	104.02 e
165	33.33 a	235	35.00 a	219	103.8 e	60	107.34 e
70	33.33 a	134	35.00 a	123	104.44 e	214	107.46 e
59	33.33 a			85	105.35 e	167	107.56 e
47	33.33 a			75	107.63 e	90	112.06 e
8	33.33 a			193	109.98 e	9	113.87 e
23	33.33 a			48	111.97 e		
60	33.33 a			45	112.40 e		

[1]Only the highest pH values are shown. [2]Titratable acidity measured in mL NaOH 100 g^{-1} dry matter.

Conclusion

The evaluated characteristics of conilon coffee cv. Robusta Tropical in the Cerrado are significantly affected by genetic variation. The genetic variance was the main component of phenotypic variation among genotypes. The high heritability, genetic coefficients of variation and selective accuracy indicate the potential to achieve genetic gains by the selection of genotypes adapted to the irrigated system in the Cerrado combined with beverage quality. The genotype CPAC 171 shows promise for selection based on total soluble solids, ether extract and protein content and total titratable acidity, while the CPAC 148 genotype shows promise for selection for low levels of caffeine.

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