Characterization of chlorogenic acids (CGA) and nine isomers in an F_2 population derived from *Coffea arabica* L.

Caracterización de ácidos clorogénicos (ACG) y nueve isómeros en una población F_2 derivada de *Coffea arabica* L.

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ABSTRACT

Chlorogenic acids (CGA) and their isomers have been associated with sensory attributes of the coffee beverage such as acidity, astringency, and bitterness. They have been linked to coffee rust resistance and acknowledged as bioactive compounds due to their antioxidant power with benefits for human health. The total chlorogenic acids (TCGA) and nine isomers of three groups, caffeoylquinic acid or CQA (5-CQA, 4-CQA, 3-CQA), dicaffeoylquinic acid or diCQA (3,4-diCQA; 3,5-diCQA, 4,5-diCQA) and feruloylquinic acid or FQA (5-FQA, 4-FQA, 3-FQA) were determined in an F_2 population of *Coffea arabica* from the crossbreed (Bourbon x Maragogype) x Timor Hybrid. TCGA contents were quantified by UV-VIS spectrophotometry and High-Resolution Liquid Chromatography - HPLC. The group of caffeoylquinic acids (CQA) represented 82% of the TCGA. From the diCQA, 4,5-diCQA showed lower contents, whereas the highest isomer was 3,5-diCQA. Results per quartile for TCGA-UV and for every isomer showed statistical differences among group averages per isomer. The population behaved as a parental Maragogype according to contents of 5-CQA, 3,5-diCQA, and TCGA-UV. TCGA contents were higher in the parental GQ956 derived from the Timor hybrid 832-1, with resistance to coffee rust. From the three groups, the first characteristic of parental Bourbon showed a higher concentration of diCQA and FQA; the second one showed a lower concentration of TCGA and CQA isomers and the third group higher TCGA and 5-CQA concentrations. This research allowed establishing the basis for plant selection in the F_2 generation of *C. arabica* due to the TCGA content and isomers derived from CQA, diCQA, and FQA.

Key words: distribution, caffeoylquinic acids, introgression, coffee quality, Timor hybrid, plant breeding.

RESUMEN

Los ácidos clorogénicos (ACG) y sus isómeros han sido asociados a los atributos en la bebida del café especialmente la acidez, astringencia y el amargo. Estos compuestos han sido reportados como relacionados a la resistencia a la roya del café y reconocidos como compuestos bioactivos en la salud humana por su capacidad antioxidante. Se determinó la distribución de ácidos clorogénicos totales (ACGT) y nueve isómeros pertenecientes a tres grupos, los ácidos cafeoilquínicos o CQA (5-CQA, 4-CQA y 3-CQA), los ácidos dicafeoilquínicos o diCQA (3,4-diCQA; 3,5-diCQA y 4,5-diCQA) y los ácidos feruloilquínicos o FQA (5-FQA, 4-FQA y 3-FQA) en una población F_2 de *Coffea arabica* proveniente del cruce de (Bourbon x Marapagogype) x Híbrido de Timor. Se cuantificó el contenido de ACGT mediante espectrofotometría UV-VIS y cromatografía líquida de alta resolución - HPLC. El grupo de los ácidos cafeoilquínicos (CQA) representó el 82% de los ACGT. De los diCQA, el 4,5-diCQA mostró los menores contenidos, mientras que el isómero mayoritario fue el 3,5-diCQA. Los resultados por cuartil para ACGT-UV y cada isómero indicaron diferencias estadísticas entre los promedios de los grupos por cada isómero. La población se comportó como el padre Maragogype según los contenidos de 5-CQA, 3,5-diCQA, y los ACGT-UV. Los contenidos de ACGT fueron mayores en el parental GQ956 derivado del híbrido de Timor 832-1, cuya característica principal es la resistencia a roya. Se formaron tres grupos de plantas de acuerdo a los isómeros analizados. El grupo uno fue característico del parental Bourbon al presentar mayor concentración de diCQA y FQA; el grupo dos presentó menor concentración de ACGT y de isómeros del CQA; y el grupo tres estuvo caracterizado por presentar mayor concentración de ACGT y 5-CQA. Este trabajo permitió establecer las bases para la selección de plantas en una generación F_2 de *C. arabica* por el contenido total de ácidos clorogénicos y los isómeros derivados de CQA, diCQA y FQA.

Palabras clave: distribución, ácidos cafeolquínicos, introgresión, calidad de café, híbrido de Timor, fitomejoramiento.

Received for publication: 19 August, 2019. Accepted for publication: 13 April, 2020. Doi: 10.15446/agron.colomb.v38n1.74338

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Introduction

The world culture of *Coffea arabica* L. has been performed mainly with traditional varieties such as Typica and Bourbon either by mutation or selection. Another important group of varieties is that with resistance to coffee rust, which has derived from the crossbreed between the traditional varieties of *C. arabica* L. and the Timor hybrid (Van Der Vossen *et al*., 2015).

The conventional method of coffee genetic improvement has been determined by the method of selection by pedigree (Van Der Vossen *et al*., 2015). After an original crossbreed, the seeds of the F_1 plants advance to a new generation F_2, which offers the first opportunity to practice a selection of only those plants that meet the desired requirements. In the development of coffee varieties, there has been a focus to select the best plants and upgrade them to F_3 or F_4 generations, placing emphasis on inheritable variables such as production and resistance to diseases (coffee rust), and assessing the quality in the last generations of the selection (Van Der Vossen, 2009).

The quality in a cup of coffee depends on the contents of chemical compounds present in the green coffee, which at the same time are influenced by factors such as species, variety, environment, crop condition, and harvest and post-harvest processes, among others (Bertrand *et al*., 2008). There are studies related to the determination of chemical compounds associated with quality that show the appearance of contrasting levels among species and at the intra-specific level (Herrera and Lambot, 2018).

Among the studied compounds, there are chlorogenic acids (CGA) and their isomers, which have been associated with sensory attributes of the beverage, especially acidity, astringency, and bitterness (Mazzafera and Melo, 2004).

CGA have been associated with coffee rust resistance (Guerra-Guimarães *et al*., 2015) and acknowledged as bioactive compounds due to their antioxidant power. These compounds have benefits on human health causing different biological effects such as free radical capture, metal chelation, enzymatic activity modulation and signal transduction way alteration (Cheng *et al*., 2016).

CGA determination in green coffee is mainly performed through analytic techniques of Ultraviolet-Visible Spectrophotometry (UV-VIS) for total concentration and High-Resolution Liquid Chromatography (HPLC) form isomer measure (Brighenti *et al*., 2017). These techniques have been used to identify the presence of chemical compounds in different plant organs, obtain the diversity of *Coffea* species, and characterize different coffee varieties (Etienne *et al*., 2018).

For CGA quantification in coffee varieties, Guerrero *et al*. (2001) found that 4,5-caffeoylquinic acid was present in all the material of Colombian varieties: varieties from *C. arabica*, three accessions of Timor hybrid, the first generation (F_1) of a crossbreed from *C. canephora* x Timor hybrid and three accessions of *C. canephora*. Therefore, it can be used to separate these two varieties from the rest of the genotypes.

Bertrand *et al*. (2008), studied the content of chlorogenic acids in four introgressed lines and the Caturra variety. Their results showed differences among genotypes for most of the studied chlorogenic acids, with the exception of 3-CQA and 3-FQA, whereas regarding the chlorogenic acids the highest effect was found in 3,4-diCQA.

The objective of the current study was to determine the concentration of chlorogenic acids and nine isomers in an F_2 population of *Coffea arabica*.

Materials and methods

Population of work and sampling

An F_2 population formed by 143 plants from the crossbreed of Maragogype parentals, Bourbon resistant to *Ceratocystis fimbriata* and Timor Hybrid (GQ956 - CIFC 832-1) was used. These plants were sown in the Station Naranjal in the municipality of Chinchina (4°59' N, 75°39' W, and altitude of 1,400 m a.s.l.).

Samples were taken during the peak week of the main harvest (September-November). Coffee cherries were collected from each of the plants and then they were washed and sun-dried until their mass reached a verified humidity from 10 to 12%. Once dried, green coffee was obtained by the dry milling process and ground in a cryogenic mill at 14000 rpm.

Extraction

The extraction of chlorogenic acids in green coffee was carried out following the methodology proposed by Bicchi *et al.* (1995). The study was carried out in two phases:

Phase 1

Consisted in the selection of the best analytic method for total chlorogenic acids (TCGA) using two methodologies:

Visible Ultra-Violet (UV-VIS) and High-Resolution Liquid Chromatography (HPLC).

A *t*-test was used for the comparison of the means of both techniques and to determine if there were statistical differences in the quantification of TCGA.

The following formula was applied for the *t*-test:

$$t = \frac{\overline{X}_{\text{ACT-UV}} - \overline{X}_{\text{ACT-HPLC}}}{\sigma\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}} \quad (1)$$

where $\sigma = \sqrt{\frac{N_1 s_1^2 + N_2 s_2^2}{N_1 + N_2 - 2}}$

$\overline{X}_{\text{TCGA- UV}}$: Mean of chlorogenic acids by UV-VIS.

$\overline{X}_{\text{TCGA-HPLC}}$: Mean of chlorogenic acids by HPLC.

N: Number of population individuals.

S^2: Variance of each of the population individuals.

If the total chlorogenic acids quantified by both techniques did not show statistical differences, then the contents of isomers 3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, 3-FQA, 4-FQA, and 5-FQA were compared, which were quantified by HPLC through the proportionality of each isomer concerning the total. This procedure was carried out following the methodology of Bastos De Maria and Alvez (2004) and using the methodologies validated in stage 1.

Phase 2

Consisted in the application of the method selected in phase 1 and the determination of isomers in the F_2 population (143 plants) and the respective parentals.

Results and discussion

The results of the *t*-test are presented in Table 1, which showed that the determination of TCGA by both techniques is the same and either of both technologies can be used. Since UV-VIS is the one with the lower cost, this method is the best option to determine TCGAs in an analytic form.

TCGA determined by UV showed an average content of 4% in the population, with a minimum and maximum 2.78% and 5.05%, respectively, while TCGA by HPLC showed an average content of 3.96% in the population, with a minimum and maximum of 2.62% and 4.93%, respectively.

TABLE 1. *T*-test for the mean of total chlorogenic acids quantified by UV-VIS and HPLC, with different variance.

Variables	HPLC	UV-VIS
Mean	3.96	4.00
Variance	0.18	0.21
Observations	143	143
Mean difference hypothesis	0	
GL	289	
t-statistic	0.81	
t critical two-tail	1.97	

Table 2 presents the contents for each one of the nine isomers tested by HPLC. Values ranged between 3.67% for 5-CQA and 0.01 for 3-FQA, in which isomer 5-CQA has the highest concentration and 3-FQA is the one with the lowest. CQA showed higher contents than diCQA and FQA, which is similar to the results obtained by Cheng *et al*. (2018).

Six isomers showed a normal distribution: three isomers of the CQA (5-CQA, 4-CQA, and 3-CQA), two isomers of the diCQA (3,5-diCQA and 4,5-diCQA) and one of the FQA (5-FQA). Isomers 3,4-diCQA, 3-FQA and 4-FQA were not adjusted to a normal distribution. The TCGA by HPLC was not adjusted to a normal distribution. The results of the frequency analysis for five isomers and the TCGA-UV (normally adjusted) are shown in Figure 1.

TABLE 2. Minimum and maximum values observed, mean, standard deviation and lowest and upper limits for the content of nine isomers of the family of chlorogenic acids expressed in dry matter percentages in an F_2 population.

Isomer Group	Isomer	Minimum	Maximum	Mean	Standard deviation	LL (95%)	UL (95%)
TOTAL CQA	UV	2.78	5.06	4.00	0.44	3.93	4.08
	HPLC	2.62	4.94	3.96	0.42	3.89	4.03
CQA	5-CQA	1.77	3.67	2.66	0.35	2.60	2.71
	3-CQA	0.13	0.42	0.25	0.04	0.24	0.26
	4-CQA	0.22	0.58	0.35	0.05	0.34	0.36
	TCQA	2.17	4.35	3.26	0.38	3.19	3.32
diCQA	3,4-diCQA	0.05	0.21	0.12	0.04	0.11	0.13
	3,5-diCQA	0.11	0.35	0.23	0.05	0.22	0.24
	4,5-diCQA	0.06	0.16	0.10	0.02	0.10	0.11
	TdiCQA	0.26	0.70	0.46	0.09	0.44	0.47
FQA	5-FQA	0.11	0.34	0.19	0.04	0.18	0.20
	4-FQA	0.00	0.08	0.04	0.01	0.04	0.05
	3-FQA	0.00	0.05	0.01	0.01	0.01	0.02
	TFQA	0.14	0.45	0.25	0.05	0.24	0.26

LL= lowest limit; UL= upper limit.

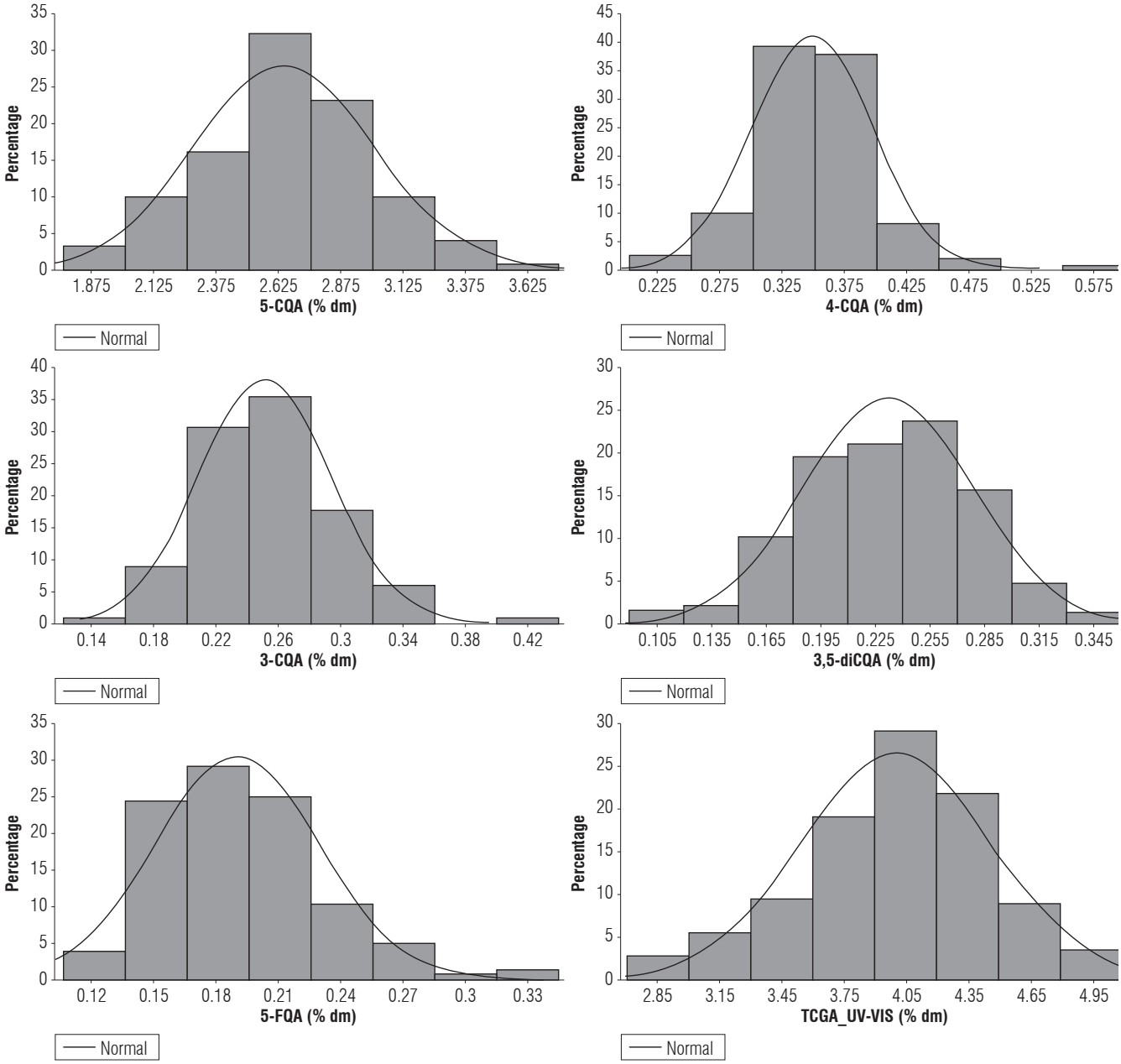

FIGURE 1. Frequency distribution for five isomers and total chlorogenic acids quantified by UV-VIS (TCGA-VIS).

Isomers of the CQA group had a concentration of 3.27%; the highest content was found for 5-CQA with 2.67% and the minimum for 3-CQA with 0.13%, whereas diCQA had a value of 0.45%. The isomer that showed the highest content was 3,5-diCQA with a 0.35%, whereas the lowest value was found for 3,4-diCQA with 0.05%.

Caffeoylquinic acids (CQA)

The content of 4-CQA and 3-CQA represented between 13% and 9% of the content of 5-CQA. 5-CQA was the isomer with the highest average content, being statistically different from the other isomers. This behavior was reported by Guerrero *et al.* (2001) and Perrone *et al.* (2008). The isomer 4-CQA is the second isomer in importance and 3-CQA is the third isomer in abundance, even though their content does not differ descriptively from 3,5-diCQA.

The behavior for the content of group CQA matches one of the majoritarian isomers reported in studies of *C. arabica* varieties by Scholz *et al.* (2016) and Barbosa *et al.* (2019).

Dicaffeoylquinic acids (diCQA)

From the diCQA group, isomer 3,5-diCQA showed the highest contents, which differs from that reported by other authors in commercial lines who found 4,5-diCQA and 3,4-diCQA as the isomers with the highest contents. The behavior of isomers found in our study for this group was the following: 3,5-diCQA > 3,4-diCQA > 4,5-diCQA.

However, Guerrero *et al.* (2001) report in an early population (F_1), low levels for 4,5-diCQA compared to 3,5-diCQA, which has the highest contents within these groups. This is because an F_1 population presents dominant characteristics in the expression of chemical compounds.

Feruloylquinic acids (FQA)

Isomer 5-FQA presented an average content of 0.19%, it is the isomer with the highest content within the FQA group, and it was superior to the content of isomers 3,4-diCQA and 4,5-diCQA. This behavior was reported by Bertrand *et al.* (2008) in four materials introgressed by Timor Hybrid. The minimum and maximum values for this isomer were 0.15 and 0.34%, respectively. For isomers 3-FQA and 4-FQA, no content was detected in the 38% of tested plants.

FQA presented a relationship of proportionality lower than 2% before total chlorogenic acids were quantified by UV-VIS (TCGA-UV). This proportion was reported by Ky *et al.* (2001) about biochemical diversity in the *Coffea arabica* L genus.

Total Chlorogenic Acid (TCGA)

Table 3 shows the descriptive statistics for the variable proportion of chlorogenic acids quantified by HPLC. The percentage participation of the CQA group (3-, 4- and 5-CQA) was 82% of the TCGA with an average content of 3.27%. Farah and Donangelo (2006) report contents for CQA of 83%, similar to those found in this study.

In the CQA group, 5-CQA was the isomer that descriptively represented the highest percentage participation with 66.27% of TCGA, which matches what was reported by Cheng *et al.* (2016). These authors showed that 5-CQA represents 66% of chlorogenic acids. The minimum and maximum values of proportionality for this isomer were 58.77 and 76.31%, respectively. Fifty percent of the tested materials are above 66.15% of 5-CQA with a standard deviation of 3.46%.

Isomer 4-CQA represented 8.77% of the TCGA-UV and is the second isomer in terms of percentage of participation. The minimum and maximum values of proportionality for this isomer were 5.41 and 17.28%, respectively. Fifty percent of the materials tested are above 8.67% and the other 50% is under this value with a standard deviation of 1.22%.

Isomer 3-CQA represented descriptively the 6.34% of the TCGA-UV, and it is the third isomer in the percentage of participation. Fifty percent of the materials tested are under 6.20%. The behavior of isomers the CQA group was the same as reported by Bertrand *et al.* (2008), where 5-CQA > 4-CQA > 3-CQA.

The diCQA group showed an average content of 0.45%, which represented 11% of the TCGA content, whereas isomer 3,5-diCQA represented 51.11% of the diCQA content.

The FQA group had an average content of 0.24%, representing el 6% of total chlorogenic acids. The isomer 5-FQA

TABLE 3. Minimum and maximum values observed, mean for the variable proportion of chlorogenic acids quantified by HPLC.

Proportion (%)	Minimum	Maximum	1st Quartile	3rd Quartile	Mean
TCQA	0.74	0.96	0.79	0.84	0.81
TdiCQA	0.07	0.16	0.10	0.13	0.11
TFQA	0.04	0.11	0.06	0.07	0.06
5-CQA	0.59	0.76	0.64	0.68	0.66
3-CQA	0.04	0.13	0.05	0.07	0.06
4-CQA	0.05	0.17	0.08	0.09	0.09
3,4-diCQA	0.01	0.05	0.02	0.04	0.03
3,5-diCQA	0.03	0.08	0.05	0.07	0.06
4,5-diCQA	0.01	0.04	0.02	0.03	0.03
5-FQA	0.03	0.08	0.04	0.05	0.05
4-FQA	0.00	0.02	0.01	0.01	0.01
3-FQA	0.00	0.01	0.00	0.01	0.00

represented descriptively 4.77% of the TCGA-UV, a value that matches the contents reported by Guerrero *et al*. (2001) for *Coffea canephora* materials with 4.76%. This is the last isomer of the five tested (5-CQA, 4-CQA, 3-CQA, 3,5-diCQA, and 5-FQA). The minimum and maximum values of proportionality for this isomer were 3.13% and 8.32% respectively.

The proportionality of the isomers was as follows: 5-CQA > 4-CQA > 3-CQA > 3,5-diCQA > 5-FQA > 3,4-diCQA > 4,5-diCQA > 4-FQA > 3-FQA. However, diCQA and FQA showed differences. It was found that the higher isomer of diCQA was 3,5-diCQA and not 4,5-diCQA. This order is different from the one reported by Perrone *et al*. (2008). They reported that in two varieties of the *Coffea arabica* species (Mundo Novo and Catuai Vermelho) and in a variety of *Coffea canephora* (Conillon) the proportionality 5-CQA > 4-CQA > 3-CQA > 4,5-diCQA > 3,4-diCQA > 3,5-diCQA > 5-FQA > 4-FQA > 3-FQA was established.

Quartiles for TCGA-UV

The value of the first group Q1 was 3.4%, which indicates an average content of approximately 25% of the 143 F_2 plants, with 32 plants in this quartile; Q2 was 3.9%, and the number of plants was 37; Q3 was 4.16% and the number of plants was 37, and Q4 was 4.53%, and the number of plants in this quartile was 37.

Quartiles for isomer 5-CQA

The average content of approximately 25% of the F_2 population, belonging to the first group or Q1 was 2.2%, where the number of plants in this quartile was 32; Q2 was 2.55% and the number of plants was 36; in the third quartile (Q3), the average was 2.75% and the number of plants was 37. The average value in Q4 was 3.09%, and the number of plants classified in this quartile was 38.

Quartiles for isomer 4-CQA

The value for Q1 was 0.29%, and the number of plants for this quartile was 32; Q2 had 0.33% as average, and the number of plants in this quartile was 36; Q3 had a value of 0.36% and the number of plants was 38. Q4 was composed of 37 plants and an average value of 0.41%.

Quartiles for isomer 3-CQA

The Q1 value indicated that approximately 25% of the population had an average content of 0.2% and 32 plants were classified in this quartile. The average value was 0.23% in Q2 with 36 plants; the average value of Q3 was 0.26% and the number of plants was 38; finally, the value for Q4 was 0.3% with 37 plants.

Quartiles for el isomer 3,5-diCQA

Q1 was 0.17% and the number of plants classified in this quartile was 34; the value of Q2 was 0.21%, with 36 plants located in this quartile; the value of Q3 was 0.25% with 36 plants located in this group; and the average value of the population in Q4 was 0.29%, with 37 plants.

Quartiles for isomer 5-FQA

The average value of Q1 was 0.14% with 32 plants; Q2 was 0.17% with 35 plants classified in this group; Q3 had an average content of 0.2% and 39 plants; finally, the average value for the population of Q4 was 0.24% with 37 plants classified.

The Duncan comparison tests showed significant differences between the average values of every inter-quartile range for each of the tested isomers as well as the content of total chlorogenic acids by UV. Inter-quartile averages, Duncan comparison and the number of plants per range are shown in Table 4.

Table 5 shows the average contents of chlorogenic acids for the three groups and five isomers of the family of chlorogenic acids, quantified in three reference materials of the subject population. In all isomers, it was possible to observe a difference between the highest isomer (5-CQA) and the other isomers. It is set that the group of CQA is highest, followed by the diCQA and FQA isomer groups.

TABLE 4. Average for the Chlorogenic Acids variable expressed in percentage of dry matter per plant in every quartile and for each of the tested isomers.

Isomer	Q1	Plants	Q2	Plants	Q3	Plants	Q4	Plants
5-CQA	2.2 D	32	2.55 C	36	2.75 B	37	3.09 A	38
4-CQA	0.29 D	32	0.33 C	36	0.36 B	38	0.41 A	37
3-CQA	0.20 D	32	0.23 C	36	0.26 B	38	0.30 A	37
3,5-diCQA	0.17 D	34	0.21 C	36	0.25 B	36	0.29 A	37
5-FQA	0.14 D	32	0.17 C	35	0.2 B	39	0.24 A	37
TCGA-UV	3.40 D	32	3.9 C	37	4.16 B	37	4.53 A	37

Different letters show average differences among isomers according to the Duncan test at 5%.

TABLE 5. Average contents (% dm) of chlorogenic acids for the three groups and nine isomers of the family of chlorogenic acids, analyzed in three reference materials and the average value of an F_2 population.

Group	Compound	Parental			Mean F_2
		Maragogype	Bourbon	GQ956	
CQA	5-CQA	1.88	1.76	2.26	2.67
	4-CQA	0.28	0.29	0.35	0.35
	3-CQA	0.21	0.23	0.27	0.25
diCQA	3,5-diCQA	0.22	0.22	0.27	0.23
FQA	5-FQA	0.16	0.12	0.14	0.19
TCGA	TCGA-UV	3.07	2.91	3.62	4.00
	TCGA-HPLC	2.97	2.84	3.54	3.96

Only 27 of the 143 plants and in one of the three reference materials (Maragogipe), the grouping of the CQA coincided with the grouping of the TCGA-UV. The low coincidence in the grouped plants shows the variation for each of these isomers and the independence of the behavior of each of the plants in the population.

The quartiles of the TCGA-UV and that of the 5-CQA isomer coincided in 94 of the 143 F_2 plants and the three reference materials evaluated.

In 42 of the 143 plants, the grouping of isomers 3,5-diCQA matched that of the TCGA-UV. Groupings of TCGA-UV and isomer 5-FQA matched in 54 of the 143 F_2 plants and in two out of the three reference materials tested.

The plants classified in the first quartile or Q1 show the lowest contents found in approximately 25% of the population. The plants found in the first quartile show a better quality cup, so they must undergo evaluations of cupping panels (plants 199, 225, 251 and 300). In Q2, just three plants complied with the group and those plants were 220, 269 and 294, which determines a high variability. Q3 shows plants that exceed the average content of CGA; these plants are 147, 175, 178, 181, 183, 219, 227, 243, 253 and 290; in quartile Q4, plant 198 was the only one whose isomers classified it totally in this quartile, showing the highest contents of CGA.

Plant 198 showed that the five isomers were found in the first quartile exhibiting low contents, while plant 199 showed that the five isomers were found in Q4. This indicates that despite being planted in the same location and plot, next to one another, under the same environmental conditions and with the same agronomic handling, there is a clear difference considering that this is the offspring that shows higher segregation of hereditary characters.

Isomer 5-CQA has a higher content in the GQ956 material with 2.26%, whereas 4-CQA has a content of 0.35 in the population. This content was equal to the one found in the material GQ956 (0.35), coming from the Timor Hybrid. In contrast, the lower material was found in Maragogype, with 0.28% (Fig. 2). Isomer 3-CQA content was 0.25 in the population and it was similar to the one found in material GQ956 (0.27) and Bourbon (0.23). In isomer 3,5-diCQA (Fig. 3), the highest content was found in the reference material GQ956 with 0.27%; in contrast, the lowest content was found in Bourbon and Maragogipe type materials both with 0.22%.

Isomer 5-FQA shows content far from the reference materials concerning the population. The average of the population was 0.19, while the average of the closest reference material was 0.16, which corresponds to Maragogype (Fig. 4). The content of TCGA-UV of the population had a value of 4%, and the reference material GQ956 had the closest content with 3.62%.

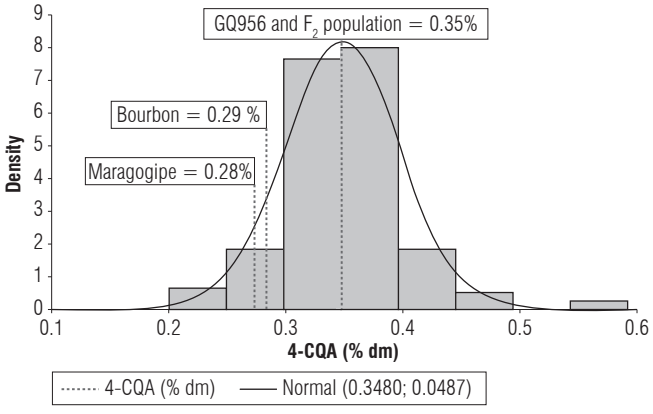

FIGURE 2. Distribution of isomer 4-CQA of the population concerning the reference parentals.

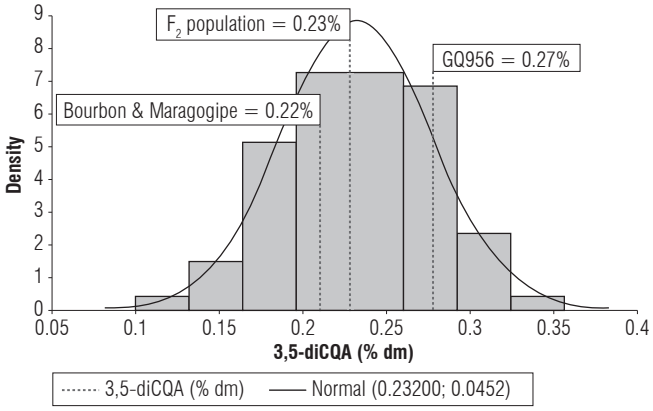

FIGURE 3. Distribution of isomer 3,5-diCQA of the population concerning the reference parentals.

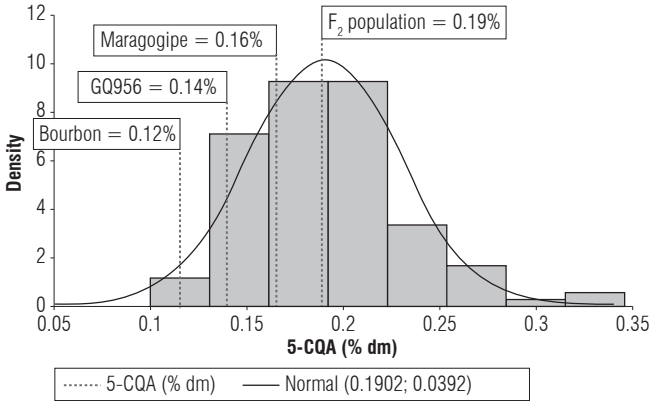

FIGURE 4. Distribution of isomer 5-FQA of the population concerning the reference parentals.

With the ascending hierarchic classification, three groups were found as presented in Figure 5. The first group (Bourbon group) was formed by 86 plants, characterized by the genotype Bourbon as parental and typified for presenting a higher concentration of diCQA and FQA; group two (Timor hybrid - Resistance to Rust) was formed by 42 plants, characterized by the parentals Timor Hybrid and Maragogype and presented a lower concentration of total CQA and the isomers 3, 4 and 5-CQA; group 3 was formed by 18 plants, characterized for presenting higher concentration of total CGA and a higher concentration of 5-CQA. The plants that made up of each group are presented in Table 6.

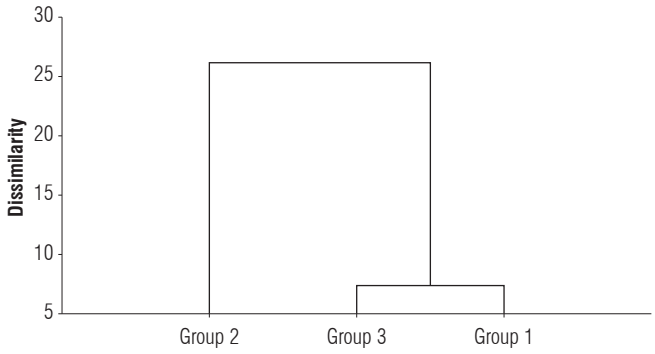

FIGURE 5. Groups formed by the content of 9 isomers of chlorogenic acids in an F_2 population and their parentals.

TABLE 6. Average values (%dm) of each class of the 9 isomer contents of the chlorogenic acids and TCGA by HPLC for the F_2 population and the parentals.

	Group		
Compound	**1**	**2**	**3**
CGA-HPLC	4.07	3.43	4.58
5-CQA	2.73	2.23	3.21
3-CQA	0.26	0.24	0.26
4-CQA	0.36	0.32	0.37
3,4-diCQA	0.13	0.11	0.13
3,5-diCQA	0.24	0.22	0.24
4,5-diCQA	0.10	0.10	0.11
5-FQA	0.20	0.16	0.20
4-FQA	0.03	0.04	0.05
3-FQA	0.02	0.01	0.02
Plants	147, 148, 149, 150, 151, 153, 158, 159, 162, 163, 165, 166, 167, 172, 173, 174, 175, 176, 178, 180, 181, 183, 185, 186, 189, 190, 192, 193, 197, 201, 202, 203, 204, 205, 209, 211, 212, 214, 215, 216, 217, 218, 219, 220, 224, 226, 227, 231, 233, 235, 237, 239, 240, 242, 243, 244, 246, 247, 252, 253, 254, 257, 259, 260, 261, 263, 265, 266, 267, 268, 269, 270, 272, 273, 275, 276, 279, 283, 285, 286, 287, 288, 290, 294, 298, Bourbon	152, 155, 169, 170, 184, 187, 194, 199, 200, 206, 210, 213, 222, 225, 232, 234, 236, 238, 245, 249, 250, 251, 255, 256, 258, 262, 264, 271, 274, 277, 278, 282, 284, 289, 292, 293, 296, 297, 299, 300, GQ956, Maragogype	154, 156, 157, 160, 164, 168, 171, 177, 188, 198, 221, 223, 228, 229, 241, 248, 281, 291

Conclusions

The contents of TCGA by the HPLC or UV methodologies are statistically equal; however, since UV-VIS has a lower cost, it is the best option for the determination of TCGA. The variability of diCQA and FQA makes it necessary to implement the HPLC technique.

The group of caffeoylquinic acids (CQA) represented 82% of the total chlorogenic acids (TCGA). Of the diCQA, 4,5-diCQA showed the lowest contents, the highest isomer was 3,5-diCQA; the proportionality of the isomers was in the order 5-CQA> 4-CQA> 3-CQA> 3,5-diCQA> 5-FQA> 3,4-diCQA> 4,5-diCQA> 4-FQA> 3-FQA, which allows an indirect estimate of each of the isomers.

The results per quartile for TCGA-UV and each isomer indicated statistical differences between groups for each isomer. The population behaved like parental Maragogype according to the contents of 5-CQA, 3,5-diCQA, and TCGA. The contents were higher in the GQ956 material derived from the Timor hybrid 832-1 whose main characteristic is the resistance to rust.

Three groups of plants were formed according to the analyzed isomers, with the Bourbon parent as the main one. The first group was characterized by a higher concentration of diCQA and FQA, whereas the second group had a lower concentration of total CQA and the CQA isomers and the third group was characterized for having a higher concentration of total CGA and a higher concentration of 5-CQA.

This work allowed establishing the bases for the selection of plants in an F_2 population by the total content of chlorogenic acids and isomers derived from CQA, diCQA, and FQA.

Acknowledgments

The authors would like to thank the Ministry of Agriculture and Rural Development for financing this research within the framework of the Coffee Genome Project.

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