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An interdisciplinary approach for evaluating beverage quality in *Coffea canephora*

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Abstract: The objective of this study was to assess the quality of Conilon coffee (Coffea canephora) based on agronomic, physicochemical, sensory profiles, and molecular markers. The dataset comprises 107 genotypes from the Incaper breeding program, evaluated in Marilândia, ES, Brazil. A total of 30 traits and 14 SSR markers were examined, revealing significant genetic variability. Most genotypes exhibited a medium June harvest cycle, uniform ripening, medium-sized beans, high processing yield, and a high percentage of flat and peaberry beans. Physicochemical variables, total titratable acidity, and potassium leaching contributed significantly to the observed variability. Chlorogenic acids (4.82%) and caffeine (2.58%) were the most abundant bioactive compounds. In sensory evaluations, 34 genotypes received scores exceeding 80. 13 markers were recommended for future association mapping studies to identify QTLs influencing the traits evaluated. Results identified genotypes with potential to maximize heterosis and genetic diversity in advancing generations. Among the evaluated genotypes, 34 were identified as promising due to their high sensory scores (≥ 80 points) and favorable agronomic, physicochemical, sensory, and genetic traits. The progeny HS17 stood out as the most divergent in agronomic and genetic traits compared to the other genotypes.

Key words: Assisted selection, Conilon coffee, genetic variability, hybrid progenies, molecular markers, physicochemical analyses.

INTRODUCTION

Coffee is one of the most widely consumed beverages globally. In Brazil, the cultivation of coffee represents a significant pillar of the national economy, with the country occupying the distinction of being the leading producer and exporter of coffee in the world. The state of Espírito Santo is distinguished as the primary producer of the *Coffea canephora* Pierre ex Froehner species, locally designated as "Conilon," with an estimated contribution of 67% to the Brazilian production of the species (CONAB 2023).

The attainment of quality among coffee genotypes represents a significant challenge within the Conilon coffee production supply chain. *C. canephora* breeding programs have focused their efforts on developing coffee varieties that align with consumer preferences and offer enhanced market value (Fonseca et al. 2019). The breeding program of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper) located in the state of Espírito Santo is primarily focused on the development of cultivars that exhibit superior agronomic performance and the subsequent dissemination of these cultivars, which are selected based on their physicochemical, sensory, and genetic traits related to quality. One advantage of this endeavor is the genetic variability of Conilon coffee, which allows for the planting of both clonal cultivars and cultivars derived from seeds.

Given the challenges of achieving quality in Conilon coffee genotypes, the characterization of genotypes developed by breeding programs becomes crucial for selecting superior clones, which may potentially be utilized in the future to develop commercial cultivars with enhanced market value. To obtain a comprehensive evaluation, it is essential to consider the variables of production, post-harvest, and metabolites involved in sensory quality, such as trigonelline, caffeine, sucrose, and chlorogenic acids (Herrera & Lambot 2017). According to Ferrão et al. (2019a), such variables are subject to the influence of the genotype × environment interaction. The majority of these traits are of complex inheritance, determined by many genes, and exhibit low heritability, presenting a significant challenge to breeding programs.

Given these challenges, the integration of molecular markers into breeding programs, as complementary tools to traditional methods, could expedite selection cycles, provided that the association between markers and Quantitative Trait Loci (QTLs) linked to traits of interest is established (Souza 2001). Consequently, the selection of genotypes for the development of new cultivars should consider not only their agronomic performance and genetic diversity, but also their superior physicochemical and sensory characteristics, in order to guarantee a distinctive final product quality (Gomes 2007).

The relationship between quantitative and qualitative traits and coffee quality, particularly beverage quality, has yet to be fully elucidated in the case of Conilon coffee. The majority of studies are based on sampling using Arabica coffee as a model species (Agnoletti et al. 2019, Barbosa et al. 2019, Lemos et al. 2020). These findings reinforce the necessity for continued efforts to elucidate these relationships in Conilon coffee. A comprehensive sampling base and an in-depth analysis of diverse quality-related attributes can serve as a foundation for more effective selection strategies in breeding programs and achieve higher prices on the market.

In view of the aforementioned information, the objective of this study was to characterize and select superior parents and hybrid progenies of Conilon coffee coming from the Incaper breeding program through evaluations of agronomic, physicochemical, and sensory properties related to beverage quality. The findings of this study could provide a basis for the establishment of reference values for Conilon coffee. An additional aim was to study the association between these evaluated properties and Simple Sequence Repeat (SSR) molecular markers, in an effort to identify potential *loci* involved in the control of the traits evaluated.

MATERIALS AND METHODS

Genotypes

A total of 107 Conilon coffee genotypes from the Coffea canephora breeding program of Incaper, in collaboration with Embrapa Café, Brazil, were evaluated. These genotypes consisted of 101 hybrid progenies (HS01 to HS101) and six parental clones (P1 = Clone 02, P2 = Clone 07, P3 = Clone 23, P4 = Clone 24, P5 = Clone 73, and P6 = Clone 153). These materials were selected from hybrid competition trials arranged in a diallel pattern (Figure 1). Data were collected from an experiment conducted during the fourth harvest in Marilândia, Espírito Santo, Brazil. The experimental design was a randomized block

Figure 1. Adult Conilon coffee plants evaluated. (a, b, and c) Clones in the fruit expansion and maturation phase (d) Clones in the flowering stage.

with three replications, each plot containing eight plants, spaced at 3.0 × 1.0 m.

Procedures for data collection and experimental evaluations

The coffee beans were harvested when the plots had more than 80% ripe fruit. Samples comprising of 3.0 kg of fruit were separated for drying on a ventilated drying yard (natural processing) and subsequently hulled. The hulled samples then passed through agronomic evaluations related to hulled coffee yield, classification by type of bean and sieve yield, and physicochemical and sensory evaluations.

Coffee bean yield was determined based on weight at harvest (3 kg of cherry coffee) and the weight of the dried cherry and hulled coffee (11% moisture). For bean classification by type and sieve size, 300 g of each hulled sample was used.

In order to evaluate the physicochemical and sensory profile of each treatment, the three replicates of the hulled beans were combined to form a single sample for each treatment. From each combined sample, only flat coffee beans larger than the 13 sieve were selected. From these samples, for each treatment, 200 g of beans were separated for physicochemical analyses, and 150 g of beans for sensory analyses.

Agronomic evaluations

A total of 9 agronomic traits were evaluated: fruit ripening period (FRP) (classified as very early (April), early (May), medium (June), late (July), or very late (August)); uniformity of ripening (UR) (uniform, moderately uniform, not uniform); coffee bean size (BS) (very small, small, medium, large, very large); ratio between cherry coffee and hulled coffee (CHR) (measured by the ratio of the weight of the cherry coffee sample (3.0 kg) and the weight of the hulled coffee obtained from the 3.0 kg sample); ratio between dry cherry coffee and hulled coffee (DCHR) (measured by the ratio of the weight of the dry cherry coffee sample and the weight of the hulled coffee obtained from the 3.0 kg sample); percentage of flat beans, sieve size 13, 15, and 17 (FLaT); percentage of flat beans, sieve size 15 and above (FLa≥15); percentage of peaberries, sieve size 10, 11, and 12 (PP); and percentage of residue (Re) or bottom of the sieve.

Physical-chemical characterization

Ten properties were evaluated in the physicochemical characterization. Bean moisture content was determined according to the Brazilian Ministry of Agriculture (Ministério de Agricultura, Pesca e Abastecimento do Brasil) (Brasil 1992). The moisture values were used to convert the calculations of the other analyses into bean dry matter. Electrical conductivity (EC, µS cm^{-1} g^{-1}) and potassium leaching (KL, ppm g^{-1}) were evaluated according to Malta et al. (2005). Measurements of pH were made at 25°C and at 96°C following to the protocol described by the Instituto Adolf Lutz (IAL 1985). Total titratable acidity (TTA, mL of 0.1 mol L^{-1} NaOH / 100 g of dry coffee) was determined according to the procedures described in AOAC (1990). The concentrations of sucrose and of the bioactive compounds, including chlorogenic acid [5-caffeoylquinic acid (5-CQA)], trigonelline (1-methylpyridin-1-ium-3-carboxylate), caffeine (1,3,7-trimethylxanthine) and *p*-coumaric acid (4-hydroxycinnamic acid), were determined by chromatographic analysis.

Sucrose was quantified by High Performance Liquid Chromatography (HPLC). Two grams of ground green coffee beans were mixed with 10 mL of Milli-Q water and placed on an orbital shaker for 10 minutes. Next, the fluids were centrifuged twice at 10,000 rpm for 10 min at 4 °C. Then, the samples were microfiltered by a 0.2 µm cellulose acetate filter and injected directly into the chromatographic column. The determination by HPLC in the Shimadzu® brand chromatograph (Prominence model, Kyoto, Japan) was under the following conditions: an Aminex® HPX-87H (300 × 7.8 mm, Bio-Rad, Hercules, CA, USA) ion exchange column, operated at 65 °C and refraction index detector (RID). The mobile phase consisted of a 5 mM sulfuric acid solution, with a flow rate of 0.5 mL min^{-1} and injection volume of 50 µL. The sucrose concentration in the samples was quantified by the external standard method with calibration curve from solutions of known concentrations (1 to 8 g/L) of a sucrose standard (Sigma-Aldrich, St. Louis, MO, USA). The calibration curve, with an R^2 value greater than 0.99, was derived from the peak areas obtained in the chromatogram at the different concentrations. The equation obtained ($y = 888155x + 106868$) was used to calculate the sucrose content in the coffee extracts.

The bioactive compounds were quantified simultaneously by Ultra Performance Liquid

Chromatography (UPLC). Extraction was performed using 0.5 g of ground green coffee beans and 100 mL of Milli-Q water at 80 °C under orbital shaking for 15 minutes. The UPLC analysis was conducted using an ACQUITY UPLC® I-Class chromatograph (Waters® Inc., Milford, MA, USA) under the following conditions: an ACQUITY UPLC® BEH C18 1.7 um column (2.1 × 50 mm); UV/visible spectrophotometric detector fixed at 272 nm. The mobile phase comprised methanol, Milli-Q water, and acetic acid suitable for UPLC (20:80:1), with a flow rate of 0.1 mL min^{-1} and injection volume of 0.1 µL. The external standard method was employed to quantify the concentration of each compound, utilizing solutions of known concentrations [6.25; 12.5; 25; 50; 100 µg mL^{-1} (ppm)] of chlorogenic acid, trigonelline, caffeine, and *p*-coumaric acid standards (Sigma-Aldrich, St. Louis, MO, USA). Calibration curves were obtained with $R^2 > 0.99$. The quantities of metabolites in the coffee extracts were calculated using the following equations: chlorogenic acid ($y = 768.12x - 1216.5$), caffeine ($y = 2473.1x + 1723.8$), trigonelline ($y = 1110.7x + 1655.4$), and *p*-coumaric acid ($y = 4521.8x – 12752$).

Sensory analyses

The sensory evaluations followed the methodology proposed by the Uganda Coffee Development Authority (UCDA 2010). The coffee beans were roasted 24 hours prior to the tasting session. The roasting process was conducted for a duration of 9 to 10 minutes using a Laboratto TGP-2 roaster with Agtron-SCAA discs, resulting in a roast level between colors #65 and #55 (SCAA 2015). Following the roasting process, the beans were permitted to rest for a period of eight hours prior to grinding. Five cups of each coffee sample were prepared using a ratio of 8.25 grams of ground coffee to 150 milliliters of water at a temperature of 92-95°C. The coffee was evaluated by four Q-Graders once the temperature of the cup reached 55 degrees Celsius, four minutes after infusion. The cupping form for the samples encompasses the following attributes: fragrance/aroma, flavor, aftertaste, acidity, sweetness, mouth-feel, uniformity, clean cup, balance, overall, defects, and final score.

Analysis with molecular markers

Preliminary association tests were conducted on the mean values of all agronomic and physicochemical traits using 14 microsatellite markers—11 derived from genomic DNA sequences and 3 from EST-SSRs (Missio et al. 2009, Silva 2013). These markers were previously selected and deemed polymorphic for the Conilon coffee genotypes in the study conducted by Souza et al. (2021). Association analysis with molecular markers was carried out aiming to identify potential loci involved in control of traits of interest, thereby providing valuable insights to guide and enhance future investigations.

Statistical analyses

An analysis of variance (ANOVA) was conducted for each of the evaluated parameters to assess differences among the 107 genotypes. Each parameter was analyzed independently, and when the F-test indicated statistical significance ($p<0.05$), the Scott-Knott clustering method ($p<0.05$) was applied to group genotypes into homogeneous clusters. This approach identified significant differences among the genotypes, providing a clear and objective interpretation of the observed variability. A multivariate statistical analysis was conducted to explore the relationships among the evaluated traits. Pearson's correlation coefficient was applied to assess the degree of association between pairs of traits, with a significance threshold set at $p < 0.05$. The relative contribution of the variables to genetic divergence was quantified in accordance with the criterion proposed by Singh (1981). Additionally, multivariate analyses were conducted on the combined data set, including agronomic variables (excluding FLa≥15), physicochemical variables, and the final sensory scores. Principal component analysis (PCA) and cluster analysis were conducted, excluding variables with strong correlations. In the cluster analysis, the similarity coefficient was estimated using Gower's algorithm (1971), with variables standardized. All analyses were conducted using Genes (Cruz 2016) and R (R Core Team 2017) software, with the 'easyanova' package (Arnhold 2013).

Association between molecular markers and the agronomic and physicochemical traits was assessed through single marker analysis by linear regression, with the coefficient of determination of the regression (R^2) estimated using the GQMOL software (Cruz 2011). The molecular data were also used in cluster analysis by the UPGMA method, with the similarity coefficient calculated from the complement of the weighted index. The resulting molecular dendrogram was then analyzed in comparison with the dendrogram of the combined analyses. To investigate association between the two hierarchical groupings, the Tanglegram algorithm was used with the 'dendextend' package (Galili 2015) of the R software, enabling a visual comparison of two dendrograms through the value of the entanglement coefficient.

RESULTS

Agronomic evaluations

The fruit ripening period (FRP) among the 107 Conilon coffee genotypes ranged from very early to late. Most genotypes exhibited a medium maturation cycle, with harvest occurring in June (50.47%). The ripening of the coffee beans (UR) was observed to be uniform for most of the genotypes (70.10%). The coffee bean size (BS) ranged from small to very large, with most being of medium size (72.90%)

The observed variation and mean values for hulling yield ratios (CHR and DCHR), bean classification by type (flat and peaberry), and sieve yield are summarized in Table I. The table also presents the range of variation, mean values, and the three genotypes with the highest and lowest mean values among the 107 Conilon coffee genotypes evaluated. This information aids in understanding the performance and quality variations within the sample set.

The values for the CHR and DCHR ratios indicate high hulling yields, with mean values of 4.15 for CHR (CV = 6.46%) and 1.98 for DCHR (CV = 3.47%). In relation to the bean type and sieve yield, a high percentage of flat beans (FLaT) and peaberries (PP) was observed. The highest mean values were for FLaT (CV = 23.21%) and FLa≥15 (CV = 23.45%) in comparison to that found for PP (CV = 27.86%). The genotypes P1, HS05, HS11, HS39, HS47, HS48, HS57, and HS94 exhibited the highest values for FLaT and FLa≥15, exceeding 60% (Supplementary Material - Table SI). Many genotypes had small beans, with a high percentage for the variable Re (CV = 21.48%). Detailed data for these traits, by genotype, are available in Table SI.

Physicochemical characterization

The mean data from the physicochemical characterization showed significant differences among the genotypes for all the properties, as determined by Scott-Knott clustering at 5% probability (Table SII). The descriptive statistics for the physicochemical properties of the Conilon coffee genotypes were presented, including the range of variation, mean values, and identification of the three genotypes with the highest and lowest mean values out of the 107 genotypes evaluated (Table II). This

Table I. Descriptive statistics for hulling yield (CHR and DCHR), bean classification (flat and peaberry), and sieve yield, showing variation range, overall mean, and the three genotypes with the highest and lowest means among 107 Conilon coffee genotypes.

Properties*	Amplitude of variation (%)	Mean	Genotypes with < mean	Genotypes with > mean
CHR	2.59 - 4.80	4.18±0.27	HS101; HS79; P3	HS77; HS78; HS87
DCHR	1.61 - 2.05	1.84±0.06	HS02; HS47; HS79	HS21; HS28; HS69
FLa≥15	0.60 - 73.90	32.63±22.96	HS23; HS76; HS84	HS05; HS47; P1
FLaT	1.09 - 74.10	33.15±23.08	HS23; HS77; HS84	HS05; HS47; P1
PP	3.66 - 51.08	22.65±18.93	HS11; HS23; HS31	HS08; HS28; HS38
Re	5.83 - 95.26	44.20±28.48	HS03; HS28; HS40	HS23; HS31; HS84

***CHR: ratio between cherry coffee and hulled coffee; DCHR: ratio between dry cherry coffee and hulled coffee; FLa≥15: total % of flat beans from 15 and 17 sieves; FLaT: total % of flat beans; PP: total % of peaberries; Re: % of beans in the bottom of the sieve. P1 to P6: parents and HS01 to HS101: hybrid progenies.**

Table II. Descriptive statistics of the physicochemical properties, considering the variation range, overall mean, and the three genotypes with the highest and lowest mean values among 107 Conilon coffee genotypes.

Property*	Amplitude of variation (%)	Mean	Genotypes with < mean	Genotypes with > mean
EC	54.72 - 137.24	95.15±5.41	HS09; HS68; HS87	HS38; HS43; HS100
KL	63.33 - 212.00	140.74±8.79	HS23; HS87; HS89	HS05; HS28; HS43
pH (25 °C)	5.48 - 6.28	5.75±0.06	HS72; HS78; HS87	HS54; HS86; HS91
pH (96 °C)	5.39 - 5.77	5.55±0.02	HS59; HS72; HS89	HS11; HS54; HS81
TTA	132.11 - 389.89	264.42±25.67	HS77; HS89; HS98	HS11; HS29; HS60
Sucrose	1.17 - 2.53	1.92±0.09	HS34; HS55; HS57	HS21; HS28; HS68
5-CQA	2.01 - 6.21	4.82±0.35	HS37; HS85; P6	HS29; HS60; HS61
Caffeine	1.53 - 3.46	2.58±0.17	HS17; HS85; HS100	HS61; HS64; HS91
Trigonelline	0.53 - 1.16	0.86±0.06	HS42; HS72; HS85	HS10; HS16; HS80
APC	0.08 - 0.29	0.16±0.01	HS48; HS63; HS85	HS28; HS31; HS62

***EC: electrical conductivity (μS cm^{-1} g^{-1} in dry basis), KL: potassium leaching (ppm g^{-1} in dry basis), TTA: total titratable acidity (mL of 0.1 mol L^{-1} NaOH 100 g^{-1} in dry basis), sucrose (% dry basis), 5-CQA (5-caffeoylquinic acid): chlorogenic acid (% dry basis), caffeine, trigonelline (% dry basis), APC: *p*-coumaric acid (% dry basis). P1 to P6: parents and HS01 to HS101: hybrid progenies.**

summary serves to illustrate the variability and performance of the genotypes with respect to the physicochemical traits.

For the EC (CV = 5.68%) and KL (CV = 6.25%) values, the progenies HS09, HS48, HS68, and HS87 exhibited the best results, with the lowest mean values (Table SII). The progeny HS87 exhibited a significantly lower mean for these variables than the other genotypes (EC = 54.72 μS $cm^{-1}g^{-1}$ and KL = 63.33 μS $cm^{-1}g^{-1}$) (Table SII).

In relation to pH, the values at 96 °C (CV = 0.42%) were lower than those at 25 °C (CV = 1.25%), indicating that the beans became slightly more acidic when heated (Table SII). For the TTA variable (CV = 9.70%), values exceeding 300 mL of 0.1 mol L^{-1} NaOH / 100 g were observed, with the highest mean value for the HS29 sample (389.89 mL de 0.1 mol L^{-1} NaOH / 100 g) (Table SII).

The genotypes exhibited low sucrose concentrations (CV = 4.89%). The progenies HS21, HS28, HS68, and HS72 stand out in exhibiting the highest percentages (2.39% - 2.53%).

For the bioactive compounds, 5-CQA (CV = 7.41%) exhibited the highest concentrations among the genotypes (Table SII). Notably, the progenies HS60 (6.18%) and HS61 (6.21%) demonstrated concentrations that were three times higher than the lowest observed value. Caffeine (CV = 6.58%) was the compound with the second greatest concentration, followed by trigonelline (CV = 7.18%) and *p*-coumaric acid (CV = 12.13%).

Sensory analysis panel

The mean scores for the sensory attributes that were analyzed, including the range of variation, the overall mean scores, and the identification of the three genotypes with the highest and lowest mean scores out of the 107 genotypes evaluated, are summarized in Table III. Most of the attributes exhibited mean scores within the range of 7.10 to 7.55. However, the mean scores for "clean cup" and "uniformity" remained constant at 10 points across all genotypes, indicating no variation.

The final sensory analysis scores for each genotype were listed in Table SII, offering a detailed overview of their performance. The genotypes were divided into two groups by means of a Scott-Knott cluster analysis. Group A, comprising genotypes with scores between 79.09 and 84.63, and Group B, comprising genotypes with scores between 73.38 and 79, had a final mean score of 78.88 points. Of the 107 genotypes, 34 had scores of 80 points or greater (Figure 2). The highest score was observed for Progeny HS89 (84.63 points), followed by P5 parent (Clone 73) and HS68 progeny (83.40 points).

Correlation between the variables

The correlation network (p < 0.05) between the variables is depicted, with the thickness of the lines indicating the strength of the correlation; thicker lines denote stronger correlations (Figure 3a). Green lines represented positive correlations, while red lines represented

Table III. Descriptive statistics of the sensory attributes, considering the variation range, overall mean, and the three genotypes with the highest and lowest mean values among 107 Conilon coffee genotypes.

Attribute*	Amplitude of variation (%)	Mean	Genotypes with < mean	Genotypes with > mean
F/A	6.63 - 8.00	7.32±0.38	HS64; HS86; HS94	HS19; HS33; P5
Flavor	6.75 - 8.38	7.55±0.39	HS21; HS91; HS94	HS63; HS89; P5
After	5.63 - 7.88	7.10±0.46	HS21; HS22; HS29	HS72; HS89; P5
Acidity	6.38 - 7.88	7.20±0.38	HS21; HS91; HS94	HS03; HS89; P5
S/B	5.63 - 8.25	7.42±0.51	HS54; HS58; HS83	HS03; HS68; HS89
Mouth	6.63 - 8.13	7.45±0.41	HS57; HS83; HS94	HS33; HS75; HS89
BAL	6.63 - 8.13	7.37±0.37	HS83; HS91; HS94	HS33; HS68; HS89
Overall	6.63 - 8.13	7.39±0.41	HS54; HS64; HS94	HS68; HS72; HS89
Score	73.38 - 84.63	78.88±2.32	HS21; HS54; HS91	HS68; HS89; P5

***F/A: fragrance/aroma; After: aftertaste; S/B: sweetness/bitterness; Mouth: mouth-feel; BAL: balance; Overall: overall impression; Score: final score. P1 to P6: parents; and HS01 to HS101: hybrid progenies.**

negative correlations. The principal agronomic correlations included BS × FLaT, BS × FLa≥15, BS × Re, FLaT × CHR, FLaT × DCHR, FLaT × Re, and FLa≥15 × Re. For the physicochemical variables, strong positive correlations were observed between Caf × 5-CQA and KL × EC.

All the sensory attributes demonstrated a significant positive correlation with one another; however, they exhibited only a limited number of significant correlations with the other variables under evaluation (Figure 3a). The sucrose variable was the only physicochemical property to demonstrate a significant positive correlation with the sensory attributes. The most significant negative correlations were exhibited by caffeine, followed by the variables EC and pH25.

Principal component analysis (PCA) and cluster analysis by the UPGMA

Due to strong correlations among the variables, only specific characteristics were included in the in PCA. For the classification variables by type and sieve, only CHaT, MoT, and Re were considered, as the other variables showed strong correlations with at least one of these. In the sensory analysis, only the global rating variable was used, as it had a strong correlation with the other sensory attributes (Figure 3a). The distribution of the genotypes and the variables used to characterize them with respect to the two principal components (PC1 and PC2) is depicted in Figures 3b1 and 3b2, respectively

A negative correlation was between the final score variable and caffeine and with pH 25 °C (opposite quadrants in PCA), confirming what was found in the Pearson correlation shown in Figure 3a. In relation to the projection of individuals, wide differentiation of the genotypes HS11, HS28, HS42, HS47, HS61, HS79, HS89, HS91, and HS101 was observed.

Genotypes with the highest concentrations of caffeine, 5-CQA, and pH also exhibited the lowest sensory scores (Table SII). This pattern

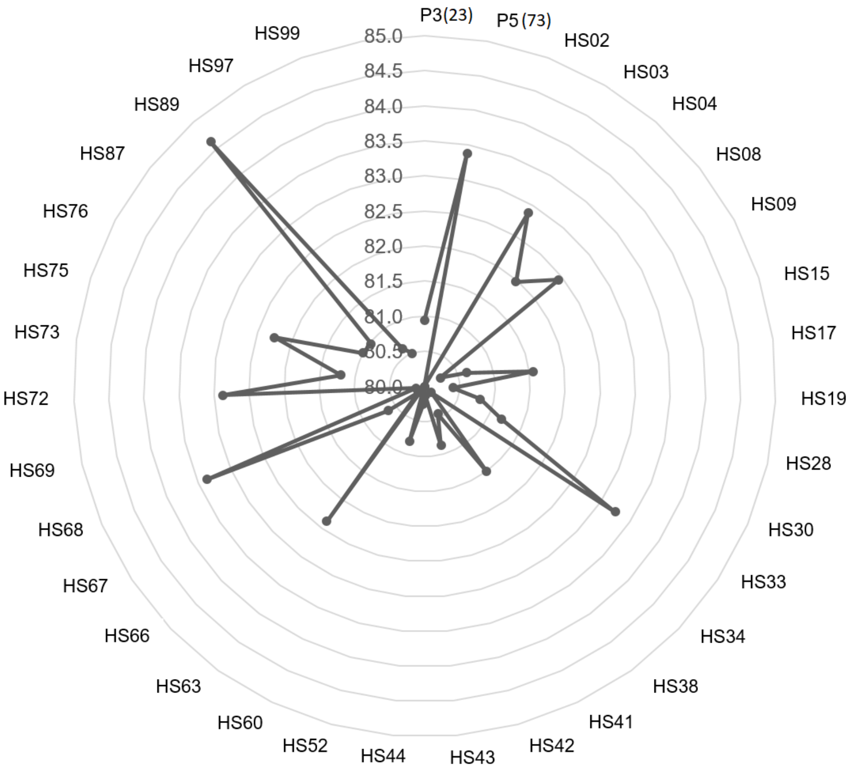

Figure 2. **Final sensory scores of the 34 Conilon coffee genotypes that received ≥ 80 points. The vertical axis represents sensory scores, while 'P' and 'HS' followed by numbers refer to the parents and progenies, respectively.**

was evident in genotypes HS91, HS54, and HS57, which recorded sensory scores below 75 points. Conversely, genotype HS89 displayed a high sensory score, which was associated with a higher concentration of sucrose and lower values of KL, pH, caffeine, and 5-CQA. These findings suggested that genotypes tend to cluster according to the relationship between the physicochemical and sensory traits. Genotypes with higher sensory scores were positioned in the principal components according to lower values of TTA, pH, EC, KL, caffeine, and 5-CQA (highlighted in red). These results corroborate those found in the Pearson correlation (Figure 3a).

In cluster analysis (Figure 4), variability among the genotypes was evident, with the formation of 11 distinct groups (G_c1 to G_c11) in the dendrogram. Among these groups, four consisted of a single individual each: G1 (HS85), G2 (HS28), G3 (HS17), and G4 (HS21). The greatest dissimilarity found was between the progenies HS28 and HS85, which formed isolated groups in the dendrogram (G2 and G1, respectively). The shortest distance was between HS30 and HS96, both of group G11.

Association analysis with molecular markers

The association analysis of the agronomic and physicochemical properties with the 14 polymorphic microsatellite markers for Conilon coffee revealed that 13 markers exhibited a significant association with at least one of the studied variables (Table IV).

The variables UR, DCHR, CHR, and trigonelline were not found to be associated with any of the markers evaluated (Table IV). The markers SSRCa018, SSRCa085, SSRCa088, and SSRCa091 were the only ones associated with agronomic traits (FRP and BS). The markers ESTCOF 21 and GENCOF29 showed significant associations exclusively with physicochemical traits, with TTA being the common variable linked to both. For this variable, these markers accounted for 6.19% and 7.46% of the phenotypic variance, respectively. Notably, SSRCa085 was significantly associated with 11 traits, while SSRCa084 and SSRCa091 were each linked to only one trait, pH 25 °C (7.31%) and BS (8.065%), respectively.

The hierarchical clustering of the combined variables (agronomic, physicochemical, and sensory) along with the molecular data and their associations is depicted in Figure 4. As previously stated, the dendrogram of the combined analyses was divided into 11 groups (G_c1 to G_c11), while that of the molecular analyses was divided into 15 groups (G_m1 to G_m15). The entanglement coefficient for the comparison of these dendrograms was 0.51 (entanglement = 0.51).

The genotypes that received scores of 80 points or higher for the sensory evaluation were grouped into different clusters, indicating both genetic and quality variability among the materials (Figure 2 and Figure 4). Genotypes P2 (Clone 07), HS03, HS10, HS19, HS23, HS41, HS55, HS58, HS69, HS73, HS84, HS89, and HS90 exhibited both genetic and quality similarity, remaining united in the same group in both dendrograms (G_c11 in the combined analyses, and G_m15 in molecular analysis). A similar pattern was observed for genotypes HS06 and HS26 (G_c14 and G_m10) and HS50 and HS51 (G_c5 and G_m6).

The progenies HS52 × HS44 and HS81 × HS79 are indicated with red lines in Figure 4, as they represent individuals that remained united in the same clade, regardless of the group in which they were arranged in the two dendrograms. This indicates a high degree of similarity for the variables evaluated. In contrast, progenies HS08 and HS09, although genetically identical with zero dissimilarity (G_m14); displayed divergence

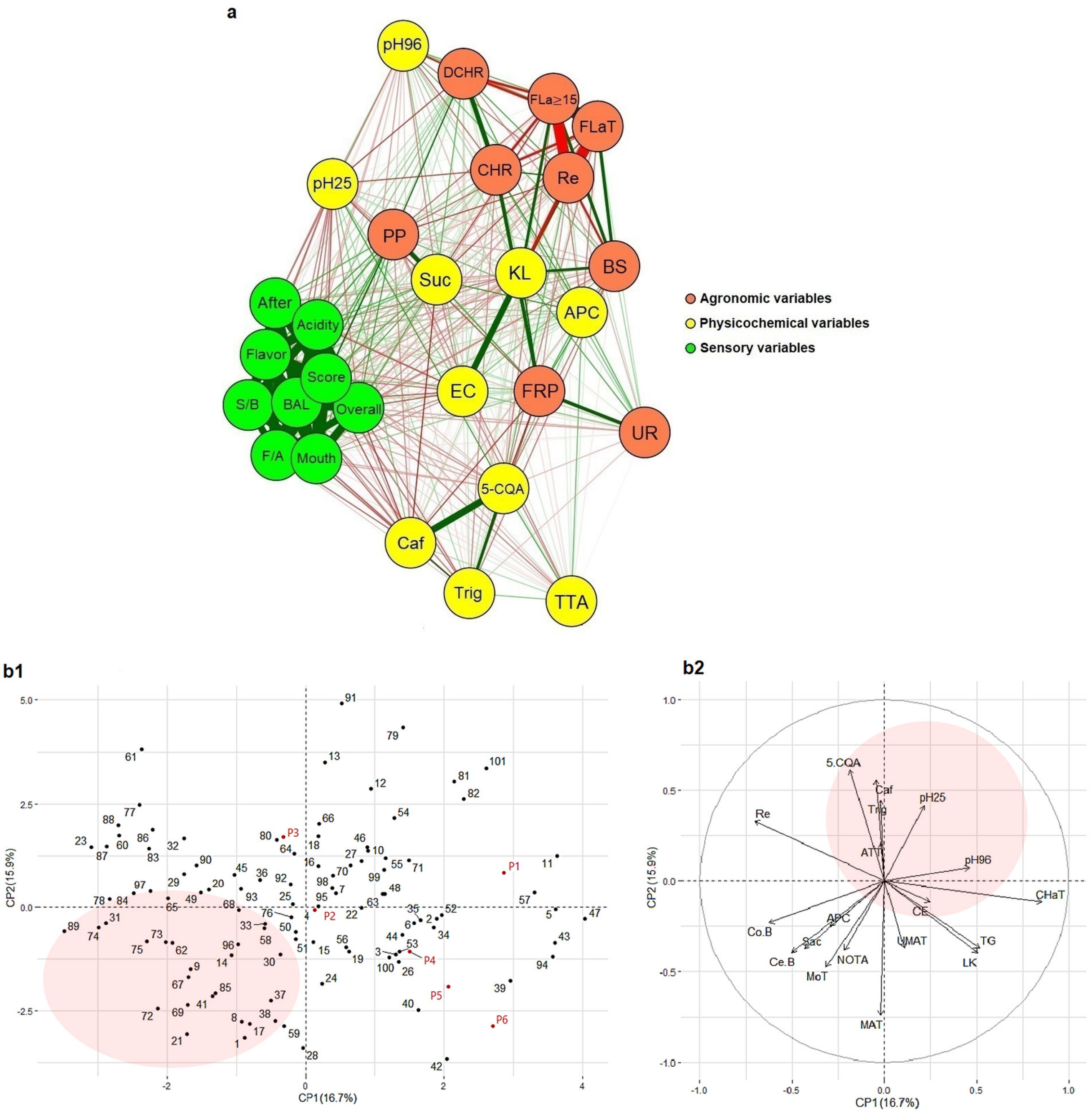

Figure 3. (a) Pearson correlation coefficients ($p < 0.05$) between evaluated properties. (b) Principal component analysis (PCA) of 107 Conilon coffee genotypes and 19 variables. (b1) Biplot of genotype distribution on the first two principal components (PC1 and PC2). (b2) Biplot of variables according to PC1 and PC2. FRP: fruit ripening period (VE = very early; E = early; M = medium; T = late); BS: coffee bean size (S = small; M = medium; L = large; VL = very large); UR: uniformity of ripening (U = uniform; MU = medium uniformity); DCHR: ratio between dry cherry coffee (Kg) and hulled coffee (Kg); CHR: ratio between cherry coffee (Kg) and hulled coffee (Kg); Re: % of coffee beans retained in the sieves below 13 and 10; FLaT: total % of flat beans from 13, 15, and 17 sieves; PP: total % of peaberries retained in the 10, 11, and 12 sieves; FLa≥15: total % of flat beans from 15 and 17 sieves; TTA: total titratable acidity (mL of 0.1 mol L^{-1} NaOH 100 g^{-1} in dry basis); Suc: sucrose (% dry basis); Caf: caffeine (% dry basis); 5-CQA (5-caffeoylquinic acid): chlorogenic acid (% dry basis); EC: electrical conductivity (µS cm^{-1} g^{-1} in dry basis); APC: *p*-coumaric acid (% dry basis); KL: potassium leaching (ppm g^{-1} in dry basis); pH25: pH at 25 °C; pH96: pH at 96 °C; Trig: trigonelline (ppm g^{-1} in dry basis); F/A: fragrance/aroma; After: aftertaste; S/B: sweetness/bitterness; Mouth: mouth-feel; BAL: balance; Overall: overall impression; Score: final score of sensory analysis. P1 to P6: parents, 1 to 101: hybrid progenies (HS01 to HS101).

for the combined variables and were placed in different clades within group G_c11.

In both the clustering of the combined variables and the molecular analysis, groups comprising a single individual were identified. Notably, genotype HS17 was highlighted, as it remained in an isolated cluster in both dendrograms (Gc3 and Gm13, Figure 4). In contrast, the progenies HS21, HS28, and HS85, constituted isolated groups in the dendrogram of the combined variables (G_c4, G_c2, and G_c1, respectively), yet were unified in the same group in the molecular analysis (G_m15).

DISCUSSION

Agronomic evaluations

The variability observed in the fruit ripening period among the genotypes can be strategically utilized, especially for clones intended for commercial planting, staggered harvesting, and improving beverage quality. Environmental factors such as elevated temperatures hasten the maturation process, thereby reducing the accumulation of metabolites that are crucial for the production of a superior quality beverage (Geromel et al. 2008). Lemos et al. (2020) demonstrated that the maturation process influences the chemical and sensory profile of coffee beans. Therefore, the genotypes that

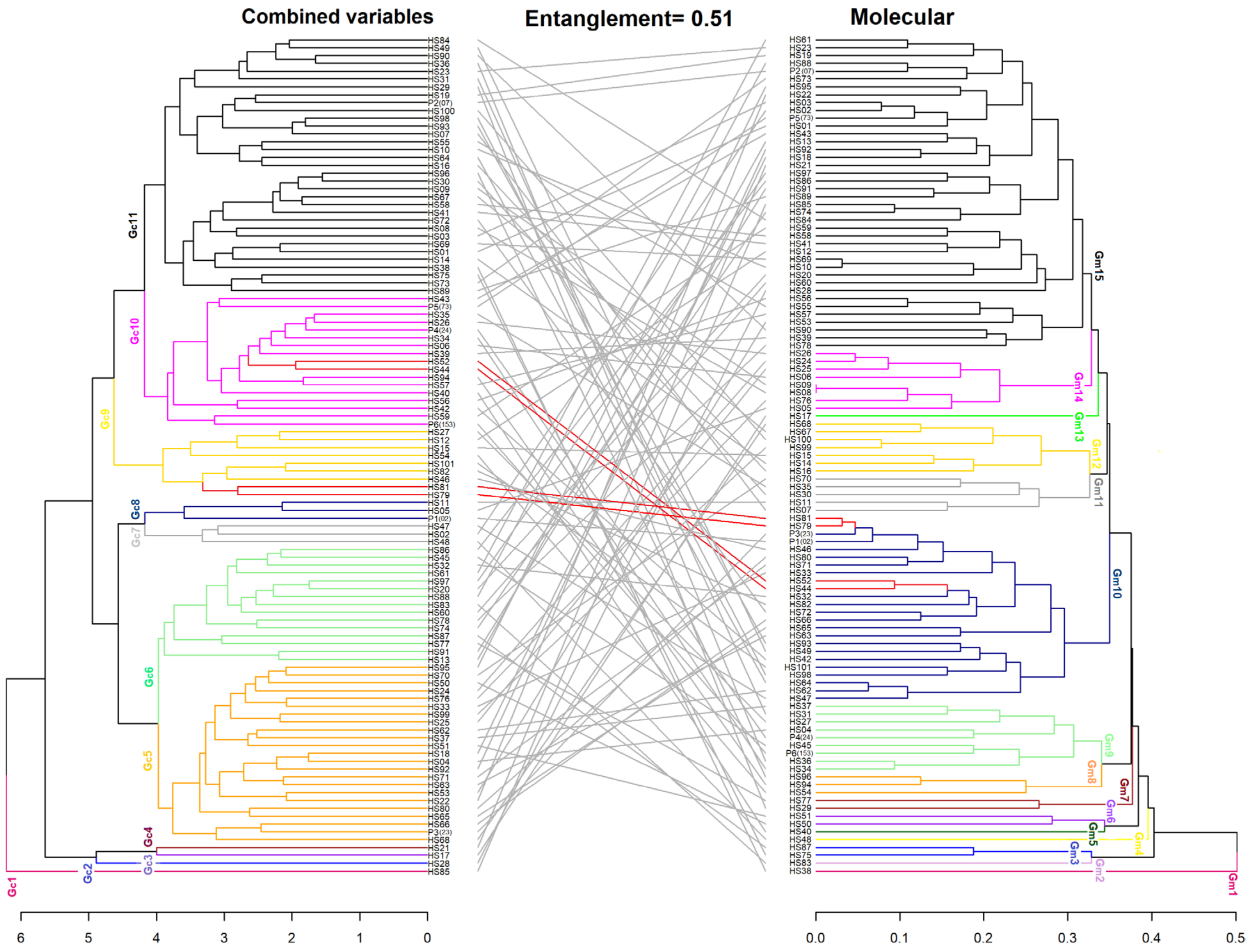

Figure 4. Comparative dendrogram representing the entanglement among 107 genotypes of Conilon coffee, based on the Gower distance and Weighted Index, considering the combined (agronomic, physicochemical, sensory) (cutoff point: 63.60%) and molecular (cutoff point: 63.93%) variables, respectively.

exhibited medium to late maturation may be more pertinent for selection in regions with higher temperatures where Conilon coffee is adapted. This approach would ensure that the accumulation of physicochemical compounds in the coffee bean is not affected, ultimately leading to the production of superior-quality coffee.

Most of the evaluated genotypes exhibited uniform ripening and medium-sized beans. These characteristics align with those of recently released clonal cultivars of Conilon coffee (Ferrão et al. 2015, 2019b), suggesting that these genotypes are promising candidates for clonal selection. Regarding yield after hulling, Conilon coffee breeding efforts focus on reducing the CHR and DCHR ratios to increase the industrial yield and, consequently, the commercial value (Ferreira et al. 2005). For Conilon coffee, the CHR ratio averages 4:1 (4.0 Kg of cherry coffee yields 1.0 Kg of hulled coffee) (Ferrão et al. 2007a). The genetic materials evaluated in this study showed an average CHR of 4.15 and a DCHR of 1.98, which were consistent with the expected values for the species. The materials of the late maturation cycle had lower yields (CHR ratios greater than 4) compared to the earlier to medium cycle materials (Table SI). Of the total materials evaluated, 32 clones (of very early to medium maturation cycle) had mean values for CHR less than 4. These materials can be recommended for gains in yield, after validation of these results with data from other crop seasons.

Sieve yield classification is crucial for commercializing coffee, as it standardizes the beans and ensures a more uniform roasting process. Selecting materials with a higher percentage of flat type beans (FLaT) from sieve sizes 15 and above is advantageous compared to peaberry beans, which are less preferred for export and specialty coffee markets (Fernandes et al. 2020, Matiello et al. 2015). In the evaluated genotypes, the mean values for FLaT and FLa≥15 were higher than those for peaberry beans (PP). The mean values of PP were found to be within the range recommended by Incaper for Espírito Santo cultivars (20% to 32%) (Ferrão et al. 2015, 2019a), indicating the suitability of the beans for export and production of specialty coffee.

The genotypes exhibited a high percentage for the variable Re, which is generally considered unfavorable for breeding of the species. However, as the data pertained to the 2017 crop year, the high percentage of Re may be attributed to drought and high temperatures in the growing regions during the preceding years, 2015 and 2016. These environmental conditions likely impacted fruit growth and development, leading to lower sieve yields (CONAB 2017).

Physicochemical characterization

The results obtained highlighted significant variability among the compounds evaluated and across the genetic materials, offering valuable insights for the breeding program of the species. This variability can be strategically used to guide the selection of superior genotypes that combine characteristics associated with higher coffee quality. The EC and KL variables are indicators of the integrity of the coffee bean cell membranes. Higher values of these variables suggest membrane degradation and loss of permeability control (Martinez et al. 2019). The typical range for EC in coffee is 56.68 to 213.19 µS cm-1 g-1, while KL values range from 15.48 to 132.30 ppm g-1, with lower values typically indicating higher coffee quality (Pinheiro et al. 2012, Partelli et al. 2014, Rodrigues et al. 2019, Araújo et al. 2020). In line with these findings, genotypes with lower EC and KL values can be considered promising, especially for regions with adverse environmental conditions that may accelerate cellular degradation. Most of the genotypes evaluated had EC and KL values within

Table IV. Association analysis of microsatellite markers with the agronomic and physicochemical variables performed through single marker analysis by linear regression.

Marker	Forward and reverse primer (5'>3')	Variable[1]	R^{2+} (%)	*p*-value
ESTCOF13	CCATCACTTCATATCGGTCC ATTCCTTCCCCTAATCTCCC	FLaT	7.774	0.015*
ESTCOF21	GACTATGGTGTGGGCTGGTA TGGTCCCGATAAGATTCAAG	TTA	6.197	0.038*
		Sucrose	7.032	0.024*
		5-CQA	7.111	0.023*
ESTCOF58	CCAAATGGGTTGAAAGCTAC GATTTCAGGGTGAAAGCAAG	5-CQA	3.7223	0.0465*
		KL	3.8174	0.0437*
		pH 96 °C	3.8141	0.0438*
		Re	22.1919	0.0 **
		FLaT	18.4699	0.0 **
		FLa≥15	18.6295	0.0 **
GENCOF29	CCTCGCTATCTGGTTCATTT CACAACTTGGGTACTTGCTG	TTA	7.463	0.018*
		APC	5.954	0.042*
		EC	5.981	0.042*
SSRCa018	GTCTCGTTTCACGCTCTCTC	APC	6.710	0.029*
	ATTTTTGGCACGGTATGTTC	BS	5.812	0.047*
SSRCa040	AGGGATGTAGAACCAGCAAA CCAATAGCTCACAACAAAGG	APC	5.4329	0.0157*
		FLaT	7.2206	0.0051 **
		PP	3.7386	0.046 *
		FLa≥15	7.0171	0.0058 **
SSRCa052	GATGGAAACCCAGAAAGTTG TAGAAGGGCTTTGACTGGAC	Caffeine	9.098	0.008**
		5-CQA	6.627	0.031*
		KL	8.665	0.01*
		Re	13.283	0.001**
		FLaT	11.938	0.002**
		FLa≥15	11.966	0.002**
SSRCa084	ATCGGAAAGATGTCAACCAT CAAATTGAAGCCAGTGGTG	pH 25 °C	7.31	0.029*

Table IV. Continuation.

Marker	Forward and reverse primer (5'>3')	Variable[1]	R^2[+] (%)	*p*-value
SSRCa085	ATGTGAAAATGGGAAGGATG CACAGGAAAGTGACACGAAG	Sucrose	6.066	0.044*
		KL	11.01	0.003**
		pH 96 °C	8.843	0.01**
		FRP	15.154	0.0**
		BS	7.428	0.021*
		Re	8.548	0.011*
		FLaT	14.093	0.0**
		PP	7.959	0.016*
		FLa≥15	13.911	0.001**
SSRCa087	TCACTCTCGCAGACACACTAC GCAGAGATGATCACAAGTCC	APC	5.3023	0.0175*
		pH 96 °C	5.4892	0.0156*
		Re	3.8009	0.0452*
		FLaT	5.1551	0.0193 *
		FLa≥15	5.2519	0.0181 *
SSRCa088	CGTCTCGTATCACGCTCTC TGTTCCTCGTTCCTCTCTCT	Sucrose	4.7324	0.0244 *
		BS	4.4434	0.0293 *
		FLaT	7.8099	0.0036 **
		PP	5.3993	0.016 *
		FLa≥15	7.831	0.0035 **
SSRCa091	CGTCTCGTATCACGCTCTC TGTTCCTCGTTCCTCTCTCT	BS	8.065	0.014*
SSRCa095	GAGAGAGCCGAGTGAAGAGA	TTA	9.456	0.009**
	GAGAGAGAAGCCATGATTTGA	APC	9.263	0.01*

FRP: fruit ripening period; BS: coffee bean size; Re: % of coffee beans retained in the sieves below 13 and 10; FLaT: total % of flat beans from 13, 15, and 17 sieves; PP: total % of peaberries retained in the 10, 11, and 12 sieves; FLa≥15: total % of flat beans from 15 and 17 sieves; TTA: total titratable acidity (mL of 0.1 mol L^{-1} NaOH 100 g^{-1} in dry basis); Suc: sucrose (% dry basis); Caf: caffeine (% dry basis); 5-CQA (5-caffeoylquinic acid): chlorogenic acid (% dry basis); EC: electrical conductivity (µS cm^{-1} g^{-1} in dry basis); APC: *p*-coumaric acid (% dry basis); KL: potassium leaching (ppm g^{-1} in dry basis); pH25: pH at 25 °C; pH96: pH at 96 °C. [+] Proportion of the phenotypic variance explained by the marker; * Significant at 5%; ** Significant at 1%.

the expected ranges for the species, suggesting superior quality, minimal deterioration, and well-structured cell membranes, as supported by the aforementioned studies.

The pH values reported in studies on *C. canephora* range from 5.27 to 6.13 (Bicho et al. 2013), 5.83 to 6.17 (Pinheiro et al. 2019), and 5.58 to 5.83 (Pereira et al. 2020). These values fall within the normal range for coffee beans, indicating appropriate processing conditions. Deviations from this range, especially lower pH levels, may signal undesirable fermentation processes that negatively affect bean quality (Pinheiro et al. 2019). Given that most of the genotypes evaluated in this study fell within these ranges, it suggests that the beans were processed without harmful fermentation.

Total titratable acidity (TTA) displayed significant variability among the evaluated genotypes, with some values (300 mL of 0.1

mol L^{-1} NaOH / 100 g) exceeding those typically reported for *C. canephora*. Previous studies on Conilon coffee have reported TTA values ranging from 105 to 175 (Mori et al. 2018), 128.33 to 220 (Pinheiro et al. 2019), and 212.50 to 238.12 mL of 0.1 mol L^{-1} NaOH / 100 g (Partelli et al. 2014). In coffee, acidity is largely driven by organic, chlorogenic, and quinic acids, which are produced through both endogenous pathways and fermentation processes. The specific acid profile, including the types and concentrations of acids present, plays a critical role in determining the overall quality of the beverage (Igamberdiev & Eprintsev, 2016). Given that the individual acids were not quantified in this study, further research is essential to identify the acids contributing to the higher TTA observed in some genotypes. Understanding this relationship is crucial for determining how TTA might influence flavor profile and, consequently, the overall quality of the coffee.

Sucrose levels in green Conilon coffee beans typically range from 3% to 7%, with previous studies reporting lower percentages, such as 1.40% to 2.49% (Pinheiro et al. 2012), 1.32% to 2.31% (Pinheiro et al. 2019), and 1.64% to 2.85% (Agnoletti et al. 2019). Our findings align with these results, showing low sucrose values. Sucrose is crucial for the formation of compounds that contribute to the coffee’s color, aroma, and flavor during roasting (Scholz et al. 2016, Farah & Lima 2019). However, lower sucrose concentrations, as seen in our results, may limit the formation of these beneficial compounds, potentially leading to reduced quality in the final beverage.

Chlorogenic acids (CGAs), particularly the 5-CQA isomer, are key bioactive compounds in green coffee, influencing sensory qualities such as bitterness, acidity, and astringency in the final beverage. The mean 5-CQA concentration found in the present study (4.82%) falls within the typical range for Conilon coffee, as reported by Bicho et al. (2013), Farah & Lima (2019), and Pinheiro et al. (2019). These compounds degrade during roasting, contributing to the formation of precursors that enhance these sensory attributes (Farah et al. 2006, Schenker & Rothgeb 2017). Genotypes with lower CGA levels, such as HS85 (5-CQA < 3%), are of particular interest, as they could reduce undesirable acidity, while genotypes with elevated 5-CQA (HS60 and HS61) also exhibited higher total titratable acidity (TTA), suggesting a direct link between CGA levels and acidity in the coffee.

Caffeine is another critical bioactive compound in Conilon coffee, with concentrations typically ranging from 2% to 4% (Ferrão et al. 2019a). Elevated caffeine levels have been correlated with increased bitterness (Cheng et al. 2016, Mori et al. 2018), making it an important trait to manage in breeding programs. Genotypes such as HS17, HS37, HS85, and HS100, with caffeine levels below 2%, may offer a potential in crosses designed for reducing bitterness in the coffee, which is desirable for enhancing beverage quality.

Trigonelline, found in lower proportions, plays a significant role in forming volatile compounds and vitamin B3 during roasting (Monteiro & Trugo 2005). The concentrations observed in this study align with those found in previous research (Fonseca et al. 2011, Agnoletti et al. 2019), indicating consistent results across different genotypes.

P-coumaric acid (p-CA), a precursor in the biosynthesis of chlorogenic acids (CGAs), was also found in low concentrations. This aligns with its role in the metabolic pathways leading to 5-CQA synthesis. Given the high levels of 5-CQA observed in the genotypes, the expected lower concentration of free p-coumaric acid supports this biosynthetic relationship. Similar findings were reported by Belguidoum et al. (2014) and Wongsa et al. (2019).

It should be noted that physicochemical studies in significant sampling numbers for Conilon coffee are still limited. Most of the associations are discussed in studies using arabica coffee as the model species. Thus, the results shown here, considering a set of 107 genotypes, offer support for the development of reference values for the physicochemical composition of Conilon coffee. The calibration curves generated for chlorogenic acid, caffeine, trigonelline, and p-coumaric acid, using solutions of known concentrations, were crucial for ensuring the reliability and accuracy of the metabolite quantification. These curves enhance the robustness of the study and provide a methodological foundation for future research, enabling precise comparisons between genotypes and environmental conditions. The use of such calibration curves contributes to the advancement of knowledge on Conilon coffee's biochemical profile and strengthens the significance of these findings within the broader context of coffee research.

Sensory analysis

The results of the sensory evaluation indicated that the genotypes assessed showed “very good” coffee quality, with scores within the range established by the Specialty Coffee Association of America (SCAA). This finding aligned with previous studies, such as Lemos et al. (2020), which also reported a variation in quality within the same scoring ranges. Genotypes that scored above 80 points displayed characteristics associated with specialty coffees, suggesting that these materials have great potential for high-quality coffee breeding programs. The uniformity and absence of undesirable flavors, with consistent evaluations, further reinforced the idea that these genotypes could meet the standards required by the specialty coffee market (SCAA 2015). However, for future research, it would be interesting to investigate the genetic factors underlying this quality, which could guide the development of more resilient cultivars with better sensory characteristics.

Combined analyses: association between the different quality attributes

Coffee quality is influenced by both intrinsic and extrinsic factors, as well as market demands. Evaluating a range of parameters is essential for selecting superior genotypes (Herrera & Lambot 2017, Lemos et al. 2020, Ribeyre 2007). Combining analyses of different quality traits can lead to better decision-making in breeding programs focused on improving beverage quality. This study identified promising genotypes with favorable agronomic, physicochemical, and sensory characteristics for quality coffee. However, few significant correlations were found between sensory attributes and other evaluated traits, reinforcing the complexity of associating sensory profiles with physicochemical parameters (Di Donfrancesco et al. 2014, Pereira et al. 2018).

The variables exhibited weak to moderate correlations overall (Figure 3) (Hu et al. 2020, Paranhos et al. 2014). Positive correlations were found between bean size (BS) and the percentage of flat beans (FLaT and FLa≥15), indicating that selecting for larger beans could also improve the proportion of flat beans. Ferrão et al. (2007b) reported a similar positive genotypic correlation between these traits. The negative correlation between FLaT and CHR/DCHR (Figure 3a) suggests that increasing the proportion of flat beans could lead to higher yields, which is advantageous for commercialization and quality. Strong correlations were also found between caffeine (Caf) and 5-CQA, as these compounds accumulate together in coffee beans and interact chemically, forming a 1:1 complex (Ky et al. 2013), consistent with the findings reported by Scholz et al. (2016). Additionally, the correlation between potassium leaching (KL) and electrical conductivity (EC) was expected, as higher EC values, which indicate greater membrane damage, are associated with higher potassium leaching (Prete 1992).

Weak correlations were found between sensory analysis and the other variables, highlighting the challenge of linking these traits. The sensory profile reflects the physicochemical properties of the beans, so stronger correlations were anticipated between sensory and physicochemical variables (Barbosa et al. 2019). However, as sensory analysis was performed on roasted beans and physicochemical analysis on green beans, this difference likely hindered correlation. Despite low sucrose concentrations in some genotypes, the levels were adequate and did not impact their classification as superior for quality beverages.

Genotypes with higher sensory scores generally exhibited lower electrical conductivity (EC) and pH values, confirming the negative correlations between EC, pH25, and sensory scores. These findings align with previous studies suggesting that EC and pH can affect coffee quality (Mori et al. 2018, Noia et al. 2017).

The data showed a negative correlation between caffeine levels and nearly all sensory attributes, indicating the compound's impact on beverage quality. Increased caffeine levels were linked to reduced sensory scores and enhanced bitterness. Similar findings were reported by Mori et al. (2018) in their evaluation of Conilon coffee genotypes in Espírito Santo, where lower caffeine concentrations led to less bitterness. However, the caffeine concentrations observed in this study did not negatively affect beverage quality, as all genotypes remained within the superior beverage parameters.

Principal component analysis (PCA) revealed a tendency for the genotypes to cluster according to the correlation between the physicochemical and sensory traits. Genotypes with higher sensory scores were positioned in the principal components in accordance with lower values of TTA, pH, EC, KL, 5-CQA, and caffeine and higher values of sucrose, indicating that the physicochemical profile of green coffee beans may serve as an indicator of the sensory quality of the beverage. These findings are in accordance with those previously reported in the Pearson correlation.

The genotypes classified as specialty and/or fine coffees exhibited mean values within that expected in the literature for most of the variables, which certainly contributed to the better sensory profile of these samples. Nevertheless, for some properties, like TTA and sucrose, some genotypes had values outside the range expected for coffee cultivars. These results suggest that the sensory profile was affected in a positive manner by the combination of the different metabolites present in the coffee bean and not by individual traits, confirming that the definition of beverage quality should be evaluated by the combination of the different metabolites and traits (Barbosa et al. 2019, Lemos et al. 2020).

The clustering pattern of the genotypes in the PCA was confirmed in hierarchical clustering. The genotypes that are united in a determined group are also near in the principal component diagram, showing the similarity between them for the properties evaluated. However, the arrangement of the genotypes in groups was not directed in accordance with quality; the genotypes with the best sensory profile were arranged in different groups, but the variables of coffee bean size and acidity were the traits that most contributed to the divergence of the groups formed.

The arrangement of the genotypes in 11 different groups in the dendrogram of the combined analyses shows the variability for the different traits. This variability can support the choice of individuals and/or group of individuals that have greater or lesser similarity for the properties evaluated. The information can also guide selection of genotypes that have specific traits, according to the breeding aims for the species.

The correlation between the analyzed variables and the SSR molecular markers suggests the potential for these loci to be closely linked to the QTLs that regulate these characteristics. According to Schuster & Cruz (2008), if a significant association is detected, it may indicate the genetic connection between the marker and a QTL. A total of 13 markers were detected as associated in a significant manner with at least one of the traits. Therefore, future studies are suggested to confirm the association of these markers with QTLs that control agronomic characteristics, FRP, BS, type of bean (flat and peaberry) and physicochemical characteristics (except trigonelline).

The R^2 values indicate a small to moderate effect of the QTLs (values below 30%). These values are expected, considering that the traits evaluated are, for the most part, controlled by many genes and greatly affected by the environment. In addition, considering that many genes can exercise small individual effects on the traits, smaller variations are expected (Schuster & Cruz 2008).

A possible pleiotropic effect was observed for some markers that had significant associations with more than one trait. Prominent among them is the marker SSRCa052, which explained 9.098% of the phenotypic variation of caffeine and 6.627% of 5-CQA, strongly correlated variables. This result indicates possible pleiotropy of genes near these markers, or that the QTLs that control these traits are strongly linked. If the pleiotropy hypothesis is confirmed and the QTLs are identified, these *loci* can be used for selections aiming at beverage quality. The colocalization of QTLs for organoleptic traits and genes involved in the biosynthesis of caffeine and CGA may be explained by the shared role of these compounds in contributing bitterness to the beverage, highlighting their interconnected influence on sensory attributes (Leroy et al. 2011).

Among the markers, ESTCOF13, ESTCOF21, and ESTCOF58 are EST-SSRs, microsatellites derived from expressed sequence tags, and they are more likely to be related to the functional portions of the genome (Missio et al. 2009). Consequently, it is highly probable that these markers are in fact near the QTLs involved in the traits of coffee bean shape and size and the variables TTA, sucrose, 5-CQA, KL, and pH96.

The markers GENCOF29 (gi|117323957) and ESTCOF58 (GenBank accession number DV677006.1) are related to sequences with functions linked to activities of ion channels (Silva, 2013) and organic cation transporters (Lin et al. 2005), respectively, which may explain their association with the KL (ESTCOF58 - R2 = 3.8174%) and EC (GENCOF29 - R2 = 5.981%) variables.

It is recommended that future studies of associations between markers and traits validate these 13 markers with respect to their location, effects, and stability across diverse environments. Furthermore, it is recommended that the scope be expanded to encompass a larger set of markers, including Single Nucleotide Polymorphisms (SNPs), in association mapping studies. Once validated, these markers could facilitate the selection of superior genetic traits in the enhancement of Conilon coffee through marker-assisted selection (MAS).

The value of the entanglement coefficient utilized in the comparative analysis of the dendrograms derived from the combined and molecular data sets is a parameter that ranges from 0 to 1. A value of 0 indicates total correspondence between the clusters (Galili 2015). The value of around 50% can be considered satisfactory, since the dendrograms were obtained by means of different algorithms and the molecular markers were not associated with all the variables, which may have favored the correspondence identified. Considering the complexity of genetic mechanisms in regulation of quantitative traits, the value found confirms the associations found among the markers and the properties evaluated. At the same time, it indicates that other sets of molecular markers would be necessary to explain the traits with better correspondence.

A comparison between the dendrograms facilitated the identification of molecular variability and the delineation of specific properties among the parents and progenies under evaluation. This information can be used in guided crosses aiming at selection of superior traits in segregating generations (Cruz et al. 2012).

Some genetic materials showed molecular similarity, though divergence for the other quality traits, indicating environmental effects on expression of the traits. The progenies HS08 and HS09 (Gm14) were considered duplicates by Souza et al. (2021) because they had zero dissimilarity. However, these progenies showed differences for the quality attributes evaluated in this study, which suggests environmental effects on these traits and shows that the differences are not hereditary.

The genotypes with sensory scores ≥ 80 points were separated into different groups in both dendrograms, showing the variability among them. The progenies HS04 and HS38 show promise for future selections aiming at beverage quality and possible genetic compatibility because they were the most divergent genetically (Souza et al. 2021) and had sensory scores above 80 points.

Some genotypes were arranged in isolated groups in the dendrogram and can be considered the most divergent in relation to the others, and they may be exploited in coffee breeding programs. The progenies HS17, HS21, HS28, and HS85 can be considered the most dissimilar for the quality attributes because they formed exclusive groups in the combined analysis dendrogram. The progenies HS38, HS40, HS48, HS83, and (once more) HS17 are the most genetically divergent, as evidenced by their isolation in the molecular dendrogram. The progeny HS17, in addition to having a sensory profile for specialty coffee (≥ 80 points), remained isolated in the two analyses, showing its greater divergence in relation to the other accessions.

The agronomic, physicochemical, sensory, and molecular characterization of 107 Conilon coffee genotypes provides valuable information for understanding beverage quality in *Coffea canephora*. The range of genotypes and traits analyzed creates a solid framework for establishing reference values for Conilon coffee, supporting the development of high-quality cultivars and continuous improvements in the sector.

Although the biochemical compounds associated with coffee quality have been previously studied, this research is among the first large-scale efforts to apply an interdisciplinary approach to a Conilon population. Promising genotypes were identified, showing strong performance in productivity, sensory quality, and physicochemical profiles. These combinations of favorable traits highlight their potential use in breeding programs. This study supports the selection of genotypes for the development of new cultivars and hybrids based on their sensory attributes and agronomic characteristics.

Combining molecular data with sensory and physicochemical analyses improves the efficiency of quality enhancement efforts and contributes to the definition of quality standards for the coffee market. With 34 genotypes achieving high sensory scores (≥ 80 points), the study offers a comprehensive resource for future research and breeding efforts focused on beverage quality in *Coffea canephora*.

These findings, along with future research on additional populations, are anticipated to support the development of marker-assisted selection strategies by focusing on the favorable alleles of markers associated with QTLs linked to quality traits.

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SUPPLEMENTARY MATERIAL

Table SI-SII.

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