

# Genetic Diversity and Agronomic Performance of Coffee Genotypes in Organic Agroforestry Systems of São Tomé

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## ABSTRACT

Despite coffee's historical and economic importance in São Tomé and Príncipe, limited knowledge of its molecular and morpho-agronomic diversity has constrained the effectiveness of breeding programs. As part of a farmer-oriented technical assistance initiative, ten coffee (*Coffea* spp.) accessions were collected across São Tomé Island and evaluated using morphological and molecular markers. The experiment, conducted from August 2023 to March 2025 at the Centre for Agronomic and Technological Research, followed a randomized block design. Morpho-agronomic characterization revealed notable phenotypic variation among accessions. Orange fruit color (60.8%), elliptical fruit shape (44.5%), and green endosperm color (73.5%) were predominant. Among the evaluated genotypes, Catuai stood out for its fresh fruit mass and dimensions, while Typica and Bourbon Vermelho showed superior performance in nursery characteristics such as height and number of leaves, respectively. Leaf morphology differentiated varieties: Bourbon vermelho, Catimor, Catuai, Caturra, Mundo Novo, and Tescik were mainly elliptical; Icatu and Típica lanceolate; and Robusta and Bourbon Amarelo oval. Preliminary molecular analysis identified two genetic clusters and suggested the existence of a potentially novel *Coffea arabica* variety within the São Tomé population. These findings provide the first integrative assessment of coffee diversity on the island, laying a foundation for future genetic improvement, germplasm conservation, and sustainable agroforestry-based coffee production in São Tomé.

**Key words:** *Coffea* spp.; morpho-agronomic characterization; molecular diversity; genetic clustering.

## 1 INTRODUCTION

Coffee (*Coffea* spp.) is one of the most socioeconomically important crops worldwide (Montagnon & Bouharmont, 1996). However, environmental fluctuations associated with climate change increasingly affect the growth and development of *Coffea arabica*, particularly during the seedling stage, which is critical for successful establishment (Castillo & Andrade, 2021). Identifying and evaluating environments that provide optimal conditions for early plant growth is therefore essential.

In major organic and agroecological coffee-producing countries such as Mexico, Costa Rica, and Colombia, as well as several African nations, the predominant cultivars include 'Típica', 'Bourbon', 'Caturra', and 'Garnica'. At the same time 'Catuai' and 'Mundo Novo' are cultivated to a lesser extent (Moura et al., 2013). Evaluation and selection of new cultivars with traits adapted to local environments remain a central objective of breeding programs, as these efforts generate region-specific recommendations (Tounekti et al., 2017; Veiga et al., 2020; Atinafu & Mohammed, 2021; Merga et al., 2023). Despite this progress, limited research has focused on identifying cultivars suitable for organic systems. Major challenges include the large number of genotypes requiring evaluation, the need for multi-location trials, and the long assessment periods typical of

perennial crops (Moura et al., 2014).

In São Tomé and Príncipe, an equatorial archipelago in the Gulf of Guinea, coffee was introduced in the early 19<sup>th</sup> century. Despite initial resistance, it rapidly became a cornerstone of the national economy (Souza, 2021). The Monte Café region, historically significant for coffee production, experienced a sharp decline following independence in 1975 (Salvador & Lucas, 2020). Today, organic agroforestry coffee systems dominate production, with the Biological Coffee Export Cooperative (CECAFEB) leading the way. CECAFEB manages approximately 560 ha planted with *C. arabica* and *C. canephora* (Robusta), engaging 552 farmers who produced an estimated 86,300 kg of cherries in the 2018/2019 harvest, valued at €48,328 (Cooperativa de Exportação de Café Biológico - CECAFEB, 2020). Initially focused on Arabica, CECAFEB has expanded into Robusta cultivation to meet both domestic and export demands, while investing in quality improvement and value addition to enhance market competitiveness (CECAFEB, 2014).

In recent years, *C. arabica* production in Roça Monte Café has declined due to the abandonment of coffee plots in favor of horticultural crops (Faisandier, 2019), irregular flowering patterns, and phytosanitary challenges (Carvalho et al., 2021; 2023). Currently, coffee ranks as the fifth most

important export product of São Tomé and Príncipe, after cocoa (*Theobroma cacao*), palm oil (*Elaeis guineensis*), coconut oil, chocolate, and black pepper (*Piper nigrum*), and thrives in volcanic soils under an equatorial climate (Banco Central de São Tomé e Príncipe -BCSTP, 2026).

Several factors influence coffee yield, including climate, genetic diversity, soil characteristics, shading, and management practices such as mulching and pruning (Zebire et al., 2021). The sustainability of coffee production is threatened by accelerated climate change. However, genetic improvement of coffee can help the production chain in the face of current global changes (Ferrão et al., 2024). To withstand the current climate change, farmers have to learn crop cultivation techniques, strategies to protect crops from diseases, and understand which type of seed varieties to use to avoid crop loss (Jayanna et al., 2021). Local observations suggest that uncontrolled natural crossing in farmers' fields has contributed to the genetic degeneration of traditional varieties in Roça Monte Café. Therefore, characterizing the genetic diversity and agronomic performance of local accessions is critical for developing a structured breeding and conservation program suited to São Tomé's agroforestry systems.

This study aimed to evaluate ten coffee accessions through molecular and morpho-agronomic characterization to identify potential parental lines for establishing a breeding program adapted to the organic agroforestry conditions of São Tomé Island.

## 2 MATERIAL AND METHODS

### 2.1 Study area

The study was conducted on São Tomé Island, located approximately at 0°N and 6°30'E, with a total area of about

859 km<sup>2</sup>. The island is dominated by a volcanic mountain rising to 2,024 m above sea level. The climate is sub-equatorial with high rainfall, ranging from 1,500–1,800 mm annually in low-altitude regions (north and northeast) to over 6,000 mm in high-altitude areas (south and southwest). The driest months are June through August, while March to May represent the wettest period. Average annual relative humidity is high, and the mean sea-level temperature is approximately 25.4 °C (Afonso, 1969; World Bank, 2017).

Field activities were conducted at the Industrial Culture Experimental Base of the Centre for Agronomic and Technological Research (CIAT-BECI) between August 2023 and March 2025.

### 2.2 Sample collection

A total of 20 ripe fruits were collected in August 2023 from each of ten coffee accessions cultivated across nine plantations located at different altitudes: CIAT-BECI (321 m), Bemposta (1,055 m), Novo Destino (673 m), São Carlos (1,238 m), and Água Coimbra (847 m). The accessions were identified by CECAFEB's socio-technicians based on local naming conventions (Table 1). All samples were transported to CIAT-BECI's laboratory for further molecular and morphological analysis.

### 2.3 Morphological data collection

Different morphological data were recorded at different stages of plant growth of the coffee, including fruit weight, shape, colour, and transverse and longitudinal diameter. After characterising the fruit, the seeds were characterised. To do this, the fruit was opened to extract the seeds, and then the weight of the seeds, the colour of the endosperm, length, width, and thickness were obtained. Characterisation followed the procedures described by the International Union for the

**Table 1:** Characteristics of the coffee plots selected to collect fruit for characterisation.

| Parcels      | Coordinates geographical |       |      |            | Altitude (m.a.s.l) | Coffee Variety   |
|--------------|--------------------------|-------|------|------------|--------------------|------------------|
|              | X                        | Y     | Zone | Hemisphere |                    |                  |
| São Carlos   | 234032                   | 37143 | 32   | N          | 1178               | Catuai           |
| Água Coimbra | 234034                   | 37140 | 32   | N          | 847                | Icatu            |
| Bemposta     | 235224                   | 34299 | 32   | N          | 1055               | Teksik           |
| Bemposta     | 235224                   | 34299 | 32   | N          | 1055               | Mundo Novo       |
| Bemposta     | 235519                   | 34542 | 32   | N          | 999                | Típica           |
| Novo Destino | 235672                   | 35140 | 32   | N          | 673                | Catura           |
| Novo Destino | 235672                   | 35140 | 32   | N          | 673                | Bourbon amarelo  |
| Novo Destino | 235672                   | 35140 | 32   | N          | 673                | Bourbon vermelho |
| CIAT-BECI    | 238448                   | 37314 | 32   | N          | 321                | Catimor          |
| Novo Destino | 235301                   | 34253 | 32   | N          | 647                | Robusta          |

Note: m.a.s.l=Height above sea level (m).

Protection of new varieties of plants (UPOV) ([https://www.upov.int/edocs/mdocs/upov/en/tc/44/tg\\_coffee\\_proj\\_7.pdf](https://www.upov.int/edocs/mdocs/upov/en/tc/44/tg_coffee_proj_7.pdf)). A Swiss-made digital scale, model XB 3200D, accurate to 0.1g, was used to weigh the fruit and seeds. To obtain length, width and thickness data, the fruit and seeds were measured using a digital calliper (Power Fix® Profi + Electronic Digital calliper. Model No. Z22855), with a measuring range (0-150 mm/0.6") with a precision of 0-100 mm  $\pm$  0.02 mm/0.001" 100-150 mm  $\pm$  0.03 mm/0.001" g, expressed in millimetres.

## 2.4 Preparing seeds for sowing

The ripe fruit was placed in a container of water in order to identify the perforated by coffee berry borer. Then all the floating seeds were discarded, and the submerged seeds were used. The fruit was then pulped to extract the seeds. The extracted seeds were washed and left to dry in a dry place, out of direct sunlight.

## 2.5. Conceptual and Methodological Framework

The present study was developed under an integrative framework combining molecular and morpho-agronomic characterization to evaluate the genetic diversity and performance of *Coffea* genotypes within organic agroforestry systems of São Tomé. The framework is based on three complementary pillars: (i) the agroforestry production context, (ii) the genetic and phenotypic evaluation of local and introduced accessions, and (iii) the establishment of a baseline for future breeding and conservation efforts. From a conceptual perspective, the study assumes that the agroforestry environment characterized by tree-crop interactions, shade gradients, and microclimatic heterogeneity acts as both a selective filter and a performance determinant for coffee genotypes. Thus, understanding genotype responses under such conditions provides a more realistic assessment of their adaptability and productivity in sustainable production systems. The methodological approach integrates both field and laboratory analyses to capture the multidimensional variation among accessions. Morpho-agronomic data were collected from on-farm and experimental plots, focusing on key yield and growth traits, including plant height, stem diameter, number of productive branches, and cherry yield per plant. Phenotypic data were complemented by molecular analyses using Diversity Array technology (DAR-Seq) markers to estimate genetic diversity and structure.

## 2.6 Sowing Seeds, Nursery Establishment, and Characterisation of the Seedlings

On 10 August 2023, twenty seeds of each coffee variety and species were sown in polyethylene bags filled with soil. After sowing, the seeds were fertilized with dried and ground cocoa husks. Germination began on 20 September 2023. Once germinated, seedlings were regularly monitored

and managed to ensure proper growth and development. Nursery management activities included watering to maintain adequate soil moisture and weeding to eliminate competing vegetation and minimize nutrient competition between the seedlings and weeds.

Data collection took place one month after the seeds germinated, and data was collected weekly for two consecutive months. The number of leaves, plant height and stem diameter were assessed. The stem diameter was measured with a digital caliper, and the data were expressed in millimetres. Plant height was measured using a ruler placed against the body of the plant from the base to the last pair of leaves. Leaf number was assessed by counting pairs of open leaves each week.

## 2.7 Experimental Design and Field Establishment Procedures

The crops were installed in mid-March 2024 at CIAT-BECI. Annual rainfall, average temperature, and average relative humidity are 3431 mm, 25.4°C, and 85%, respectively (Figure 1).

Ten varieties of coffee were planted, nine of which were arabica (Mundo Novo, Típica, Icatu, Catimor, Bourbon amarelo, Bourbon vermelho, Catuai, Catuara, Teksik) and robusta. The crops were planted in randomised blocks, with each parcel measuring 2 m wide and 5 m long for arabica and 4 m wide and 7.5 m long for robusta. The spacing used differed from the usual spacing used by farmers and varied according to the variety, with the arabica varieties being spaced 1 m apart in the row and between rows, and the robusta varieties 2 m apart in the row and 1.5 m between rows. 20 plants per variety were planted, with two replicates for each treatment. The size of each block is 120 m<sup>2</sup> for arabica and 340 for robusta m<sup>2</sup>.

After the crop was installed in the field, it was pruned to ensure the plants were growing well. Data collection took place 9 months after the crop was installed, and data were collected monthly for 3 months. During this period, plant shape, plant height, stem diameter, leaf colour, leaf shape, number of plagiotropic branches, length of the first plagiotropic branch, and number of nodes on the first plagiotropic branch were assessed. The stem diameter was measured with a digital caliper, and the data were expressed in millimetres. Leaf colour and plant shape were characterised according to the UPOV protocol. Plant height was measured with a tape measure held against the plant body from the base to the last pair of leaves. The number of nodes was determined by counting those along the branch. The length of the first plagiotropic branch was obtained with a tape measure held against the initial part of the branch up to the terminal part of the branch according to the coffee descriptors of the International Plant Genetic Resource Institute (IPGRI, 1996). Leaf number was assessed by counting pairs of open leaves over the course of each week.

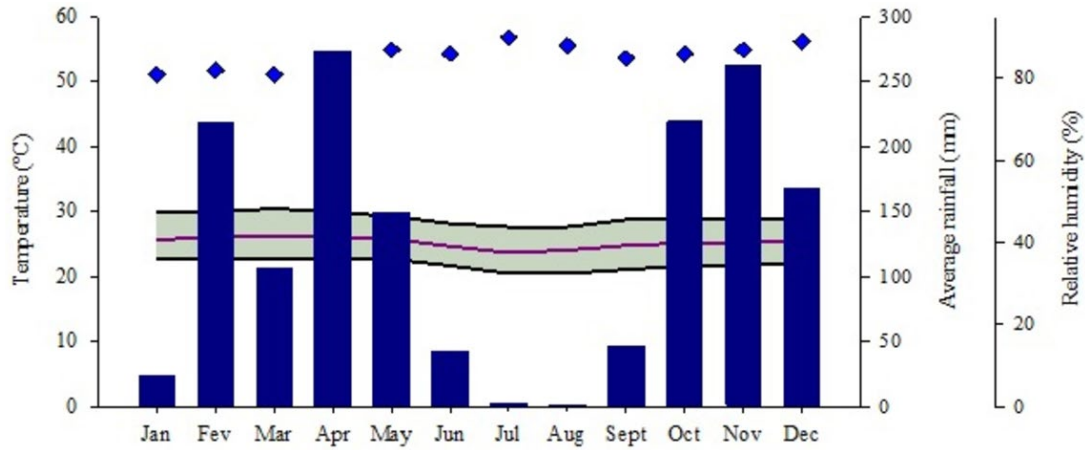


**Figure 1:** Average minimum, mean, maximum temperature, annual rainfall and relative humidity during the years 2023 to 2024 in CIAT-BECL.

## 2.8 Sample collection and genotyping

To properly identify arabica coffee varieties, plant material was collected for molecular analysis on 19 February 2025. For each coffee variety, about 10 grams of young coffee leaf were collected and stored in silica gel for about 96h, then transferred to an empty bottle.

After collection, the material was placed in a properly labelled thermal plastic bag with a small amount of silica to absorb moisture and sent to the laboratories of the International Institute of Tropical Agriculture (IITA) for molecular analysis (Table 2). In this study, 10 coffee genotypes were genotyped using the diversity array technology (DArT) platform, and 5,275 SNP markers were filtered for call rate ( $CR > 89\%$ ), minor allele frequency ( $MAF < 0.05$ ), and missing data rate ( $MDR > 20\%$ ). Data cleaning resulted in the retention of 3,467 SNP markers.

## 2.9 Data analysis

Phenotypic data were subjected to statistical analysis using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA). Analysis of variance (ANOVA) was performed to evaluate the effects of species and variety as fixed factors on the measured quantitative traits. Where significant differences were detected, mean separation was conducted using Tukey's Honestly Significant Difference (HSD) test at a significance threshold of  $P < 0.05$ .

Qualitative and categorical variables were analysed and visualized using the ggplot2 package implemented in R software (version  $\geq 4.0$ ), allowing clear graphical representation of trait distributions and relationships. To explore associations among categorical variables and to identify patterns of correspondence among their respective modalities, a Multiple Correspondence Analysis (MCA) was conducted using three selected qualitative variables. The MCA enabled dimensional reduction and visualization of the

relationships among variables, providing insights into trait structuring and multivariate interactions across species and varieties.

For the genotypic data, a dosage format was generated from the raw data and pairwise genetic dissimilarity distances based on identity-by-state (IBS) were estimated using the plink1.9 version Linux command. The IBS matrix was used to generate an hierarchical cluster dendrogram and visualized using Phylogenetics and Evolution (APE) package in R (Paradis et al., 2004).

**Table 2:** List of Coffee varieties collected.

| Codification | Local name      | Common name | Species                 |
|--------------|-----------------|-------------|-------------------------|
| Co1          | Icatu           | Coffee      | <i>Coffea arabica</i>   |
| Co2          | Catimor         | Coffee      | <i>Coffea arabica</i>   |
| Co3          | Burbon vermelho | Coffee      | <i>Coffea arabica</i>   |
| Co4          | Bumbom Amarelo  | Coffee      | <i>Coffea arabica</i>   |
| Co5          | Teksik          | Coffee      | <i>Coffea arabica</i>   |
| Co6          | Típica          | Coffee      | <i>Coffea arabica</i>   |
| Co7          | Robusta         | Coffee      | <i>Coffea canephora</i> |
| Co8          | Mundo Novo      | Coffee      | <i>Coffea arabica</i>   |
| Co9          | Caturra         | Coffee      | <i>Coffea arabica</i>   |
| Co10         | Catuai          | Coffee      | <i>Coffea arabica</i>   |

## 3 RESULTS

### 3.1 Fruit Characterisation

For the fruit-related descriptors, both fruit colour and fruit shape showed limited variability among the evaluated coffee varieties and species. Orange (60.8%) and dark red (27.2%) were the most common colours. The predominant fruit

shapes were elliptical (44.5%) and round (40%), with oblong shapes (15.5%) occurring less frequently. Coffee variety had a significant effect on the average fresh mass per fruit ( $df = 200$ ;  $F = 22.072$ ;  $P < 0.001$ ), transverse fruit diameter ( $df = 200$ ;  $F = 14.243$ ;  $P < 0.001$ ), and longitudinal fruit diameter ( $df = 200$ ;  $F = 29.159$ ;  $P < 0.001$ ). The Catuai variety recorded the highest average fresh mass per fruit ( $1.9 \pm 0.2$  g; mean  $\pm$  SE), transverse fruit diameter ( $12.6 \pm 0.8$  mm), and longitudinal diameter ( $17.4 \pm 0.8$  mm). Bourbon amarelo had the lowest average fresh mass per fruit ( $0.9 \pm 0.2$  g) and the smallest transverse diameter ( $10.4 \pm 0.6$  mm), while Robusta exhibited the smallest longitudinal fruit diameter ( $14.1 \pm 0.5$  mm) with no differ statistically from Catimor. For average fresh mass per fruit, Catuai and Icatu were significantly different from the

other coffee varieties ( $P < 0.001$ ). Significant differences in transverse fruit diameter ( $P < 0.001$ ) were observed between Catuai and the other varieties, except for Caturra ( $P = 0.097$ ), Bourbon vermelho ( $P = 0.070$ ), Icatu ( $P = 0.771$ ), Tecsik ( $P = 0.324$ ), and Mundo Novo ( $P = 0.139$ ). Similarly, for longitudinal fruit diameter, significant differences ( $P < 0.001$ ) were found between Catuai and other varieties, except for Caturra ( $P = 1.000$ ), Bourbon vermelho ( $P = 0.414$ ), Icatu ( $P = 0.600$ ), Típica ( $P = 0.403$ ), and Mundo Novo ( $P = 0.999$ ) (Table 3).

For the seed-related descriptors, endosperm colour exhibited limited variability among the evaluated coffee varieties and species. Green was the predominant endosperm colour (73.5%), followed by yellow (26.5%).

**Table 3:** Fruit colour, fruit shape and mean values for average fresh mass per fruit (MMFF), transverse diameter (DT) and longitudinal diameter (DL) in 20 cherries per variety: Bourbon amarelo, Bourbon vermelho, Catuai, Caturra, Icatu, Mundo Novo, Tecsik, Típica and Robusta coffee.

| Variety          | Fruit colour<br>Frequency (%)              | Fruit shape<br>Frequency (%)                 | MMFF<br>(g)                    | DT<br>(mm)       | DL<br>(mm)       |
|------------------|--------------------------------------------|----------------------------------------------|--------------------------------|------------------|------------------|
|                  |                                            |                                              | Mean $\pm$ standard error (SE) |                  |                  |
| Bourbon amarelo  | Yellow (100)                               | Round (20)<br>Elliptical (35)<br>Oblong (45) | $0.9 \pm 0.2^d$                | $10.4 \pm 0.6^b$ | $15.2 \pm 0.8^b$ |
| Bourbon vermelho | Orange (75)<br>dark red (25)               | Round (15)<br>Elliptical (60)<br>Oblong (25) | $1.4 \pm 0.3^{bc}$             | $11.8 \pm 0.5^a$ | $16.7 \pm 0.9^a$ |
| Catuai           | Orange (55)<br>dark red (45)               | Round (35)<br>Elliptical (60)<br>Oblong (5)  | $1.9 \pm 0.2^a$                | $12.6 \pm 0.8^a$ | $17.4 \pm 0.8^a$ |
| Caturra          | Orange (85)<br>dark red (15)               | Round (15)<br>Elliptical (60)<br>Oblong (25) | $1.5 \pm 0.2^b$                | $11.8 \pm 0.7^a$ | $17.3 \pm 0.8^a$ |
| Icatu            | Orange (90)<br>dark red (10)               | Round (15)<br>Elliptical (80)<br>Oblong (5)  | $1.8 \pm 0.3^a$                | $12.1 \pm 0.9^a$ | $16.4 \pm 1.1^a$ |
| Mundo Novo       | Orange (85)<br>dark red (15)               | Round (55)<br>Elliptical (40)<br>Oblong (5)  | $1.5 \pm 0.3^b$                | $11.8 \pm 0.9^a$ | $17.1 \pm 1.0^a$ |
| Robusta          | Orange (8)<br>dark red (92)                | Round (100)                                  | $1.3 \pm 0.1^c$                | $10.6 \pm 0.6^b$ | $14.1 \pm 0.5^c$ |
| Tecsik           | Orange (70)<br>Yellow (5)<br>dark red (25) | Round (80)<br>Elliptical (20)                | $1.3 \pm 0.3^c$                | $11.9 \pm 1.0^a$ | $15.8 \pm 1.3^b$ |
| Catimor          | Orange (60)<br>dark red (40)               | Round (30)<br>Elliptical (40)<br>Oblong (30) | $1.2 \pm 0.3^c$                | $10.7 \pm 1.2^b$ | $14.9 \pm 1.2^c$ |
| Típica           | Orange (80)<br>Yellow (15)<br>dark red (5) | Round (35)<br>Elliptical (50)<br>Oblong (15) | $1.5 \pm 0.4^b$                | $11.7 \pm 1.0^a$ | $16.7 \pm 0.5^a$ |

†ANOVA (average fresh mass per fruit:  $df=200$ ,  $F=22.072$ ,  $P=0.000$ ; transverse fruit diameter:  $df=200$ ,  $F=14.243$ ,  $P=0.000$  and longitudinal fruit diameter:  $df=200$ ,  $F=29.159$ ,  $P=0.000$ ).

†Averages followed by the same letter in the column are not significantly different by the test Tukey HSD ( $\alpha=0.05$ ).

The Icatu and Catuai varieties had the highest average fresh mass per seed ( $1.0 \pm 0.2$  g), whereas Bourbon amarelo had the lowest ( $0.6 \pm 0.2$  g) (Table 4).

For seed length, Caturra and Mundo Novo had the longest seeds ( $13.9 \pm 0.6$  mm), while Catimor had the shortest ( $12.3 \pm 0.9$  mm). In terms of seed width, Robusta had the widest seeds ( $5.7 \pm 0.2$  mm), and Tecsik had the narrowest ( $4.9 \pm 0.4$  mm) (Table 4).

### 3.2 Plant characterisation

Among the evaluated varieties, Típica had the highest average plant height ( $12.9 \pm 3.5$  cm), while Catimor recorded the lowest ( $8.2 \pm 1.8$  cm). Típica also exhibited the highest average stem diameter ( $1.7 \pm 0.3$  mm), whereas Bourbon Amarelo and Tecsik had the lowest ( $1.3 \pm 0.2$  mm). Tecsik and Bourbon Vermelho had the highest average leaf numbers ( $2.7 \pm 0.8$ ), while Robusta had the lowest ( $1.7 \pm 0.6$ ) (Table 5).

In the field-grown coffee plantation, coffee variety significantly affected average plant height ( $df = 119$ ;  $F = 10.793$ ;  $P < 0.001$ ), number of primary plagiotropic branches ( $df = 119$ ;  $F = 11.145$ ;  $P < 0.001$ ), number of nodes on the first plagiotropic branch ( $df = 119$ ;  $F = 10.291$ ;  $P < 0.001$ ), and length of the first plagiotropic branch ( $df = 119$ ;  $F = 10.918$ ;  $P < 0.001$ ), but had no significant effect on stem diameter ( $df = 119$ ;  $F = 0.874$ ;  $P = 0.551$ ).

Catuai recorded the highest average plant height ( $85.0 \pm 22.3$  cm), while Bourbon Amarelo had the lowest ( $41.2 \pm 7.9$  cm). Significant differences ( $P < 0.001$ ) were observed between Catuai and most other varieties, except Robusta ( $P = 0.090$ ) and Bourbon Vermelho ( $P = 0.068$ ). Caturra had the highest average

number of primary plagiotropic branches ( $11.3 \pm 3.0$ ), while Bourbon Amarelo had the lowest ( $3.8 \pm 2.4$ ). No significant differences ( $P > 0.151$ ) were observed between Caturra and most other varieties, but differences were significant between Robusta and Bourbon Amarelo ( $P < 0.001$ ).

Mundo Novo exhibited the highest average number of nodes on the first plagiotropic branch ( $7.4 \pm 1.3$ ), while Robusta had the lowest ( $2.4 \pm 0.8$ ). Catuai had the longest first plagiotropic branch ( $34.6 \pm 9.4$  cm), whereas Bourbon Vermelho had the shortest ( $12.7 \pm 1.5$  cm) (Table 6).

The results showed that for a two-dimensional graph, the correspondence map's percentage of variance was almost 30%. Bourbon vermelho, Catimor, Catuai, Caturra, Mundo Novo, and Teksik accessions resemble one another based on their elliptical leaf shapes. Despite being very different from the other types, Icatu and Típica have nearly identical lanceolate leaf shapes. The situation between Robusta and Bourbon amarelo is the same in terms of oval leaf shape. At the moment of data collection (January to March 2025), almost all the plants were at the vegetative phenological stage (Figure 2).

### 3.3 Coffee Molecular Germplasm Characterization

The genetic distance among the 10 coffee genotypes was generally low. Hierarchical clustering based on genetic distance (complete linkage) grouped the genotypes (Co1 to Co10) into two major clusters. Cluster 1 comprised Co8 (Mundo Novo), Co9 (Caturra), and Co10 (Catuai). These three genotypes clustered tightly, indicating high genetic similarity. This cluster separated early from the remaining

**Table 4:** Endosperm colour and mean values for average fresh mass per seed (MMFS), length, width, and thickness of 20 seeds per variety.

| Variety          | Endosperm colour<br>Frequency (%) | MMFS<br>(g)                    | Length<br>(mm)   | Width<br>(mm)   | Thickness<br>(mm) |
|------------------|-----------------------------------|--------------------------------|------------------|-----------------|-------------------|
|                  |                                   | Mean $\pm$ standard error (SE) |                  |                 |                   |
| Bourbonamarelo   | Green (60) yellow (40)            | $0.6 \pm 0.2^c$                | $12.6 \pm 0.7^b$ | $5.4 \pm 0.9^a$ | $8.0 \pm 0.6^a$   |
| Bourbon vermelho | Green (60) yellow (40)            | $0.8 \pm 0.2^a$                | $13.8 \pm 0.7^a$ | $5.6 \pm 0.8^a$ | $8.8 \pm 0.5^a$   |
| Catuai           | Green (80) yellow (20)            | $1.0 \pm 0.2^a$                | $13.7 \pm 0.8^a$ | $5.6 \pm 0.9^a$ | $8.8 \pm 0.5^a$   |
| Caturra          | Green (80) yellow (20)            | $0.8 \pm 0.2^a$                | $13.9 \pm 0.6^a$ | $5.1 \pm 0.4^a$ | $8.1 \pm 0.3^a$   |
| Icatu            | Green (85) yellow (15)            | $1.0 \pm 0.2^a$                | $13.0 \pm 1.0^a$ | $5.3 \pm 0.3^a$ | $8.7 \pm 0.3^a$   |
| Mundo Novo       | Green (90) yellow (10)            | $0.9 \pm 0.2^a$                | $13.9 \pm 0.9^a$ | $5.3 \pm 0.6^a$ | $8.5 \pm 0.5^a$   |
| Robusta          | Green (85) yellow (15)            | $0.9 \pm 0.1^a$                | $11.6 \pm 0.5^c$ | $5.7 \pm 0.2^a$ | $8.2 \pm 0.4^a$   |
| Tecsik           | Green (65) yellow (35)            | $0.6 \pm 0.2^b$                | $12.5 \pm 1.3^b$ | $4.9 \pm 0.4^b$ | $7.9 \pm 0.6^a$   |
| Catimor          | Green (65) yellow (35)            | $0.7 \pm 0.2^b$                | $12.3 \pm 0.9^c$ | $5.4 \pm 0.9^a$ | $8.5 \pm 0.7^a$   |
| Típica           | Green (65) yellow (35)            | $0.9 \pm 0.3^a$                | $13.8 \pm 0.6^a$ | $5.2 \pm 0.6^a$ | $8.8 \pm 0.4^a$   |

†ANOVA (average fresh mass per seed:  $df=200$ ,  $F=9.758$ ,  $P=0.000$ , seed Length:  $df=200$ ,  $F=20.017$ ,  $P=0.000$ , seed Width:  $df=200$ ,  $F=3.047$ ,  $P=0.002$ , seed Thickness:  $df=200$ ,  $F=1.006$ ,  $P=0.437$ ).

†Averages followed by the same letter in the column are not significantly different by the test Tukey HSD ( $\alpha=0.05$ ).

**Table 5:** Plant height, Plant Stem diameter and Leaf number in 5 plants per variety: Bourbon amarelo, Bourbon vermelho, Catuai, Caturra, Icatu, Mundo Novo, Tecsik, Típica and Robusta coffee in the nursery during October to December 2023.

| Variety                    | Plant height (cm)      | Plant Stem diameter (mm) | Leaf number           |
|----------------------------|------------------------|--------------------------|-----------------------|
| Mean ± standard error (SE) |                        |                          |                       |
| Bourbon vermelho           | 12.8±3.8 <sup>a</sup>  | 1.4±0.2 <sup>c</sup>     | 2.7±0.8 <sup>a</sup>  |
| Bourbon amarelo            | 9.0±2.5 <sup>b</sup>   | 1.3±0.2 <sup>c</sup>     | 2.0±0.8 <sup>ab</sup> |
| Catuai                     | 10.4±2.4 <sup>ab</sup> | 1.6±0.2 <sup>a</sup>     | 2.5±0.9 <sup>ab</sup> |
| Caturra                    | 8.8±2.3 <sup>b</sup>   | 1.5±0.1 <sup>b</sup>     | 2.2±1.0 <sup>ab</sup> |
| Icatu                      | 10.0±4.2 <sup>ab</sup> | 1.5±0.3 <sup>b</sup>     | 2.3±1.1 <sup>ab</sup> |
| Mundo Novo                 | 10.9±2.1 <sup>ab</sup> | 1.5±0.2 <sup>b</sup>     | 2.3±1.0 <sup>ab</sup> |
| Robusta                    | 11.7±2.1 <sup>a</sup>  | 1.5±0.1 <sup>b</sup>     | 1.7±0.6 <sup>b</sup>  |
| Tecsik                     | 8.5±3.4 <sup>b</sup>   | 1.3±0.2 <sup>c</sup>     | 2.7±1.4 <sup>a</sup>  |
| Catimor                    | 8.2±1.8 <sup>b</sup>   | 1.4±0.1 <sup>b</sup>     | 2.6±0.7 <sup>a</sup>  |
| Típica                     | 12.9±3.5 <sup>a</sup>  | 1.7±0.3 <sup>a</sup>     | 2.4±1.0 <sup>ab</sup> |

†ANOVA (plant height:  $df=278$ ,  $F=7.483$ ,  $P=0.000$ , plant stem diameter:  $df=278$ ,  $F=13.187$ ,  $P=0.000$ , Leaf number:  $df=278$ ,  $F=2.897$ ,  $P=0.003$ ).

†Averages followed by the same letter in the column are not significantly different by the Tukey HSD test ( $\alpha=0.05$ ).

genotypes at ~0.85, suggesting it is genetically distinct from the others. Cluster 2 included Co1 (Icatu) to Co7 (Robusta) and was more diverse, forming two sub-clusters: Sub-cluster A: Co1 (Icatu) and Co2 (Catimor), which showed moderate

genetic similarity. Sub-cluster B: Co3 (Bourbon vermelho) to Co7 (Robusta), which displayed a gradient of relatedness. Within this group, Co6 (Típica) and Co7 (Robusta) were the most closely related. Co3 (Bourbon vermelho), Co4 (Bourbon amarelo), and Co5 (Tecsik) showed moderate similarity and merged sequentially at increasing heights. Overall, Co8 (Mundo Novo) to Co10 (Catuai) likely represent a genetically distinct group (Figure 3).

## 4 DISCUSSION

We investigated ten coffee (*Coffea spp.*) accessions using both molecular and morpho-agronomic characterization to inform the potential establishment of a structured coffee breeding program on São Tomé Island.

Based on our findings, fruit-related descriptors, such as color and shape, showed limited variability across the evaluated coffee varieties and species. Roundish and elliptical fruit shapes with a persistent orange color were dominant within the population. This observation is partly consistent with Weldemichael (2020), who reported a higher proportion of roundish and elliptical fruit shapes and red fruit color among Ethiopian coffee accessions. In contrast, Akperterey, Anim-Kwapong and Ofori (2019) observed a greater proportion of roundish fruit and light-green color among *Coffea robusta* genotypes, while Ferrão et al. (2021) found a predominance of oblong fruit shapes and medium-red coloration in *C. robusta* genotypes.

**Table 6:** Plant height, Stem diameter, Number of primary plagiotropic branches (NPPB), Number of nodes on first plagiotropic branch (NNFPB), and Length of first plagiotropic branch (LFPB) in 5 plants per variety: Bourbon amarelo, Bourbon vermelho, Catuai, Caturra, Icatu, Mundo Novo, Tecsik, Típica, and Robusta coffee in the field during January to March 2025.

| Variety                    | Plant height (cm)       | Stem diameter (mm)    | NPPB                   | NNFPB                 | LFPB (cm)              |
|----------------------------|-------------------------|-----------------------|------------------------|-----------------------|------------------------|
| Mean ± standard error (SE) |                         |                       |                        |                       |                        |
| Bourbon amarelo            | 41.2±7.9 <sup>c</sup>   | 7.0±1.4 <sup>a</sup>  | 3.8±2.4 <sup>c</sup>   | 3.7±2.2 <sup>cd</sup> | 17.0±9.1 <sup>c</sup>  |
| Bourbon vermelho           | 57.9±6.3 <sup>bc</sup>  | 6.0±0.6 <sup>a</sup>  | 6.0±2.0 <sup>b</sup>   | 3.0±0.0 <sup>cd</sup> | 12.7±1.5 <sup>c</sup>  |
| Catuai                     | 85.0±22.3 <sup>a</sup>  | 9.3±1.7 <sup>a</sup>  | 11.1±3.1 <sup>a</sup>  | 6.9±3.2 <sup>a</sup>  | 34.6±9.4 <sup>a</sup>  |
| Caturra                    | 59.8±8.9 <sup>bc</sup>  | 9.7±1.2 <sup>a</sup>  | 11.3±3.0 <sup>a</sup>  | 5.8±1.3 <sup>c</sup>  | 20.5±5.5 <sup>c</sup>  |
| Icatu                      | 50.1±16.2 <sup>bc</sup> | 10.0±1.5 <sup>a</sup> | 10.0±2.4 <sup>ab</sup> | 6.7±2.1 <sup>a</sup>  | 31.9±9.4 <sup>a</sup>  |
| Mundo Novo                 | 54.6±7.5 <sup>bc</sup>  | 8.4±1.1 <sup>a</sup>  | 10.2±2.0 <sup>ab</sup> | 7.4±1.3 <sup>a</sup>  | 24.6±4.2 <sup>b</sup>  |
| Robusta                    | 69.8±14.3 <sup>b</sup>  | 9.5±1.3 <sup>a</sup>  | 5.1±1.8 <sup>c</sup>   | 2.4±0.8 <sup>d</sup>  | 22.9±7.1 <sup>ab</sup> |
| Tecsik                     | 56.9±21.1 <sup>bc</sup> | 9.9±1.5 <sup>a</sup>  | 10.3±4.8 <sup>ab</sup> | 6.5±1.8 <sup>ab</sup> | 32.7±7.0 <sup>a</sup>  |
| Catimor                    | 52.9±15.5 <sup>bc</sup> | 8.3±2.0 <sup>a</sup>  | 10.2±5.4 <sup>ab</sup> | 4.5±2.1 <sup>cd</sup> | 14.0±3.1 <sup>c</sup>  |
| Típica                     | 58.1±8.4 <sup>bc</sup>  | 10.3±2.2 <sup>a</sup> | 10.4±3.6 <sup>ab</sup> | 6.4±1.5 <sup>b</sup>  | 26.6±4.7 <sup>ab</sup> |

†ANOVA (plant height:  $df=119$ ,  $F=10.793$ ,  $P=0.000$ , number of primary plagiotropic branches:  $df=119$ ,  $F=11.145$ ,  $P=0.000$ , number of nodes on first plagiotropic branch:  $df=119$ ,  $F=10.291$ ,  $P=0.000$ , Length of first plagiotropic branch:  $df=119$ ,  $F=10.918$ ,  $P=0.000$ , stem diameter:  $df=119$ ,  $F=0.874$ ,  $P=0.551$ ).

†Averages followed by the same letter in the column are not significantly different by the test Tukey HSD ( $\alpha=0.05$ ).

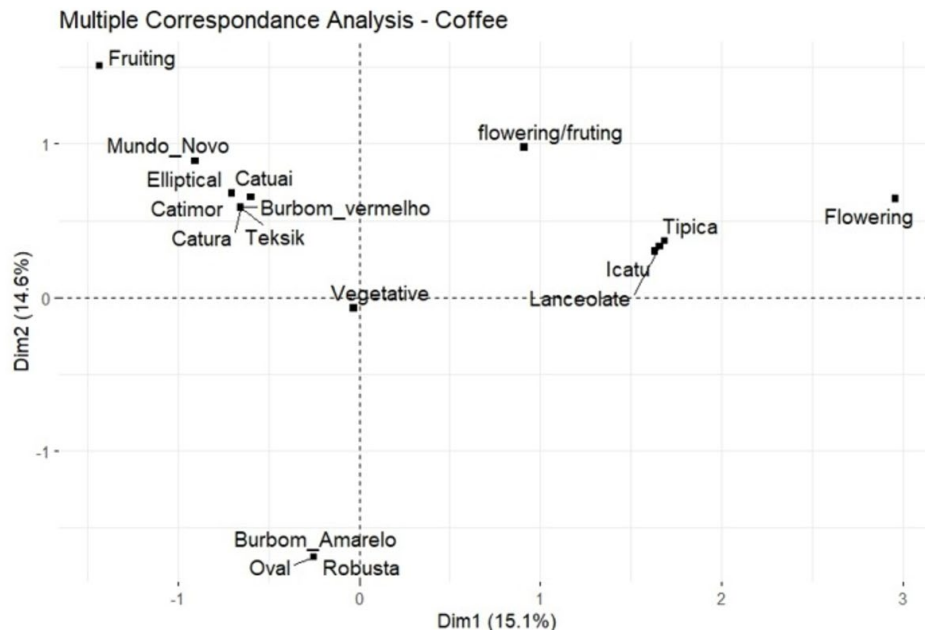


**Figure 2:** Varietal categories for the evaluation of qualitative parameters (leaf shape and phenology) and the Multiple Correspondence Analysis of 5 plants per variety: Bourbon amarelo, Bourbon vermelho, Catuai, Caturra, Icatu, Mundo Novo, Teksik, Típica and Robusta in the field from January to March 2025.

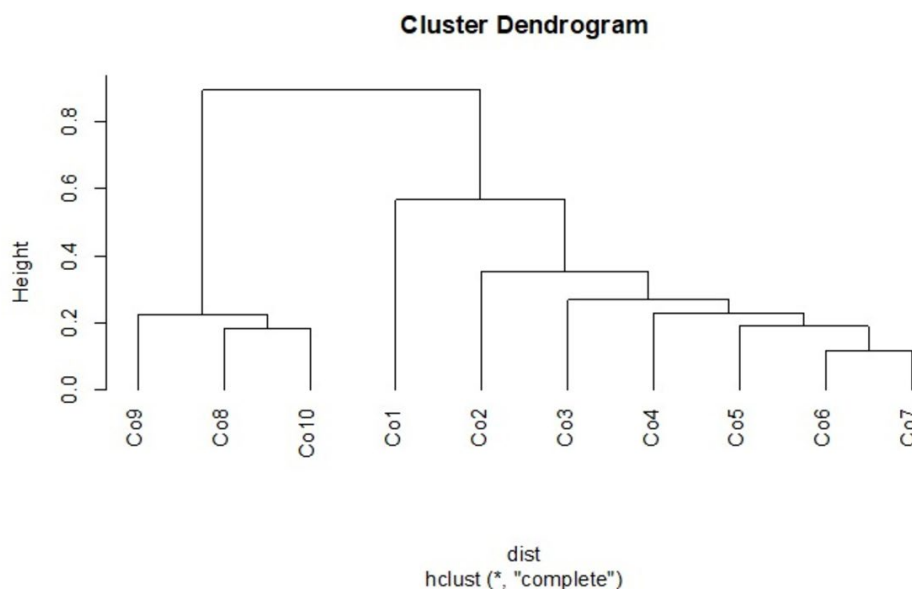


**Figure 3:** Coffee Hierarchical Clustering of Genotypes Based on Genetic Distance Using Complete Linkage.

Endosperm color also showed limited variation, with green being the most common, followed by yellow, in agreement with the results of Ferrão et al. (2021). Coffee variety and species significantly influenced plant height, stem diameter, and leaf number, corroborating findings by Paredes-Espinosa et al. (2023). The significant differences observed in these quantitative traits are further consistent with earlier studies by Kitila et al. (2011), Beksisa, Benti and Weldemichael (2021), and Regassa et al. (2024).

Leaf morphology analysis revealed that the ten accessions exhibited predominantly elliptical (60.0%), oval (20.0%), and lanceolate (20.0%) leaves. Multiple correspondence analysis (MCA) indicated that Robusta and Bourbon Amarelo were closely related in terms of leaf traits (Figure 3). These results align with Weldemichael (2020), who reported a predominance of elliptical (91.8%), lanceolate (6.1%), and ovate (2.0%) leaf shapes among Ethiopian coffee accessions. Morphological characteristics remain essential indicators of genetic diversity in coffee (Maxiselly et al., 2025).

Cluster analysis further clarified the relationships among accessions based on morphological traits (Gebrese, Tesfaye & Gedebo, 2024). Overall, Co8 (Mundo Novo) to Co10 (Catuai) likely represent a genetically distinct group, reflecting a different population origin or selection history. Co6 (Típica) and Co7 (Robusta) may be clones or closely related breeding lines. Despite their genetic similarity, these two cultivars displayed distinct phenotypes, suggesting that a new variety may have arisen through natural hybridization in farmers' fields.

Given that coffee growers in the Monte Café region face persistent challenges with the coffee berry borer, this finding could have positive phytosanitary implications. Notably, studies carried out in the same locations and using the same varieties have reported that the Co7 cultivar, also known locally as "false robusta coffee," has high resistance to the coffee berry borer, *Hypothenemus hampei* (Ferrari, 1867), a major constraint in organic coffee production on São Tomé Island (Carvalho et al., 2021, 2023). Understanding the genetic variability of coffee populations at the local level is therefore essential for effective conservation and utilization of genetic resources (Yirga et al., 2021).

## 5 CONCLUSIONS

In this study, we observed that the variability should be strategically exploited to enhance desired traits in line with specific breeding objectives. Molecular analysis further confirmed the existence of a new variety of Arabica coffee adapted to the agroecological conditions of Roça Monte Café. These findings open new opportunities for coffee growers in the region, particularly given this variety's productivity and resistance to pests. Overall, it is recommended that these accessions be properly conserved for future breeding programs, as significant variability was observed across most evaluated parameters.

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## 7 AUTHOR CONTRIBUTIONS

Conceptual Idea: Miclay, C.; Methodology design: Miclay, C.; Data collection: Alex, L.; Lewis, E.; Meyer, A.; Francisca, A.; Luís, S.; Data analysis and interpretation: Paterne Agre and Miclay Carvalho.; Writing and editing: Miclay, C.; Severino, E. S.; Gaspar, G.; Paterne, A.

## 8 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

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