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Biometric traits as a tool for the identification and breeding of *Coffea canephora* genotypes

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Genet. Mol. Res. 19 (2): gmr18541
Received December 18, 2019
Accepted April 30, 2020
Published May 30, 2020
DOI http://dx.doi.org/10.4238/gmr18541

ABSTRACT. Cross-pollination and gametophytic self-incompatibility reduce the stability of *Coffea canephora* genotypes. This is an important crop for Brazil, the largest producer of this type of coffee and also a major exporter. The study of biometric characteristics is essential to assist in the selection of promising plant materials. We examined the diversity of morpho-agronomic traits of genotypes of *C. canephora* cv. Conilon through the evaluation of branch and leaf parameters. Assessments included plagiotropic

branch length, number of nodes in plagiotropic branches, distance between nodes in plagiotropic branches, orthotropic branch length, number of nodes in orthotropic branch, distance between nodes in orthotropic branch, plant height, canopy diameter, leaf length, leaf width, and leaf area in two periods. The data from the 43 coffee genotypes were tested by multivariate and cluster analyses. Six groups were formed by the Tocher optimization method, and five groups by the unweighted pair group method with arithmetic mean (UPGMA) hierarchical method, suggesting an important genetic variability among plant materials. Both Tocher optimization and UPGMA hierarchical methods were consistent for clustering the genotypes, ordering them in six and five dissimilar groups, respectively, with genotypes 25 and 37 standing out with the greatest dissimilarity, constituting isolated groups by both methods. Pearson's correlation ranged from very weak to very strong, positive and negative, among the characteristics, as also shown by principal component analyses. These analyses indicated the morpho-agronomic traits with a greater degree of correlation, assisting in the choice of promising plant materials. The genetic parameters estimates demonstrate genetic variability and thus breeding potential within the Conilon coffee genotypes studied. These results emphasize the usefulness of biometric evaluations as a tool for the identification and breeding of genotypes to compose new Conilon coffee cultivars.

Key words: Biometrics; Clustering; Conilon coffee; Multivariate analysis; Breeding

INTRODUCTION

Coffee is one of the most valuable and traded agronomic commodities worldwide and included in the main stock exchanges, such as London and New York. It is a highly labor-intensive crop, based on the species *Coffea arabica* and *C. canephora*. Coffee is grown in more than 80 tropical countries, being responsible for the livelihoods of about 25 million farmers, mainly smallholders, and about 100 million people are estimated to be involved in this crop production chain (Martins et al., 2017; Ramalho et al., 2018). Brazil stands out as the world's largest coffee producer, where both Arabica (*Coffea arabica*, ca. 65%) and Robusta (*Coffea canephora*, ca. 35%) are grown (CONAB, 2020).

Breeding strategies have substantially contributed to the development of new coffee genotypes, resulting in noticeable advances achieved in coffee fields during recent decades (Dalcomo et al., 2015; Lima et al., 2016; Rodrigues et al., 2017; Partelli et al., 2019, 2020). However, there is always a need for new cultivars with desirable agronomic characteristics and a suitable performance in different environments. Productive cultivars adapted to various farming systems are among the principal components of both the competitiveness and the sustainability of coffee fields (Carvalho et al., 2016).

Data from morphological and biometric characteristics are very useful for the breeding process of coffee trees (Freitas et al., 2007; Carvalho et al., 2010; Nogueira et al.,

2012; Rodrigues et al., 2013; Rodrigues et al., 2014; Moura et al., 2016; Rodrigues et al.; 2016; Giles et al. 2019, Vieira et al., 2019). Among these morphological traits, plant height, plagiotropic branch length, number of nodes, and vegetative vigor are considered to be strongly related to the crop yield (Carvalho et al., 2010; Teixeira et al., 2012; Assis et al., 2014; Pereira et al., 2016). Additionally, leaf characteristics should be considered in the breeding processes (Dubberstein et al., 2019; Martins et al., 2019a), as they are important in for plant growth and development assessments, including physiological parameters, such as transpiration and net assimilation rates (Fascella et al., 2013; Schmidt et al., 2014). Such assessments may provide early information related to posterior crop performance (Brinate et al., 2015).

Coffea canephora is an allogamous and diploid species, with gametophytic self-incompatibility (Conagin and Mendes, 1961; Tran et al., 2017; Moraes et al., 2018). Therefore, natural reproduction, as well as propagation by seeds, results in a highly diverse population, wherein each plant may differ from others in relation to its architecture, shape and size of both grain and leaves, maturation pattern, and susceptibility or tolerance to biotic and abiotic environmental stresses, among others. Therefore, there is a need for coffee breeding programs to identify homogeneous and stable traits for commercial coffee fields. Conventional breeding methods require up to 30 years to obtain a new coffee cultivar with genetically stable agronomic characteristics and commercial interest. On the other hand, the clonal propagation method requires only about one third of this time, allowing hybrid vigor exploration and the multiplication of outstanding genotypes with the characteristics of interest still in segregation, which could hardly be naturally find in a cultivar propagated by seeds (Carvalho et al., 2011). Clone plants are identical to their parent plant, assuring homogeneity in their development, as well as higher crop yield, and better coffee bean quality than plants propagated by seeds, allowing one to breed crop cultivars with a distinct maturation cycle duration (Bragança et al., 2001; Carvalho et al., 2011; Covre et al., 2013; Partelli et al., 2014; Ramalho et al., 2016, Martins et al., 2019; Partelli et al. 2019, 2020). After numerous assessments, including genetic compatibility tests, selected clones are grouped to form a new clonal cultivar according to specific objectives, and thereafter maintained in an Germplasm Active Bank and other breeding programs.

Several predictive methods can be used to study genetic divergence, including 1) multivariate analysis, where means of dissimilarity are calculated from the Euclidean distance and the generalized Mahalanobis distance (D^2); 2) clustering methods involving hierarchical methods, such as the unweighted pair group method with arithmetic mean (UPGMA) and the Tocher optimization method; and 3) dispersion techniques involving principal components analysis and canonical variables (Cruz et al., 2012).

In this context, this study aimed to evaluate the genetic diversity through morphological and biometric characteristics of leaves and branches of 43 genotypes of *C. canephora* cv. Conilon, which is the most widely plant cultivar grown in Brazil for Robusta type of coffee.

MATERIAL AND METHODS

Plant material and experimental design

The assessments were performed in a field with 43 genotypes of *C. canephora* cv. Conilon (Table 1), most of which were selected by regional coffee farmers due to yield and quality performance. Therefore, currently these are genotypes with importance on a regional scale but they with potential to grow in other coffee regions. Seedlings were transplanted in April 2014 in the municipality of Nova Venécia, northern Espírito Santo State, Brazil (18°39'43" S, 40°25'52" W; 199 m above sea level, and annual mean temperature of 23°C). The soil at the site is a Latossolo Vermelho-Amarelo, distrófico, with clayey texture and a wavy relief (Santos et al., 2018). The region has a tropical climate, characterized by warm and humid summers and dry winters, classified as Aw according to Köppen (Alvares et al., 2013).

Table 1. Identification of the 43 genotypes of *Coffea canephora* cv. Conilon in Nova Venécia, ES, Brazil.

Identification	Name	Identification	Name	Identification	Name
1	Verdim R	16	Pirata	31	Cheique
2	B01	17	Peneirão	32	P2
3	Bicudo	18	Z39	33	Emcapa 02
4	Alecrim	19	Z35	34	Emcapa 153
5	700	20	Z40	35	P1
6	CH1	21	Z29	36	LB1
7	Imbigudinho	22	Z38	37	122
8	AD1	23	Z18	38	Verdim D
9	Graudão HP	24	Z37	39	-
10	Valcir P	25	Z21	40	Emcapa 143
11	Beira Rio 8	26	Z36	41	Ouro negro 1
12	Tardio V	27	Ouro Negro	42	Ouro negro 2
13	AP	28	18	43	Clementino[T]
14	L80	29	Tardio C	-	-
15	Bamburral	30	A1	-	-

Genotype 33 belongs to cv. Emcapa 8111 and genotypes 34 and 39 to cv. Emcapa 8131 (Bragança et al., 2001). Genotypes 1, 11, 15, 16, 30 and 43 belong to cv. Tributun (Partelli et al., 2020) and 30 and 35 to cv. Andina (Partelli et al., 2019).

The genotypes were arranged in a randomized block design with three replicates and seven plants of each genotype per replicate. The seedlings were transplanted with a spacing of 3 m between coffee rows and 1 m between plants in each row, resulting in a density of 3,333 plants per hectare. All genotypes were propagated by cuttings, with the exception of genotype 39, propagated by seed. Coffee pruning was performed in order to maintain four orthotropic branches per plant. The entire experimental area was irrigated by a drip irrigation system. The treatments received 500, 100, and 400 $kg.ha^{-1}$ $year^{-1}$ of N, P_2O_5, and K_2O, respectively, applied depending on plant requirements and phenological stages. Soil micronutrients were corrected by applying 2 $kg.ha^{-1}$ $year^{-1}$ Zn, 1.0 $kg.ha^{-1}$ $year^{-1}$ B, 2.0 $kg.ha^{-1}$ $year^{-1}$ Cu, and 10 $kg.ha^{-1}$ $year^{-1}$ Mn.

Leaf and branch evaluations

Leaf area was assessed from 20 leaves per genotype, sampled from the third and/or fourth newly developed pair of plagiotropic branches located in the plants' middle third. Assessments were performed during period 1 (October 2016) and period 2 (February 2017). The leaves' maximal length (LLT1 and LLT2) and maximum width (LWT1 and LWT2) were measured by using a graduated ruler (Partelli et al., 2006), and leaf area (LAT1 and LAT2) was measured using a leaf area meter (Model LI-3100, Li-Cor, Lincoln, NE, USA).

Plant biometric analyses included plant height (Hgt, measured from base to top, in cm); canopy diameter (Diam, measured from one end to the other, in cm); length of productive plagiotropic branch (PBL, measured from the insertion in the orthotropic branch to the plagiotropic branch apex, in cm); orthotropic branch length (OBL, measured from the insertion in the evaluated plagiotropic branch to the orthotropic branch apex, in cm); number of internodes per plagiotropic (NNP) and orthotropic (NNO) branches; and distance between nodes in the plagiotropic (DBP) and orthotropic branches (DBO). The productive plagiotropic branches (in the production stage) were located in the plant's lower third.

Initially, the degree of multi-collinearity for the mean X'X correlation matrix was evaluated (Montgomery and Peck, 1981). In order to identify the variables that contributed to the multi-collinearity emergence, eigenvalues and eigenvectors analyses were performed. The multi-collinearity is classified according to the condition number (CN) values as follows: weak (CN < 100), moderate (100 < CN < 1,000) or strong (CN > 1,000) (Teixeira et al*.,* 2012). The CN value in this study was above acceptable, thus some variables were removed by means of principal components, eliminating the component with highest weight from the high vector of the smallest vector. By achieving a CN value below 100, further analyses were performed.

A multivariate analysis (MANOVA) was performed from the variance components, in which the following parameters were estimated for each characteristic: coefficient of environmental variation (CVe); coefficient of genetic variation (CVg); variation index (VI), corresponding to the CVg and CVe ratio; and heritability (h^2). Differences between mean values were compared by the Scott-Knott test at 5% probability. As a dissimilarity method, the generalized Mahalanobis distance matrix (D^2) was used and genotype cluster analyses were performed using both the Tocher optimization method, and the hierarchical clustering method unweighted pair group method with arithmetic mean (UPGMA). Subsequently, the variables were subjected to Pearson's correlation analysis. Principal components analysis in a dispersion plot of biplot type was also performed. Statistical analyses were carried out with the aid of the R software (R Core Team, 2018).

RESULTS AND DISCUSSION

The multi-collinearity test indicated that the first CN value was above acceptable, so it was necessary to remove some variables. First, we eliminated the length of plagiotropic branches, which resulted in a CN of 1,405. Then, leaf area at T1 was also removed, lowering the CN to 841.67. After removing the distance between the nodes in orthotropic branches, the CN reached 445, and with the removal of leaf area at T2, CN decreased to 71, an acceptable value. Therefore, the analysis of variance and clustering methods was performed only with the 10 remaining variables.

Through the analysis of variance, we found a difference among the studied genotypes for all the characteristics evaluated at the 1% level of significance (Table 2), suggesting the occurrence of genetic variability among the population regarding the evaluated characteristics. This is a promising result, as such variability is a basic condition to obtain gains with genotype breeding (Rodrigues et al., 2012; Carias et al., 2016).

Among the estimated genetic parameters, the coefficient of environmental variation (CVe) and coefficient of genetic variation (CVg) showed values ranging from 6.43 to 11.09% and 7.75 to 12.37%, respectively, which can be considered low (<10%) or moderate

(from 10 and 20%) according to Gomes (1985). From residual coefficient of environmental variation (CVe) estimative, it is possible to indicate a high experimental accuracy and precision for the characteristics being studied. The presence of genetic variability is confirmed and quantified through the coefficient of genetic variation, which expresses the intensity of genetic variation in relation to the character mean (Resende, 1991). On the other hand, coefficients of genetic variation (CVg) above 7% are considered high by Sebbenn et al. (1998). Thus, in this study, the CVg was low only for plant height, with a value of 5.74%. The others characteristics were in accordance with the proposed classification and could be considered as useful criteria for genotype selection.

Table 2. Summary of the analysis of variance for biometric and leaf-related characteristics, and their genetic and environmental parameters, of 43 genotypes of *Coffea canephora* cv. Conilon.

Variables	MS		Mean	CVe (%)	CVg	VI	h^2 (%)
	Genotype	Residue					
NNP	11.55**	2.94	15.45	11.09	7.75	0.69	74.54
DBP	0.90**	0.086	3.80	7.74	9.71	1.25	90.41
OBL	250.67**	44.45	71.63	9.31	8.18	0.87	82.22
NNO	29.95**	3.84	19.85	9.87	10.50	1.06	87.16
Hgt	534.41**	90.52	149.72	6.35	5.74	0.90	83.06
Diam	1438.53**	258.98	162.14	9.92	8.65	0.87	82.09
LLT1	28.08**	0.74	13.39	6.43	10.07	1.56	97.35
LWT1	6.50**	0.18	5.32	8.16	12.18	1.49	97.06
LLT2	23.68**	1.17	14.55	7.44	8.41	1.29	95.03
LWT2	9.30**	0.31	6.25	8.99	12.37	1.37	96.59

** Significant at 1% by F test; CV_e: Coefficient of environmental variation; CV_g: Coefficient of genetic variation; VI: Variation index (CV_g/CV_e); h^2: Heritability; NNP: number of nodes in plagiotropic branches; DBP: distance between nodes in plagiotropic branches; OBL: orthotropic branch length; NNO: number of nodes in orthotropic branches; Hgt: plant height; Diam: canopy diameter, LLT1: leaf length at time 1, LWT1: leaf width at time 1; LLT2: leaf length at time 2, LWT2: leaf width at time 2.

The variation index given by the CVg to CVe ratio ranged from 0.69 to 1.56. Values between 0.70 and 2 were previously reported for most traits, considered as suitable indicators for genetic variation over the environmental variation (Ferrão et al., 2008). In fact, the CVg/CVe ratio indicates which part from the total variance is explained by the genotype (Vasconcelos et al., 2012), and when the ratio is greater than or equal to 1, the available genetic variation is the most responsible for the estimated experimental data variation (Leite et al., 2016).

Heritability was satisfactory for all variables, ranging from 74.5% (NNP) to 97.1% (LWT1). Similarly, Dalcomo et al. (2015) found heritability values from 67.12 to 93.21% for most evaluated variables in 22 Conilon coffee genotypes. The heritability in the character genetic study has a predictive role, expressing the reliability which the phenotypic value represents the genetic value (Ferrão et al., 2008; Dalcomo et al., 2015; Silva et al., 2015; Carias et al., 2016). High values for this parameter indicate the possibility of selecting superior genotypes with a greater accuracy (Oliveira et al., 2015), as well as high values of CVg and CVg/CVe ratio (Rodrigues et al., 2012; Oliveira et al., 2015; Leite et al., 2016). These results suggest the predominance of genetic components over environmental components in six out of 10 variables, thus characterizing favorable conditions for breeding from the evaluated traits.

The Scott-Knott test enabled the detection of variability among the genotypes for all evaluated characteristics (Table 3). Each evaluated characteristic presented at least three groups, reaching seven groups in the leaf parameters.

Table 3. Average biometric and leaf-related characteristics according to Scott-Knott test of 43 genotypes of *Coffea canephora* cv. Conilon.

Gen	NNP	DBP	OBL	NNO	Hgt	Diam	LL1	LW1	LL2	LW2	LA1	LA2
1	14.66c	3.71d	71.00b	18.00d	149c	138d	14.47c	6.22c	13.62c	7.49b	57.56b	65.48c
2	14.66c	3.72d	74.66b	17.83d	154b	135d	10.61g	4.41h	11.14e	5.43g	28.35g	38.89g
3	16.16b	4.01c	78.50b	18.50c	167a	181b	13.24e	5.32f	14.98b	6.77d	43.04e	65.95c
4	15.50b	4.32b	77.33b	19.66c	144c	168c	15.12b	7.63a	15.76a	8.62a	71.42a	87.98a
5	14.33c	3.54d	64.66d	19.83c	141c	155c	12.59f	5.47e	15.86a	7.44b	43.80e	76.46b
6	17.40a	3.71d	75.83b	19.50c	153b	200a	13.94d	5.30f	16.40a	7.00c	46.97d	75.02b
7	14.00c	4.06c	61.50d	18.83c	138c	163c	12.66f	5.38f	13.37c	5.98e	42.23e	51.01f
8	17.50b	3.97c	80.16b	21.50b	156b	166b	14.72c	5.49e	12.40d	5.00g	48.44d	38.96f
9	14.16c	3.78d	59.66d	16.66d	148c	175b	13.82d	5.63e	14.33b	5.76f	47.54d	53.27e
10	13.16c	4.44b	75.16b	19.16c	151b	168b	13.49e	5.24f	14.50b	6.60d	42.63e	60.71d
11	13.66c	4.48b	70.50b	17.33d	146c	151d	15.18b	5.77d	15.36a	7.02c	53.94c	67.41c
12	14.50c	3.44e	62.33d	19.16c	147c	137d	11.04g	4.57h	13.20c	6.07e	31.04g	51.25f
13	15.00c	3.42e	70.66b	19.16c	155b	160c	12.82e	5.06f	14.61b	6.04e	39.56e	53.11e
14	13.66c	4.49b	68.50c	15.83d	149c	161c	14.57c	4.78g	15.91a	6.29e	41.09e	60.21e
15	13.66c	3.72b	76.00b	22.16b	160b	164c	13.11e	5.26f	14.16b	6.46d	42.45e	56.51e
16	16.66b	3.98c	76.83b	20.77c	160b	160c	12.50f	4.73h	16.01a	6.41d	36.22f	67.15c
17	15.83b	3.40e	72.33b	19.66c	152b	162c	12.55f	5.12f	14.54b	6.33e	39.73e	59.42d
18	14.66c	4.28b	73.00b	19.00c	149c	162c	12.08f	4.87g	15.82a	7.18c	34.91f	72.13b
19	14.66c	3.99c	78.50b	20.33c	166a	184b	15.14b	6.22c	12.83c	5.67f	58.33b	47.57f
20	15.83b	3.60d	67.00c	19.83c	138c	147d	11.33g	4.60h	13.84c	6.26e	30.42g	55.89e
21	15.83b	3.77d	63.83d	18.66c	136c	146d	14.58c	6.48b	14.92b	6.74d	57.89b	63.67c
22	16.83a	3.29e	72.83b	24.66a	141c	141d	13.26e	5.29f	12.25d	5.04g	42.67e	39.43g
23	16.66a	3.42e	72.33b	22.83b	144c	148d	12.54f	5.07f	12.79c	5.01g	40.11e	39.98g
24	17.33a	3.58d	74.50b	23.50b	155b	164c	13.66d	4.97g	14.76b	5.78f	40.60e	55.76e
25	17.33a	4.95a	94.16a	19.33c	174a	179b	14.72c	4.92g	14.75b	5.59f	43.31e	55.47e
26	16.33b	3.90c	79.83b	20.33c	148c	171b	11.34g	4.68h	15.93a	6.47d	32.69g	66.32c
27	16.66a	3.58d	68.66c	19.50c	145c	154c	12.26f	5.53e	13.26c	5.76f	42.01e	49.54f
28	17.83a	3.00 e	70.33b	22.33b	145c	173b	11.42g	4.60h	14.44b	5.85f	31.40g	53.69e
29	15.83b	3.63d	64.33d	18.66c	145c	156c	13.2e	4.95g	15.70a	6.54d	40.45e	61.10d
30	14.67c	3.89c	70.33b	17.66d	154b	160c	14.30d	4.88g	16.10a	6.70d	40.97e	67.43c
31	15.00c	4.24b	73.50b	17.50d	156b	164c	14.48c	5.62e	15.70a	7.66b	51.02	74.66b
32	14.83c	3.79d	74.00b	20.66c	142c	162c	15.33b	6.29c	15.96a	7.18c	60.52b	75.80b
33	15.83a	3.44e	75.33b	20.66c	154b	158c	12.5f	4.90g	14.63b	5.99e	35.70f	53.33e
34	14.66c	4.10c	75.16b	21.50b	143c	175b	12.52f	5.26f	15.07b	6.76d	41.00e	65.53c
35	15.00c	3.75d	71.50b	19.00c	156b	171b	14.25d	5.22f	16.31a	6.00e	46.19d	63.67c
36	17.33a	3.86d	72.83b	19.66c	140c	153c	12.92e	5.61e	14.76b	6.39d	44.51d	60.24d
37	12.00c	4.04c	68.33c	20.66c	147c	158c	17.41a	6.68b	14.83b	5.89f	70.97a	53.92e
38	15.66b	3.27e	68.00c	25.66a	141c	147d	13.19e	4.60h	13.03c	4.73g	35.65f	36.39g
39	15.50b	3.57d	74.83b	20.50c	166a	168b	13.04e	5.30f	13.04c	5.08g	42.74e	42.31g
40	15.00c	3.30e	59.16d	19.16c	136c	155c	12.24f	4.64h	15.42a	6.25e	33.55f	58.49e
41	15.83b	3.60d	61.16d	17.50d	131c	148 d	13.86d	5.92d	13.62c	5.80f	49.66c	49.10f
42	15.00c	3.74d	72.00b	25.16a	138c	145d	12.93e	4.74h	14.69b	5.71f	36.45f	51.99f
43	18.33a	3.50e	69.33c	15.83d	158b	183b	15.12b	5.77d	15.27a	6.18e	52.23c	57.88e

Means followed by the same letter in the column do not differ between themselves by Scott-Knott test with 5% probability. NNP: number of nodes in plagiotropic branches; DBP: distance between nodes of plagiotropic branches; OBL: orthotropic branch length; NNO: number of nodes in orthotropic branches; Hgt: plant height; Diam: canopy diameter, LLT1: leaf length at time 1, LWT1: leaf width at time 1; LLT2: leaf length at time 1, LWT2: leaf width at time 2; LA1: leaf area at time 1, LA2: leaf area at time 2.

For NNP the 43 genotypes were separated into three groups, with genotype 43 and 37 showing the highest (18.33) and lowest (12) number of nodes, respectively. For DBP, 5 groups were formed, in which the longest (4.95 cm) and shortest (3 cm) distance between nodes of plagiotropic branches were find in genotypes 25 and 28, respectively. The orthotropic branch length (OBL) separated the genotypes into four groups, with values from

59.2 cm (genotype 40) to 94.2 cm (genotype 25). NNO also formed four groups, with genotype 38 comprising the highest number of nodes (25.7), and genotype 14 the smallest (15.83). Plant height (Hgt) formed only three distinct groups, with genotype 25 accounting for the tallest plants (174 cm) and genotype 41 the smallest ones (131.33 cm). Canopy diameter (Diam) formed four groups, with higher (207 cm) and lower (135 cm) canopy values found in genotypes 16 and 2, respectively.

A considerable variability was found for leaf characteristics, allowing the establishment of higher number of groups. For leaf length at time 1 (LLT1) seven groups were formed, ranging from 17.41 cm (genotype 37) to 10.61 cm (genotype 2). Leaf width at time 1 (LWT1) was divided into eight distinct groups, varying from 7.63 cm (genotype 4) to 4.41 cm (genotype 2). Leaf length at time 2 (LLT2) formed five groups, from a maximal value of 16.40 cm in genotype 6, to the minimum value of 11.14 cm in genotype 2. Leaf width at time 2 (LWT2) was separated into seven groups, ranging from 8.62 cm in genotype 4 to 4.73 cm in genotype 38.

The clustering of our genotypes was than performed using the Tocher optimization method, and the generalized Mahalanobis distance (D^2) as a genetic dissimilarity measure. This allowed the formation of six distinct genotype groups (Table 4), thus suggesting wide genetic variability among the genotypes, as the method recommends minimizing the intra-group distance and maximizing the inter-groups distance. Group I was the most represented group, as it included 35 genotypes. The other five groups represented only eight genotypes, divided by three (group II), two (group III), or one (groups IV, V, VI) genotypes.

Table 4. Clustering between by the Tocher method, considering 10 biometric and leaf-related characteristics of 43 genotypes of *Coffea canephora* cv. Conilon.

Group	Genotypes
I	17 33 13 39 15 32 35 24 5 29 9 40 30 1 12 27 23 20 21 28 34 7 41 36 10 26 18 4 6 3 31 16 11 8 19
II	22 38 42
III	14 43
IV	25
V	2
VI	37

The Tocher clustering method was previously used in *C. canephora*. Studies included assessments from 32 clones that comprise three clonal cultivars (Fonseca et al., 2006), 21 progenies of half-siblings (Ivoglo et al., 2008), and 34 (Covre et al., 2016) and 30 (Giles et al., 2018, 2019), promising genotypes, which formed three, four, eight, and three groups, respectively.

The cluster analysis performed by the UPGMA hierarchical method using as a measure of genetic dissimilarity the generalized Mahalanobis distance (D^2), resulted in the dendrogram that illustrates the genetic distance among the studied genotypes. By establishing the maximum limit of 40% of dissimilarity among the genotypes following the principles of Mojema (1977), we observed a formation of five distinct groups (Figure 1).

The first and second groups were composed by genotypes 25 and 43, respectively. The third group gathered the highest number of genotypes, totaling 36 (83.7%), which are as follows: 31, 11, 14, 4, 10, 34, 18, 26, 3, 6, 16, 1, 2, 28, 12, 20, 29, 5, 40, 9, 7, 36, 27, 21,

41, 30, 35, 24, 32, 15, 13, 17, 33, 8, 19, and 39. The fourth group was composed only by genotype 37; and the fifth was represented by four genotypes, 22, 23, 38, and 42 (Figure 1).

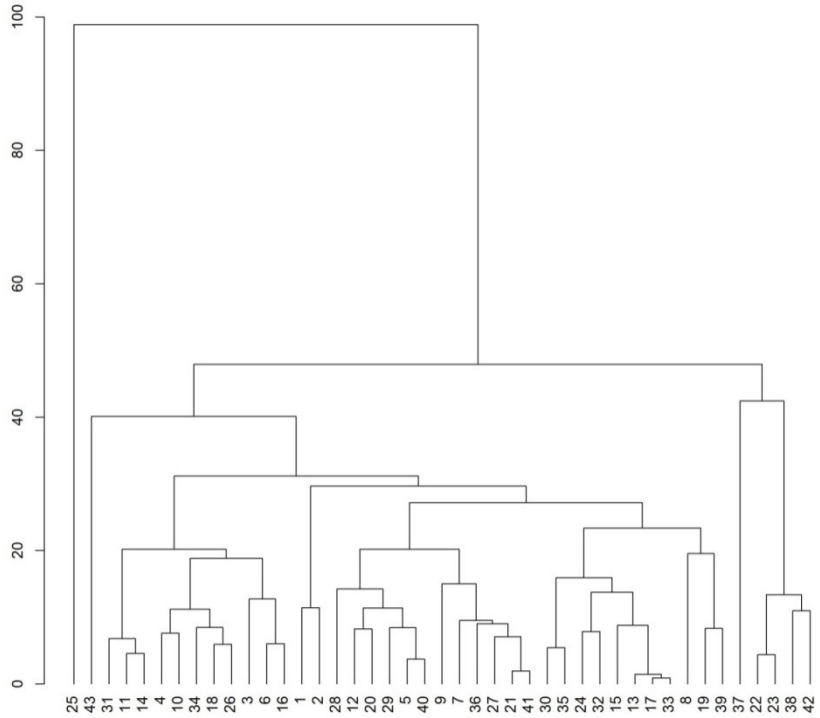

Figure 1. Dendrogram representing the genetic dissimilarity among 43 genotypes of *Coffea canephora* cv. Conilon obtained by the UPGMA clustering method, considering 10 biometric and leaf-related characteristics.

A similar study analyzed the dissimilarity of 21 progenies of *C. canephora* half-siblings by the UPGMA method with a cut-off point at 45% found the formation of nine groups (Ivoglo et al., 2008). Guedes et al., (2013) studied 12 plant materials from *C. arabica* L. var. Maragogipe Hoert. exFrohner found seven distinct groups with a cut-off point at 15%. Similarly, seven and three groups in a population of 34 (Covre et al., 2016) and 30 (Giles et al., 2018, 2019) Conilon coffee genotypes were previously reported. Additionally, the evaluation of 17 morphoagronomic traits of 22 Conilon coffee genotypes formed six groups with cut-off point at 45% (Dalcomo et al., 2015). As this study's results, the above-mentioned words obtained groups composed of progenies/plant materials/isolated genotypes, evincing their dissimilarity.

The cluster analyses from the Tocher optimization method and UPGMA hierarchical method were somehow similar in the group's composition (with only a few particularities), wherein such similarity has been previously reported by other works (Ivoglo et al., 2008; Guedes et al., 2013; Covre et al., 2016; Giles et al., 2018, 2019). For a better understanding and discussion of the main characteristics used in the UPGMA cluster analysis, we further analyzed the mean values of each variable (Table 5).

Table 5. Means of the biometric and leaf characteristics per group formed by the UPGMA method using data from 43 genotypes of *C. canephora* cv. Conilon.

Group	NNP	DBP	OBL	NNO	Hgt	Diam	LLT1	LWT1	LLT2	LWT2
I	12.00	4.04	68.33	20.67	147.83	158.33	17.41	6.69	14.83	5.89
II	18.33	3.50	69.33	15.83	158.00	183.33	15.09	5.77	15.27	6.18
III	15.35	3.81	71.21	19.43	149.81	163.06	13.25	5.33	14.67	6.41
IV	17.33	4.95	94.17	19.33	174.00	179.33	14.73	4.92	14.75	5.59
V	16.04	3.43	71.25	24.58	141.33	145.33	12.98	4.93	13.17	5.13

NNP: number of nodes in plagiotropic branches; DBP: distance between nodes in plagiotropic branches; OBL: orthotropic branch length; NNO: number of nodes in orthotropic branches; Hgt: plant height; Diam: canopy diameter, LLT1: leaf length at time 1, LWT1: leaf width at time 1; LLT2: leaf length at time 2, LWT2: leaf width at time 2.

Genotype 25, the only member of group I, presented the lowest number of nodes in plagiotropic branches, lowest orthotropic branch length, and larger leaf width at T1. Such properties should be carefully analyzed, as productive coffee fields usually tend to have a shorter distance between nodes and a higher number of nodes (Tomaz et al., 2005), diverging from this study's results. The larger leaf width may favor photosynthetic processes in favorable environments due to a larger area for intercepting of luminous energy (Brinate et al., 2015; Khan et al., 2016).

Group II included only the genotype 43, which had the highest number of nodes in plagiotropic branches, greater canopy diameter, and greater leaf length in both evaluated times. These characteristics are of great interest in coffee trees because they may be correlated to a greater productive potential, which one of the main objectives of coffee breeding, along to other agronomic traits (Carvalho et al., 2010, 2016).

The third group was formed by a large number of genotypes (36), and presented intermediate values for most of the evaluated traits, except leaf width at T2, which stood out with the highest mean. Group IV was composed only by the genotype 37, which was separated due to its longer distance between nodes in plagiotropic branches, greater orthotropic branches length, and higher plant height. Such characteristics are usually not interesting for commercial fields and thereafter may describe a less productive plant.

The fifth group was composed of genotypes 22, 23, 38, and 42. These genotypes differed from others due to their higher number of nodes in orthotropic branches, shorter distance between nodes in plagiotropic branches, lower plant height, smaller canopy diameter, shorter leaf length, and shorter leaf width at both T1 and T2. These genotypes have important characteristics because current breeders recommend smaller coffee trees. Higher number of nodes in orthotropic branches is linked to higher number of productive branches (Tomaz et al., 2005; Dubberstein et al., 2017), while lower distance between nodes may provide a higher number of nodes, which is a remarkable characteristic of plants with greater productive potential (Freitas et al., 2007).

The correlation analysis with all variables resulted in a total of 91 correlations, 42 of which were significant, with values ranging from very weak to very strong, positive and negative (Figure 2). Values from 0.00 to 0.19 are classified as very weak correlation; from 0.20 to 0.39, weak correlation; from 0.40 to 0.69, moderate correlation; from 0.70 to 0.89, strong correlation; and from 0.90 to 1.00, very strong correlation (Devore, 2006). Three

aspects should be considered in the interpretation of correlations: magnitude, direction, and significance (Nogueira et al., 2012).

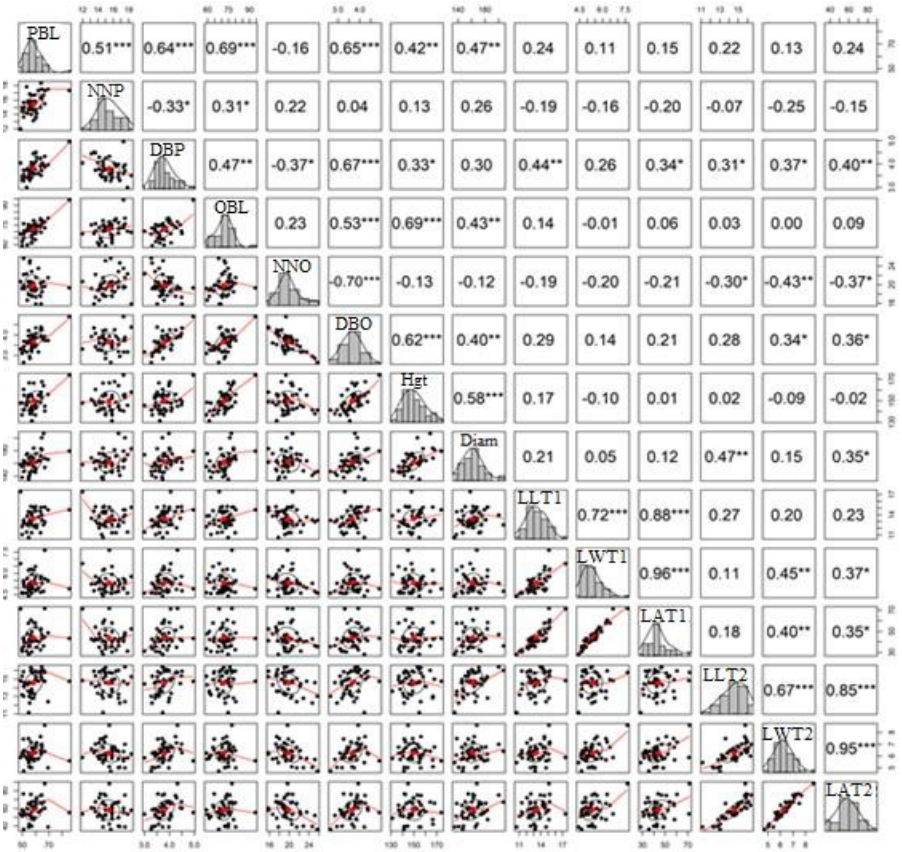

Figure 2. Correlation between biometric and morphological characteristics of coffee plant leaves (PBL: plagiotropic branch length, NNP: number of nodes in plagiotropic branches, DBP: distance between nodes in plagiotropic branches, OBL: orthotropic branch length, NNO: number of nodes in orthotropic branches, DBO: distance between nodes in orthotropic branches, Hgt: plant height, Diam: canopy diameter, LLT1: leaf length at time 1, LWT1: leaf width at time 1, LAT1: leaf area at time 2, LLT2: leaf length at time 2, LWT2: leaf width at time 2, LAT2: and leaf area at time 2) of 43 *Coffea canephora* cv. Conilon genotypes (*, **, and *** correspond to significances of $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively).

The plagiotropic branch length was positively moderate-correlated with the number of nodes in plagiotropic branches, distance between nodes in plagiotropic branch, orthotropic branch length, canopy diameter, and plant height. Teixeira et al. (2012) found a high correlation between plagiotropic branch length and number of nodes and plant height, and also that the plagiotropic branch length had a significant direct effect on crop production. Another study also indicated a moderate correlation between length and number of nodes in plagiotropic branches, and a high correlation value between plagiotropic branch length and plant height (Carvalho et al., 2010). Moreover, studies suggested that plagiotropic branch length was an indicative of canopy diameter (Freitas et al., 2007), and

that the number of nodes and plagiotropic branch length were strongly correlated (Teixeira et al., 2013).

The number of nodes in plagiotropic branches showed a weak negative correlation with distance between nodes of plagiotropic branches and a positive correlation with orthotropic branch length. Such results suggest that a longer distance between nodes decreases the number of nodes, which can be used to predict a lower coffee production (Tomaz et al., 2005; Freitas et al., 2007; Teixeira et al., 2012).

The distance between nodes in plagiotropic branches was positively weakly-correlated with plant height, leaf area at T1, leaf length at T2, and leaf width at T2. It was negative correlated with the number of nodes in orthotropic branches. Moderate correlations were found for orthotropic branch length, distance between nodes in the orthotropic branches, leaf length at T1, and leaf area at T2. Orthotropic branch length showed moderate correlations with distance between nodes in orthotropic branches, plant height, and canopy diameter. These results demonstrate the importance of these characteristics for the plant's architecture, thus greater orthotropic branches lengths suggest taller plants. However, such outcome is not necessarily interesting, because short-sized plants is desirable and thereafter aimed in breeding programs because it facilitates the overall crop management and manual or mechanized harvesting (Carvalho et al., 2013). In fact, the positive correlation between plant height and distance between nodes in plagiotropic branches indicates the possibility of selecting plants of smaller size and shorter distance between nodes (Rocha et al., 2013). In high-density coffee fields, there is a preference for short-sized cultivars due to higher yield (Freitas et al., 2007). However, there may be positive correlations between plant height and crop yield (Carvalho et al., 2010; Valadares et al., 2016), as proven by Teixeira et al. (2012) and Bitika and Sakiyama (2017), who found correlations of 0.73 and 0.42, respectively.

The number of nodes in orthotropic branches was strong correlated with the distance between nodes in orthotropic branches and negative weakly-correlated with leaf length, leaf width, and leaf area at T2, in accordance with other reports of correlations of 0.29 and 0.32 for number of nodes in relation to both leaf length and width, respectively (Teixeira et al., 2013). The Distance between nodes in orthotropic branches was positively and moderately-correlated with plant height and canopy diameter, and negative and weakly-correlated with leaf width and area at T2. According to Paulo et al. (2005), the plant height is determined mainly by the growth/length between nodes.

The correlation between plant height and canopy diameter was moderate and positive (0.58), in line with the positive correlations of 0.65 (Teixeira et al., 2013), and 0.87 (Bitika and Sakiyama, 2017), although in some cases a negative correlation was also found (-0.8102) (Freitas et al., 2007). Moderate and weak correlations were found between canopy diameter and leaf length at T1 and T2, respectively. Teixeira et al. (2013) found a correlation value of 0.46 between canopy diameter and leaf length. In addition, leaf length at T1 was strongly correlated with leaf width and area at T1. Leaf width at T1 was very strongly correlated with leaf area at T1. Leaf length at T2 was moderately and strong correlated with leaf width and leaf area at T2, respectively. Leaf width and area at T2 were very strongly correlated (0.95).

Strong and very strong correlation values between leaf length and width with leaf area in both assessed periods suggest dependence between these variables. Similar results were verified by Teixeira et al. (2012), Teixeira et al. (2013) and Schmildt et al. (2015), which found values of 0.88 between leaf length and leaf width from the fourth leaf pair for

269 *C. arabica* plant materials. The leaf surface of a coffee plant is an indicative for the crop yield potential, wherein larger leaf areas implies in larger surfaces for light interception, which may result in higher photosynthetic rates and carbohydrates availability to coffee development (Valadares et al., 2016; Walia and Kumar, 2016). Therefore, breeding programs should choose plant materials with larger leaves.

Principal component analysis demonstrated that the first two components PC1 and PC2 explained 56.77% of the total variation (Figure 3). It is not a very high value, as the first two principal components should ideally concentrate the greater amount of data variance in order to explain the divergence among the genotypes groups (Cruz et al., 2011). In the Biplot chart, the variables are represented by vectors and the genotypes by numbers. The larger the vector, the greater the influence of the variable in the cluster. The smaller the angle between the vectors, the greater the correlation between the variables. Therefore, it is possible to note that many genotypes are dispersed, indicating considerable divergence within the evaluated characteristics.

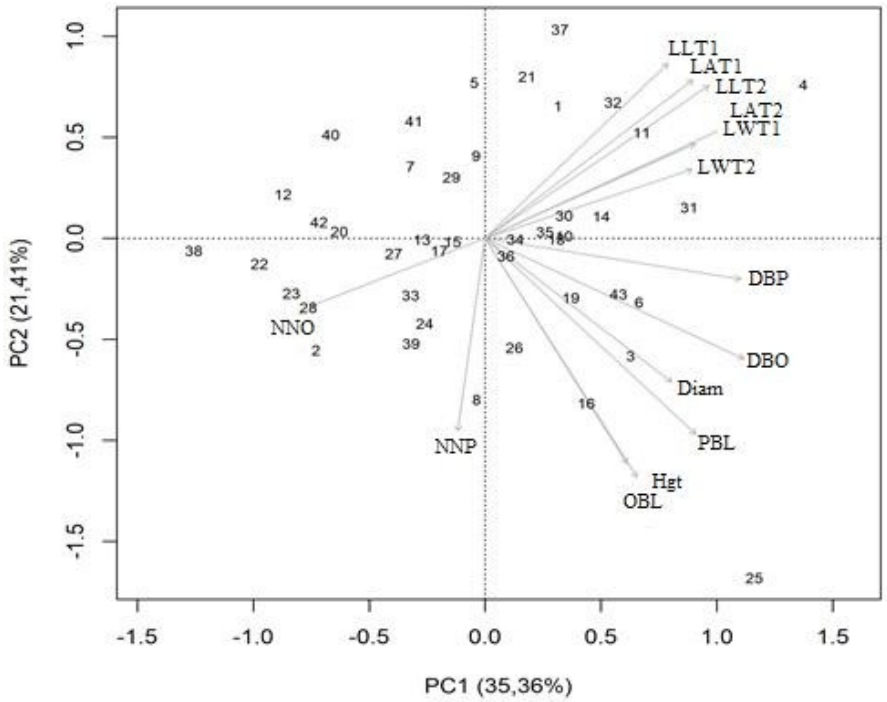

Figure 3. Principal component analysis for 14 biometrics and leaf-related variables of 43 genotypes of *Coffea canephora*. cv. Conilon (PBL: plagiotropic branch length; NNP: number of nodes in plagiotropic branches; DBP: distance between nodes in plagiotropic branches; OBL: orthotropic branch length; NNO: number of nodes in orthotropic branches; DBO: distance between nodes in orthotropic branches; Hgt: plant height; Diam: canopy diameter, LLT1: leaf length at time 1, LWT1: leaf width at time 1; LAT1: leaf area at time 1; LLT2: leaf length at time 1, LWT2: leaf width at time 2; LAT2: leaf area at time 2).

From PC1 it can be observed that there is a large distance between genotypes 25 and 37. This behavior certainly occurred because genotype 25 presented the longest distance between nodes in plagiotropic branches, greatest length of orthotropic branches

(94.16 cm), and highest plant height (174 cm). Due to its position and distance from the others, it can be considered an outlier. Genotype 37 is distinguished for presenting the lowest number of nodes in plagiotropic branches. Both formed isolated groups in the dendrogram, thus, these characteristics are very specific compared to the other genotypes.

Regarding PC2, the longest distance was found between genotypes 4 and 38. The characteristics that mostly separated the genotype 38 were the higher number of nodes in orthotropic branches and the lower leaf width values. In contrast long and the larger leaves were observed in genotype 4 in relation to other genotypes.

In relation to leaf characteristics, there are large positive factorial loadings in components 1 and 2, in which all are concentrated, confirming the high degree of correlation among them. Plant height, canopy diameter, orthotropic branch length, plagiotropic branch length, distance between nodes in both orthotropic and plagiotropic branches have also large positive factorial loadings in PC1 and negative factorial loadings in PC2. Therefore, all these traits are related and thus contribute to the plant size and structure. The number of nodes in both branches remained isolated, with large negative factorial loadings in PC 1 and 2. We noted an overlap for orthotropic branch length plant height overlap. Similarly, the plagiotropic branch length was very close to the canopy diameter, suggesting that these variables are positively correlated.

It is important to note that the genotypes distribution is related to the position and direction of vectors that most influenced and differentiated each genotype from the other. For example, genotype four presented larger leaf area and this was plotted exactly above this characteristic. The same happened with genotype 25, the highest plant height, plotted below this trait. Other genotypes were plotted close to a given variable as they presented similar characteristics in their structure. These analyses confirmed the existing correlations between variables, and the distribution of the genotypes indicates different aspects in their constitution, thus assisting in the breeding process of plants with desirable characteristics.

CONCLUSIONS

The estimates of genetic parameters indicated the existence of genetic variability and breeding potential among the Conilon coffee genotypes, especially for leaf area, orthotropic branch number and length, number of nodes and canopy diameter. Both Tocher optimization and UPGMA hierarchical methods were consistent for clustering the genotypes, ordering them in six and five dissimilar groups, respectively, with genotypes 25 and 37 standing out with the greatest dissimilarity, constituting isolated groups in both methods. The correlation and principal components analyses indicated the characteristics with a greater degree of correlation, assisting in the choice of more promising plant materials.

ACKNOWLEDGMENTS

The authors wish to thank the coffee breeders, i.e., the farmers who performed the initial selection of most superior genotypes currently available and the farmer Mr. Thekson Pianissoli. They also thank the Universidade Federal do Espírito Santo - UFES for funding this research, and for providing experimental resources; the Fundação de Amparo à Pesquisa e Inovação do Espírito Santo - FAPES (grants nº. 84320893); the Coordenação de

Aperfeiçoamento de Pessoal de Nível Superior - CAPES (Finance Code 001); and the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (grants nº. 420789/2016-2 and nº. 304687/2017-0). Funding from FAPERJ (grants E-26/202.323/2017, W.P.R.) is also gratefully acknowledged. Support from Fundação para a Ciência e a Tecnologia (FCT), Portugal, through the research units UID/04129/2020 (LEAF), and UIDP/04035/2020 (GeoBioTec) are also greatly acknowledged. This work is from the first author's Ph.D. thesis.

AUTHOR CONTRIBUTIONS

D.D., F.L.P. and J.C.R. conceived and designed the experiments.; D.D., F.L.P, J.H.S.G., W.P.R., J.C.R. and A.I.R.-B. collected and analyzed the data. D.D., F.L.P, J.H.S.G., W.P.R., J.C.R. and A.I.R.-B. wrote the paper.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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