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BIOLOGY

Morpho-agronomic and leaf anatomical traits in *Coffea canephora* genotypes

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ABSTRACT: Genetic variability is the basis for coffee genetic breeding. This study evaluated the potential of leaf anatomy and morpho-agronomic traits in studies of genetic variability in *C. canephora* cultivars. Ten genotypes were distributed in randomized block designs with three replicates. Significant differences among genotypes were detected by F-test ($P < 0.05$) for 13 of 15 evaluated traits. These results evidenced the heterogeneity of the studied cultivars, which is essential in composition of genetic basis in breeding programs. The Scott-Knott test detected variability among genotypes, grouped into up to four mean groups. Leaf anatomy traits presented the largest variations. Five out of seven leaf anatomy traits presented heritability higher than 80%, with emphasis on stomatal density (95.69%) and stomatal pore length (92.72%). Positive correlations were observed among morpho-agronomic and anatomic traits. Cluster analysis used the Mahalanobis general distance (D^2) as a measure of genetic dissimilarity and divided the genotypes into two distinct groups. The inclusion of leaf anatomic traits to characterize *C. canephora* genotypes may assist plant breeders with better genetic discrimination and with greater security in plant selection when composing cultivars.
Key words: Conilon coffee, Electron Microscopy, Leaf Microscopy, Plant breeding.

Caracteres morfoagronômicos e anatômicos foliares em genótipos de *Coffea canephora*

RESUMO: A variabilidade genética é a base para o progresso genético em café. Este estudo teve como objetivo, avaliar o potencial de caracteres morfoagronômicos e anatômicos foliares em estudos de variabilidade genética entre cultivares de *Coffea canephora*. Dez genótipos foram distribuídos em um experimento seguindo delineamento em blocos ao acaso com três repetições. Foram detectadas diferenças significativas entre os genótipos pelo teste F ($P < 0,05$) para 13 dos 15 caracteres avaliados. Esses resultados evidenciaram a heterogeneidade entre as cultivares estudadas, o que é essencial para a composição da base genética e avanço dos programas de melhoramento. O teste de Scott e Knott detectou as diferenças entre os genótipos, separando-os em quatro grupos. Os caracteres anatômicos foliares apresentaram as maiores variações. Cinco, dentre os sete caracteres anatômicos foliares, apresentaram estimativas de herdabilidade superiores a 80% com destaque para a densidade estomática (95,69%) e comprimento do poro estomático (92,72%). Correlações positivas foram observadas entre caracteres morfoagronômicos e anatômicos foliares. A análise de agrupamento, considerando a distância de Mahalanobis como medida de dissimilaridade (D^2), apontou a distinção de dois grupos de genótipos. A inclusão de caracteres anatômicos foliares na caracterização de genótipos de *C. canephora* pode auxiliar os melhoristas de plantas na melhor discriminação entre genótipos e maior segurança na seleção de clones para geração de novas cultivares.
Palavras-chave: café conilon, microscopia eletrônica, microscopia foliar, melhoramento de plantas.

INTRODUCTION

Coffee is widely cultivated and consumed in the world. In Brazil, the coffee production had produced 40 million sacks in recent years and this crop contributes significantly for economy stability (ICO, 2021). According to the Brazilian Council of Coffee Exporters (Cecafé), Brazilian coffee exports reached 44.706 million 60 kg-sacks, yielding US$ 5.6 billion in foreign exchange revenue.

One hundred and twenty four coffee species have been identified (DAVIS et al., 2011) however, *Coffea arabica* L. and *C. canephora* Pierre ex A. Froehner represent the total coffee cultivation in Brazil. Besides the diversity among coffee species, intraspecific variety is also reported (ALKIMIM et al., 2018; SILVA et al., 2020). *C. canephora* is an allogamy species due the genetic self-incompatibility, and such mechanisms block flower fertilization by pollen from the plant itself. So, these features

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are fundamental for *C. canephora* reproductive success and ensures a wide genetic base in this crop (MORAES, et al. 2018).

Genetic variability is the basis of coffee breeding programs. Several methods are used to evaluate genetic diversity, including morphologic, physicochemical, and molecular markers. Morphological markers are primordial to selection and have been substantially used to explain diversity among coffee accesses (AKPERTEY et al., 2019; NGUGI & ALUKA, 2019).

Microscopy techniques, including light and electron microscopy, allow evaluation of morpho-anatomy diversity in distinct plant tissues (LUSA et al., 2018; SINGH et al., 2020). Electron microscopy, in particular, is an efficient versatile tool in plant anatomy studies by producing high-resolution images (STABENTHEINER et al., 2010; GUL et al., 2019).

Among plant organs, microscopy studies with leaves have been conducted in several crops (SCHOLLERT et al., 2015; FARALLI et al., 2019; SINGH et al., 2020). Leaves are essential to plants, since they are responsible for supplying photoassimilates; and therefore, are one of the first organs responding to unbalances in plant metabolism (TAIZ & ZEIGER, 2017). Among the leaf structures studied, stomata are structures that arouse interest, as they are highly variable between species and within species (GUL et al., 2019). Such variations are considered as immediate response to environmental conditions (CASTRO 2009; MATTHEWS & LAWSON, 2019) and to genetic components acting on stomata development (CASSON & GRAY, 2008; CHATER et al., 2017; ZOULIAS et al., 2018) both contributing to changes in stomatal development.

Few studies of divergence in coffee have been carried out under light microscopy (GILES et al., 2019), and efforts are needed to reaffirm the potential of these anatomical traits. Moreover, studies must still demonstrate possible associated genetic components and advance in inference precision with the aid of enhanced tools, including electron microscopes. The identification of new phenotypic traits capable of discriminating genotypes will contribute to greater efficiency in genetic diversity studies. Thus, this study evaluated the potential of leaf anatomic traits, along with agronomic traits, in the evaluation of genetic variability in two *C. canephora* registered cultivars.

MATERIALS AND METHODS

Plant material and experimental design

Ten *C. canephora* genotypes were sampled from two cultivars, registered in the Ministry of Agriculture, Livestock and Food Supply (MAPA, Brazil): Andina and Tributun (Table 1), both consisting of five genotypes each. In C. *canephora*, commercial cultivars are composed of different genotypes due to genetic self-incompatibility. Although, these cultivars had already reached ideal productivity levels under antagonistic conditions, new additions may be explored by investigating leaf anatomy peculiar traits. Andina is recommended for cultivation at c.a. 850 m of altitude and low temperatures (PARTELLI et al., 2019), whereas Tributum is recommended for regions lower than 500 m (PARTELLI et al., 2020).

The genotypes were cultivated after vegetative propagation in June 2018 at the Experimental Farm of Federal University of Espírito Santo (UFES), located at São Mateus, Espírito Santo, Brazil (coordinates 18º 42’ 58” S / 39º 51’ 32” W; altitude 36 m; temperature annual mean 24 °C;

Table 1 - Identification of ten *Coffea canephora* genotypes grown in the Fazenda Experimental of UFES is the city of São Mateus, state of Espírito Santo.

ID	Genotype	ID	Genotype
1	Pirata*	6	Beira Rio 8*
2	Verdim R*	7	P1**
3	Bamburral*	8	Verdim TA**
4	A1***	9	NV2**
5	Clementino*	10	NV8**

*Tributum cultivar (Partelli et al., 2020); ** Andina cultivar (PARTELLI et al., 2019).

precipitation annual mean 1.240 mm). The region presents tropical climate characterized by warm wet summers and a short dry period, which is classified as Am by Köppen (ALVARES et al., 2013). Spacing was 2 m between lines and 1 m between plants (5.000 plants.ha^{-1}). Crop formation was managed by conduction of two rods per plant (10.000 rods. ha^{-1}), and a supplementary irrigation by dripping was adopted. The treatments were constituted by the distinct genotypes, and each experimental unit was composed by three plants. The genotypes were arranged in random blocks, with three repetitions.

Morpho-agronomic traits

Two plants per experimental unit were evaluated, totalizing six plants per genotype. The number of rosettes (NROS) was counted, and a measure tape was used to evaluate the plant height (H – cm), plant diameter (D – cm), and length of plagiotropic branches (LPB – cm). Six leaves were collected from each plant medium third for evaluation of leaf area (LA – cm), petiole length (PL – cm) and central vein length (CV – cm), and then dried at 60 °C until constant mass for determination of specific dry mass per leaf square centimeter (SDM – g).

Leaf anatomic traits

Six leaves per experimental unit of each coffee genotype were collected during morning from the third or fourth leaf pairs from the top of plagiotropic branches. Fragments of collected leaves (1 cm^2) were fixed with 50% FAA (formaldehyde, glacial acetic acid and 50% ethanol, 1:1:9) for 48h. The samples were preserved by fixation with 2.5% glutaraldehyde, 2% formaldehyde and 0.1 M sodium cacodylate buffer. Next, they were transferred to a recipient covered with laminated paper and submerged during 1h at room temperature in aqueous solution containing 2% OsO_4 (osmium tetroxide), 0.2 M sodium cacodylate buffer and 2.5% potassium ferrocyanide. After this period, the samples were washed with the same buffer and milliQ water, dehydrated with ethanolic series (30, 50, 70, 90 and 100%), and critical point dried. The dried material was mounted on stubs and covered in gold.

Images from leaf surface were obtained in Scanning Electron Microscope (SEM), using a 50 µm scale, for measurements of stomata polar diameter (PD – µm), equatorial diameter (ED – µm), stomatal pore length (SPL – µm) and stomatal pore width (SPW – µm). These anatomic traits enabled estimations of stomatal functionality (SF), stomatal area index (SAI) and stomatal density (SD) (CASTRO et al., 2009). Image analyses were conducted with ImageJ software (PAPADOPULOS et al. 2007).

Data analysis

Data was subjected to analysis of variance using F-test ($P < 0.05$). The following genetic parameters were evaluated using variance components of each evaluated trait: genetic variation coefficient (VCg), environmental variation coefficient (VCe), variation index (VI) and heritability (h^2). After identifying significant differences among genotypes, means were grouped by the Scott-Knott test using 5% significance level.

For analysis of dissimilarity of the studied traits, the dissimilarity was estimated using the Mahalanobis distance. After that, the cluster analysis used the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method. The relative importance of traits for diversity was evaluated according to SINGH (1981).

All statistical procedures were conducted with Genes® software (CRUZ, 2013). The Pearson linear correlation analysis were processed, with the R software (RCORE TEAM, 2021) and "corrplot" package, to verify possible correlations among agronomic and anatomic traits.

RESULTS

Morpho-agronomic traits, leaf anatomic traits and genetic parameters

The F-test detected significant differences ($P < 0.05$) among *C. canephora* cultivars in 13 of 15 traits (Table 2). The VCe for those traits varied between 3.55-24.08%. VCg, which quantifies the influence of genetic components in each trait, varied between 5.75-23.36%, and the highest VCg values were found in LA and SD. The trait LA exhibited the highest VCe and VCg values (24.08 and 23.36%, respectively). Moreover, SD, PD and ED presented the highest IV (VCg/VCe) values, which were higher than one.

The heritability coefficient measures the proportion of variability caused by genetic effect. In all traits exhibiting significant differences among genotypes, heritability varied between 73.85-95.66% (Table 2), and five out of seven leaf anatomic traits presents a value higher than 80%, emphasizing SD and SPL. Moreover, whereas the mean heritability of morpho-agronomic traits was 64.98%, it increased to 87.14% in leaf anatomic traits.

Table 2 - Analysis of variance with morpho-agronomic traits and leaf anatomic traits from *Coffea canephora* genotypes, and their respective genetic parameters.

Variables	----------QM----------						
	Genotype	Residue	Means	VCe (%)	VCg (%)	VI	h² (%)
LA	1810.61**	473.49	90.37	24.08	23.36	0.97	73.85
PL	0.289**	0.047	1.44	15.09	19.78	1.31	83.75
CV	10.26**	2.48	15.25	10.33	10.56	1.02	75.83
H	264.29ns	168.65	80.71	16.09	6.99	0.43	36.18
LPB	200.85*	62.24	54.72	14.41	12.42	0.86	69.01
NROS	5.53ns	4.18	10.32	19.8	6.50	0.32	24.45
D	210.78**	56.2	63.15	11.87	11.36	0.95	73.34
SDM	0.061**	0.010	1.07	9.37	12.14	1.30	83.43
PD	2.82**	0.30	13.88	3.96	6.61	1.67	89.33
ED	0.93**	0.10	9.09	3.55	5.75	1.62	88.67
SPL	3.42**	0.25	9.75	5.12	10.55	2.06	92.72
SPW	0.44**	0.089	2.31	12.93	14.85	1.14	79.84
SF	0.03**	0.006	1.53	5.31	6.11	1.15	79.85
SAI	661.32**	106.05	128.65	8.00	10.58	1.32	83.96
SD	0.025**	0.001	0.425	7.86	21.33	2.71	95.66

** and * indicate significance at 1 and 5% probability by F-test, respectively; ns = non-significant. VCe: environmental variation coefficient; VCg: genetic variation coefficient; VI: Variation Index; h2: Heritability; LA: leaf area (cm); PL: petiole length (cm); CV: leaf central vein length (cm); H: plant height (cm); LPB: length of plagiotropic branches (cm); NROS: number of rosettes; D: plant diameter (cm); PD: stomata polar diameter (µm); ED: stomata equatorial diameter (µm); SPL: stomatal pore length (µm); SPW: stomatal pore width (µm); SF: stomatal functionality; SAI: stomatal area index; SD: stomatal density; SDM: specific dry mass per leaf square centimeter (g).

The Scott-Knott test grouped the genotypes in two, three or four distinct mean groups to depend on the trait (Table 3). Morpho-agronomic traits presented lower variations compared to leaf anatomic traits, as LA, PL, H, D, and SDM formed only two groups of means. The leaf anatomic traits exhibited higher variations, and up to four mean groups were distinguished. Such variations are easily spotted by SEM images (Figure 1).

Four mean groups were found for SPL (Table 3). This trait was highly variable between NV2 and NV8. Moreover, SD and PD formed three mean groups, the first varied between 0.290-0.563 µm, whereas the latest varied between 12.79-15.85 µm, respectively. Two mean groups were formed for ED, SAI and SF. The highest means for ED and SAI were found in NV8 (10.44 µm and 157.87 µm, respectively). The genotypes with the highest and the lowest SF were, respectively, P1 and NV2, due to the smaller polar diameter found in NV2. High PD/ED ratios indicate greater stomata functionality, since stomata have an ellipsoid shape (Figure 2a, b). SPW values formed a single mean group; although, significant differences have been reported for this trait. Leaves from all genotypes are hipostomatous (Figure 2a, b).

Cluster analysis

After analyzing simultaneously multiple traits at a 99% maximum threshold (significant point from the dissimilarity distances matrix among genotypes), two groups were distinguished (Figure 3). Group I was formed by eight genotypes, whereas group II clustered only two genotypes. According to the evaluated traits, Bamburral and Beira Rio 8 are the most similar genotypes, exhibiting a 36.36 dissimilarity (Mahalanobis Distance). Verdim TA and NV8, which formed group II, presented a distance of 294.17. Groups I and II presented genetic distance of 490.13. The cophenetic correlation coefficient between Mahalanobis general distance and the cophenetic distance matrix was 88%. PD, SF and SD were the most contributing traits considering the divergence (Figure 4), being responsible for 56.69% of diversity among genotypes. Notably, the genotypes that formed group II were the ones that

Table 3 - Mean values of morpho-agronomic and leaf anatomic traits for 43 *Coffea canephora* genotypes, according to Scott-Knott test.

Genotypes	LA	PL	CV	LPB	D	SDM	PD
Pirata	79.97[b]	1.83[a]	16.5[a]	62.67[a]	76.17[a]	0.011[a]	13.37[c]
Verdim R	57.3[b]	1.06[b]	12.83[b]	51.33[b]	58.5[b]	0.012[a]	12.93[c]
Bamburral	110.4[a]	1.1[b]	14.0[b]	48.5[b]	62.5[b]	0.012[a]	14.17[b]
A1	100.4[a]	1.2[b]	18.5[a]	61.83[a]	71.33[a]	0.008[b]	13.22[c]
Clementino	135.13[a]	1.53[a]	17.83[a]	68.5[a]	73.67[a]	0.011[a]	13.41[c]
Beira Rio 8	108.87[a]	1.13[b]	15.0[b]	55.0[b]	61.33[b]	0.012[a]	13.96[b]
P1	87.27[b]	1.33[b]	15.5[b]	58.83[a]	69.5[a]	0.011[a]	14.00[b]
Verdim TA	55.37[b]	1.63[a]	14.33[b]	46.67[b]	47.67[b]	0.009[b]	15.85[a]
NV2	78.23[b]	1.63[a]	13.5[b]	42.17[b]	58.5[b]	0.010[b]	12.79[c]
NV8	90.77[b]	1.9[a]	14.5[b]	51.67[b]	56.33[b]	0.010[b]	15.10[a]
Genotypes	ED	SPL	SPW	SF	SAI	SD	
Pirata	9.17[b]	8.87[d]	2.44[b]	1.47[b]	122.53[b]	0.563[a]	
Verdim R	8.65[b]	8.85[d]	2.36[b]	1.50[b]	111.45[b]	0.403[b]	
Bamburral	8.91[b]	9.41[c]	2.22[b]	1.59[a]	126.35[b]	0.413[b]	
A1	9.03[b]	9.25[c]	2.27[b]	1.47[b]	140.67[a]	0.417[b]	
Clementino	8.83[b]	9.73[c]	2.09[b]	1.52[b]	117.77[b]	0.327[c]	
Beira Rio 8	8.84[b]	10.04[b]	2.42[b]	1.58[a]	123.96[b]	0.397[b]	
P1	8.84[b]	10.51[b]	3.10[a]	1.66[a]	117.89[b]	0.370[b]	
Verdim TA	8.42[b]	11.36[a]	2.40[b]	1.71[a]	146.93[a]	0.563[a]	
NV2	9.41[b]	8.12[d]	1.53[b]	1.36[b]	121.08[b]	0.290[c]	
NV8	10.44[a]	11.30[a]	2.25[b]	1.47[b]	157.86[a]	0.506[a]	

Means followed by the same letter in columns belong to the same group, after Scott-Knott test with 5% probability.LA: leaf area (cm); PL: petiole length (cm); CV: leaf central vein length (cm); LPB: length of plagiotropic branches (cmD: plant diameter (cm); PD: stomata polar diameter (µm); ED: stomata equatorial diameter (µm); SPL: stomatal pore length (µm); SPW: stomatal pore width (µm); SF: stomatal functionality; SAI: stomatal area index; SD: stomatal density; SDM: specific dry mass per leaf square centimeter (g).

showed the highest PD values, which is a determining characteristic for clustering.

Correlations among morpho-agronomic and leaf anatomic traits

Pearson correlation coefficients emphasized significant relationships among traits, including positive-high and negative-high associations (Figure 5). A positive correlation was reported for PD and SPL ($r = 0.9^{*}$), in this sense, the higher PD is, the higher SPL. Since SPW is perpendicular to ED, a similar trend was expected for both. However, correlation between ED and SPW was negative ($r = -0.4$). Significant positive correlations ($r = 0.9^{*}$) were also found between ED and SAI.

Among morpho-agronomic and leaf anatomic traits, only CV and ED had a significant positive correlation ($r = 0.7^{*}$). Conversely, SDM and SAI were negatively correlated ($r = -0.6^{*}$), indicating that both traits are inversely correlated.

DISCUSSION

Stomata are related to diverse physiological functions Besides being crucial to plant survival, stomata are fundamental in photosynthesis, since they balance gas exchanges between the plants and the atmosphere; and therefore, contributing strongly to the agricultural production (POMPELLI et al., 2010; ZOULIAS et al., 2018; ENDO & TORII, 2019).

Stomata were found only at the abaxial leaf surface in all genotypes. Thus, such leaves are hipostomatous, which is possibly a strategy to minimize water transpiration, since the lower surface is less exposed to sun radiation (RAMALHO et al., 2013; ZOULIAS et al., 2018).

The ANOVA results evidenced the heterogeneity of the studied clonal population, essential for composition of the genetic base in breeding programs. Similar results were reported by GAMA et al., (2017) and GILES et al., (2019)

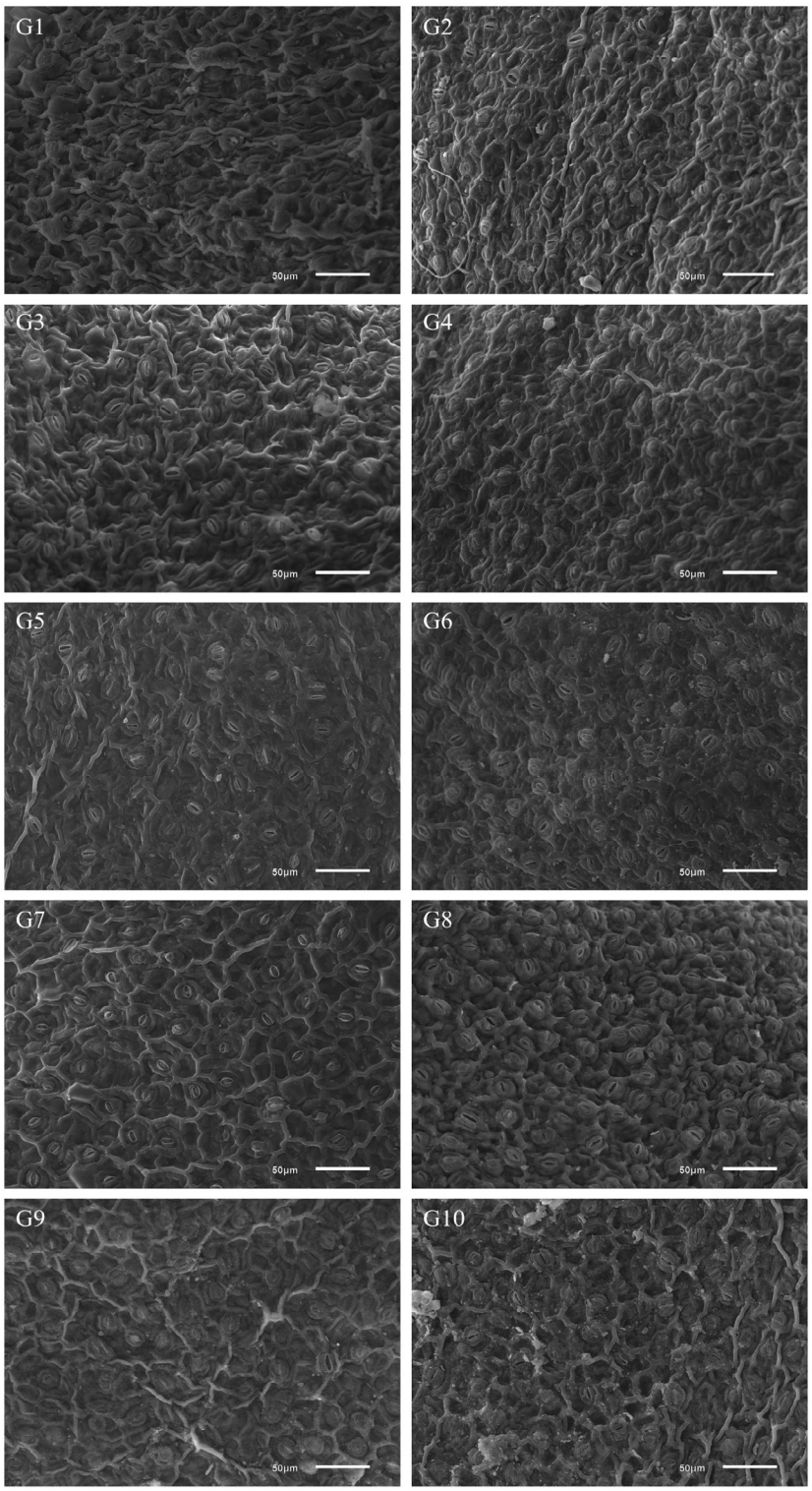

Figure 1 - Scanning Electron Microscopy (SEM) images of *Coffea canephora* leaf surfaces. G1:Pirata*; G2: Verdim R*; G3: Bamburral*; G4: A1*,**; G5: Clementino*; G6: Beira Rio *; G7: P1**; G8: Verdim TA**; G9: NV2; G10: NV8**. * Tributum cultivar (Partelli et al., 2020); ** Andina cultivar (Partelli et al., 2019). Scale bar = 50 µm

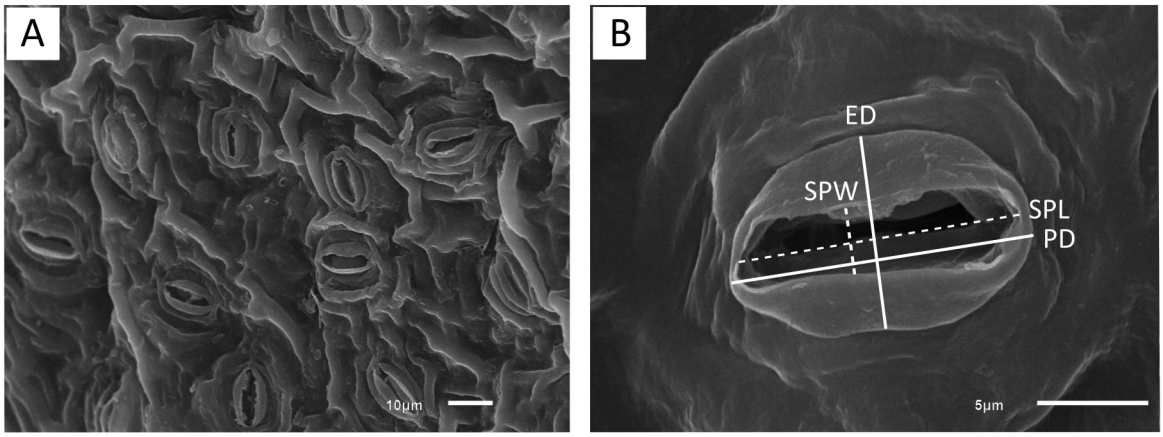

Figure 2 - Scanning electron nicroscopy (SEM) images of *Coffea canephora* ellipsoid stomata. Scale bar = 10µm (A) and 5µm (B). ED: stomata equatorial diameter (µm); PD: stomata polar diameter (µm); SPL: stomatal pore length (µm); SPW: stomatal pore width (µm).

evaluating anatomical characteristics of coffee leaves, although these studies analyzed images with optical microscopy.

The leaf anatomic traits may be related to environmental responses and hormonal stimulation, and therefore a high variation level is expected among individuals from the same species (QI & TORII, 2018). Although, this study had been carried at a single site, the results support that variation among genotypes are mainly genetic-based rather than environmental-based. Thus, the studied traits can be safely used as target selection traits in breeding programs.

All VCe values in this study are acceptable, since only LA presented VCe > 20%. These results indicate high experimental precision (CRUZ et al., 2012). The highest VCg values were found for LA, SD and CV. The determination of VCg allows the breeders to have a greater precision during selection, since this parameter gives a real vision of the existent genetic variation (FERREIRA et al., 2016). All traits, except H and NROS, presented IV values close or higher than one, indicating that the genetic effects predominate over environmental effects (CRUZ et al., 2012; GILES et al., 2019).

The heritability values showed that the leaf anatomic traits were less affected by environment when compared with morpho-agronomic traits. The adoption of traits with greater heritability in studies of genetic divergence is desirable in breeding programs. The higher the heritability and the lower the environmental effect, the greater the safety for the breeder to select favorable alleles by phenotypic data.

Lower variations in morpho-agronomic traits were expected, since the genotypes had already passed through a selection process to compose the two cultivars to which they belong (PARTELLI et al., 2019; PARTELLI et al., 2020). Similarly, and as a reflex of this selection process, the lowest percentages of relative contribution of the morpho-agronomic traits for diversity were also already expected.

The morpho-agronomic traits are the first to be used in distinguishing phenotypes, since the phenotypic expressions are easily distinguishable. Thus, such traits are common targets in enhancing programs (AKPERTEY et al., 2019; PARTELLI et al., 2019; PARTELLI et al., 2020). However, even though these genotypes had already passed through selection by morpho-agronomic traits, the high variability for leaf anatomic traits suggests their use in future strategies inside the breeding program. Studies with some of the studied genotypes had also detected variability in vegetative and nutritional traits (MARTINS et al., 2019; MARTINS et al., 2020).

The knowledge on plants from measurements of stomata size and density is fundamental in plant biology, since they are related with stomatal conductance (SACK & BUCKLEY, 2016). In this study, Pirata and Verdim TA presented the highest SD values, whereas NV2 had the lowest mean values, which is next to half than in Pirata and Verdim TA. According to CASTRO et al., (2009), leaves adapted to drought present higher stomatal density and smaller stomata, correlated to a more

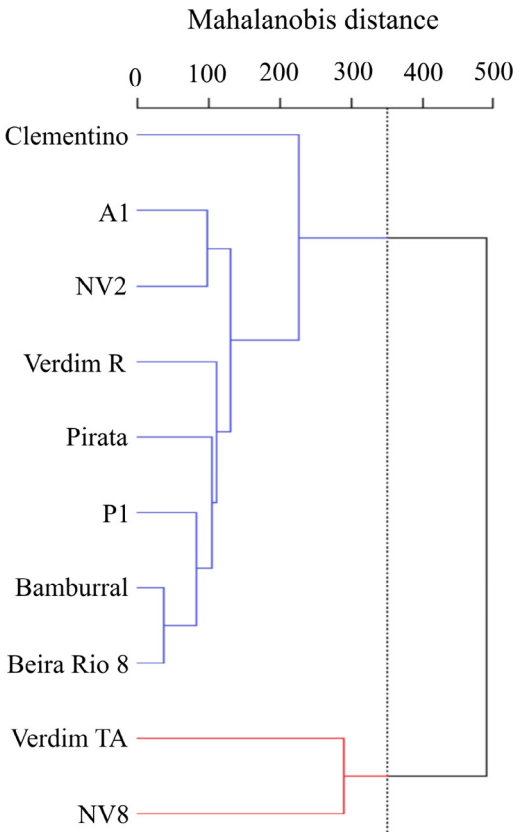

Figure 3 - Dendrogram of genetic dissimilarity among 10 *Coffea canephora* genotypes, obtained by UPGMA clustering method and Mahalanobis general distance. Fifteen morpho-agronomic and anatomic traits were considered.

efficient stomatal conductance. The understanding of the development base of those traits allows the targeting of plant enhancing towards grain productivity (SACK & BUCKLEY, 2016).

The dissimilarity analysis showed that clones of Tributum cultivar were grouped into the same cluster, whereas the Andina clones, Verdim TA and NV8, were placed in a distinct group. Thus, the variability of the studied traits was higher within the Andina cultivar when compared with Tributum. According to PARTELLI et al., (2019), Verdim TA and NV8 adapted well and presented high productive capacity at 850 m altitude, which in theory would be a stressing condition for *C. canephora*. Possibly, these clones present higher capacity in changing stomata attributes after distinct climate conditions.

To guarantee a greater production of conilon coffee crops, cultivars must be composed by genetically distant genotypes, due to its gametophytic self-incompatibility (MORAES et al., 2018; PARTELLI et al., 2020; TEIXEIRA et al., 2020). Therefore, the assembly of genetic variation and water use efficiency, which is one of the stomata major roles, may improve crop yield in environments without water (BERTOLINO et al., 2019; ENDO &TORII, 2019).

In this study, the estimated maximum distance between groups was 490.13 (Mahalanobis distance). Such a high value reinforces the potential of leaf anatomical traits, together with morpho-agronomic traits, to increase the level of precision in estimating genetic divergence between genotypes.

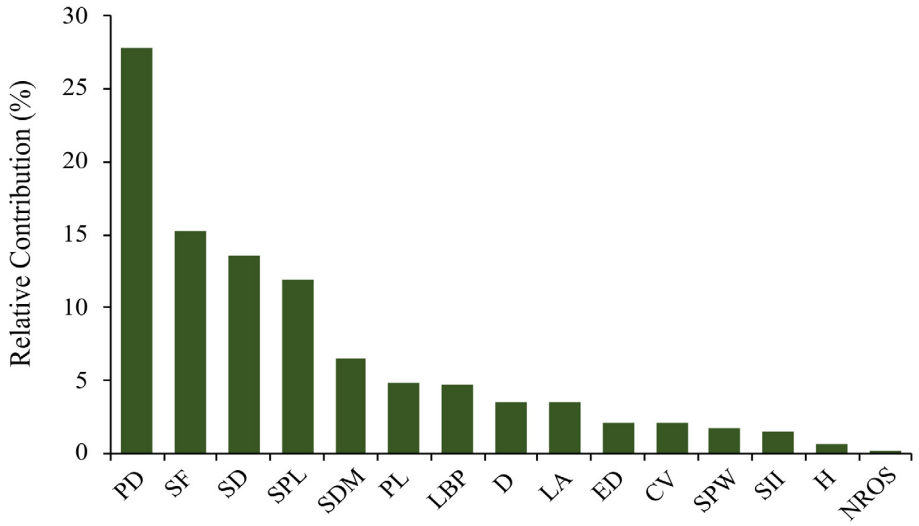

Figure 4 - Trait relative contribution for divergence by SINGH (1981) method.

LA: leaf area (cm); PL: petiole length (cm); CV: leaf central vein length (cm); H: plant height (cm); LPB: length of plagiotropic branches (cm); NROS: number of rosettes; D: plant diameter (cm); PD: stomata polar diameter (µm); ED: stomata equatorial diameter (µm); SPL: stomatal pore length (µm); SPW: stomatal pore width (µm); SF: stomatal functionality; SAI: stomatal area index; SD: stomatal density; SDM: specific dry mass per leaf square centimeter (g).

GILES et al., (2019) evaluated the genetic divergence among 34 coffee genotypes by observing agronomic and leaf anatomic traits obtained by light microscopy and obtained 71.41 (Mahalanobis Distance) of distance maximum estimate among groups.

Considering the precision of the electron microscopy and the high heritability values, our results support that the Scanning Electron Microscopy further higher precision in phenotyping from leaf anatomic traits (BUDEL et al., 2018; GUL et al., 2019). In a study with *Citrus* sp. using SEM (SINGH et al., 2020), the authors could distinguished genotypes using agronomic and leaf anatomic traits, which play an important role in studying diversity. According to ENDO & TORII (2021), molecular mechanisms allowing exploration of those traits in big crops, including coffee, are still needed to be fully understood.

High correlations among ED and SAI with the morpho-agronomic traits showed that such traits may be used in indirect selection strategies when morpho-agronomic traits have reduced heritability. This strategy ensures breeders greater efficiency in the selection process (BARBOSA et al., 2019; CHESEREK et al., 2020). Conversely, in cases which correlation was negative (between SAI and SDM, in example), simultaneous trait selection is hindered. The application of selection indexes may assist breeders during development of higher cultivars (CRUZ et al., 2012).

Besides the aforementioned possibilities, the potential use of leaf anatomic traits may enable coffee breeders to conduce early selection of their materials, since inferences on those traits may be conducted at early phases of the culture development. Such opportunities are important due to their agility and the consequent potentiation of genetic gains during cultivar development of perennial cultures, including coffee.

Along with previous literature data, it is expected that our results may foment utilization of leaf anatomic traits, besides the commonly used morpho-agronomic traits, in characterization and selection of coffee genotypes in genetic breeding programs.

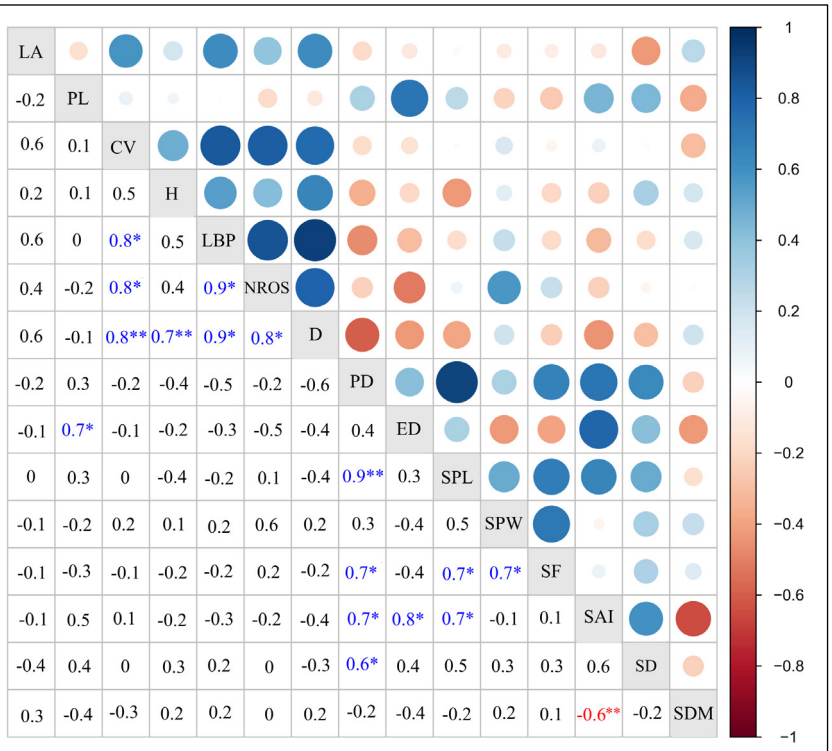

Figure 5 - Correlation matrix (correlogram) of 15 morpho-agronomic and leaf anatomic traits in different *Coffea canephora* genotypes.

LA: leaf area (cm); PL: petiole length (cm); CV: leaf central vein length (cm); H: plant height (cm); LPB: length of plagiotropic branches (cm); NROS: number of rosettes; D: plant diameter (cm); PD: stomata polar diameter (µm); ED: stomata equatorial diameter (µm); SPL: stomatal pore length (µm); SPW: stomatal pore width (µm); SF: stomatal functionality; SAI: stomatal area index; SD: stomatal density; SDM: specific dry mass per leaf square centimeter (g).

CONCLUSION

There is genetic variability in leaf anatomic traits among clones from Andina and Tributum cultivars. All leaf anatomic traits related to stomatal density and biometry presented high heritability values. The inclusion of leaf anatomic traits, related to stomata for characterization of *C. canephora* genotypes, may potentially guide plant breeders in better genetic discrimination of individuals and in greater security during plant selection for cultivar composition.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by LOESilva, RS and RNA. The first draft of the manuscript was written by LOES and all authors commented on previous versions of the manuscript. All authors read and approved the final version.

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