DOI: 10.5965/223811711732018547

Revista de Ciências Agroveterinárias 17(4): 2018
Universidade do Estado de Santa Catarina

Genetic divergence between coffee genotypes resistant to rust based on anatomical features

Divergência genética de genótipos de cafeeiros resistentes à ferrugem com base em características anatômicas

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Submission: 15/09/2017 | Acceptance: 13/06/2018

ABSTRACT

The objective of this study was to evaluate the genetic divergence of coffee genotypes resistant to rust, of the Germplasm Bank from Minas Gerais, based on anatomical features. A total of 12 foliar anatomical characteristics were assessed, and Mahalanobis distance was used to quantify the genetic divergence between genotypes. The Tocher method and the hierarchical method of UPGMA (Unweighted Pair-Group Method Using Arithmetic Averages) were used as group strategy. Moreover, relative contribution analyses of the variables were made. There was genetic variability between genotypes, and the analysis of Tocher and UPGMA grouping separated the genotypes into five and seven distinct groups, respectively. The MG 0582 genotype stood out as the most divergent based on the evaluated anatomical features. There is great genetic variability for the characteristics of foliar anatomy among the 15 genotypes evaluated, allowing to select superior genotypes based on these characteristics.

KEYWORDS: c*offea arabica,* germplasm bank, improvement, leaf anatomy.

RESUMO

Objetivou-se com esse trabalho avaliar a divergência genética de genótipos de cafeeiros resistentes à ferrugem do Banco de Germoplasma de café de Minas Gerais, com base em características anatômicas. Foram avaliadas 12 características anatômicas foliares e a distância generalizada de Mahalanobis foi usada para quantificar a divergência genética entre os genótipos. Foram empregados como estratégia de agrupamento, o agrupamento de Tocher e o método hierárquico UPGMA (Unweighted Pair-Group Method Using Arithmetic Averages), além disso, foi feita a análise da contribuição relativa das variáveis. Houve variabilidade genética entre os genótipos, sendo que a análise do agrupamento de Tocher e UPGMA separaram os genótipos em cinco e sete grupos distintos, respectivamente. O genótipo MG 0582 se destacou como o mais divergente dentre os demais com base nas características anatômicas avaliadas. Conclui-se que existe uma grande variabilidade genética para as características da anatomia foliar entre os 15 genótipos avaliados, possibilitando a seleção de genótipos superiores com base nessas características.

PALAVRAS-CHAVE: *coffea arábica,* banco de germoplasma, melhoramento, anatomia foliar.

INTRODUCTION

The success of genetic breeding programs allowed producers to achieve a more adapted, productive and high-quality coffee. Although these cultivars have already reached high levels of productivity, new features may arise from the development of cultivars with better resistence to pests, diseases or with specific characteristics that are adapted to new agricultural frontiers or product quality. These characteristics are found in *Coffea arabica* L. and in wild species of *Coffea*, thus highlighting the importance of a germplasm bank (EIRA et al. 2007).

One of the most important collections of *Coffea arabica* L. in Brazil is located in the Experimental Farm at Patrocínio, MG, from EPAMIG, and it has approximately 1500 genotypes (GUEDES et al. 2013a). Among these genotypes, 15 came from the crossbreeding between “Hibrido de Timor” x *Dilla & Alghe,* whose morphological characterization is based on the main descriptors of Arabica cultivars by EPAMIG researchers. In Brazil, arabica coffee cultivars derived from "Hibrido de Timor" have been shown to present resistance to rust (DEL GROSSI et al. 2013).

The material called "Hibrido de Timor" is an Arabica coffee carrier of *Coffea canephora pierre*'s genes, considered a source of resistance to rust. This hybrid and its progenies derived from its crossbreeding with other cultivars have been studied in several regions of the world (VÁRZEA et al. 2002), with valuable contribution from breeding programs, aiming to acquire this resistance to rust. The genotypes *Dilla & Alghe* came from Ethiopia and have resistance to *Pseudomonas syringae* pv. Garcae (the causal agent of the aureolated spot) and to some breeds of *Hemileia vastatrix* (fungus responsible for the orange rust in coffee) (GUEDES et al. 2013a).

Studies on plant anatomy aim for special characteristics, mainly resistance to rust, since pesticides are commonly used in pathogen control, and research on structural resistance has shown promising techniques to minimize the use of these products. In addition, these studies show concern for understanding the dynamics of the vegetable when facing the conditions imposed by handling (MUSSURY et al. 2012).

One of the main functions of anatomical structures such as cuticle and abaxial epidermis is to block the entrance of infectious inocula. These structures function as mechanical protection for the plant, preventing reproductive structures of phytopathogens from reaching the interior of the plant with ease. The stomata, however, are small apertures through which plants may exchange gases with the outer environment. Nonetheless, higher stomatal density may provide resistance to diseases. Cultivars that present thicker parenchyma tissues have been shown to be more resistant to pathogens and may present greater photosynthetic capacity (SILVA et al. 2005).

The quantification and localization of the stomata, trichomes, parenchyma, epidermis and the presence of lignin are important characteristics for verifying the relationship between the arrangement of these structures and the incidence and severity of the disease. If these differences are recognized, one expects to obtain information that can be used in several areas of agriculture, contributing to the sustainability of the production process, with expectations of increased productivity (LOURENÇO et al. 2011).

Characterization of plant species consists in establishing an identity for each access, using data that allow studying the genetic variability of the material (BARRETO et al. 2015). This characterization is based on morphological, physiological, cytological, biochemical, or molecular characteristics. Coffee has a very narrow genetic base, and it is important to characterize not only its external morphology, as is commonly done, but also its internal morphology, so that they can be associated (ANTHONY et al. 2002). In some cases, it is not possible to identify or classify genetic material based simply on characteristics of external morphology, and in such cases, the use of internal morphology characteristics may be employed.

Analysis of genetic divergence and multivariate techniques can characterize genotypes of a Germplasm Bank using structural characteristics of the leaves. This characterization helps in the development of work strategies by providing knowledge on the genetic variability of genotypes, as well as monitoring Germplasm Banks, generating useful information for the preservation and use of these genotypes in breeding programs (GUEDES et al. 2013a).

Therefore, the aim of this study was to evaluate the genetic divergence between coffee genotypes resistant to rust based on leaf anatomy characteristics, using material from the Minas Gerais Germplasm Bank.

MATERIAL AND METHODS

The coffee section of the Germplasm Bank in the state of Minas Gerais is located at the EPAMIG Experimental Farm in Patrocínio – MG, located in the region of Alto Paranaíba, at 18º59'26"S latitude and 48º58'9,5"W longitude, local altitude of approximately 1000 meters above sea level. The soil is of type dystrophic Red-Yellow latosol. The topography of the area is flat with a slight slope. The climate of the municipality of Patrocínio is classified as Temperate Subtropical Mesothermal Climate with summer rains, dry winter and hot summer (Wca), according to KÖPPEN (1948).

A total of 15 genotypes of coffee trees coming from the cross between Dilla & Alghe x "Hibrido de Timor" considered rust resistant were selected. The *Dilla & Alghe* accessions represented in the Germoplasm Active Bank of Minas Gerais came from Ethiopia and are characterized by their resistance to

Pseudomonas syringae pv. garcae (agent of aureolated spot), and some breeds of *Hemileia vastatrix* (fungus responsible for the orange rust in coffee). In addition, the accessions for “Hibrido de Timor” are characterized by the presence of rust resistance, carrying the resistance factors SH, 5, 6, 7, 8 and 9 and others not yet identified (EIRA et al. 2007).

These genotypes presented potential characteristics for immediate use in breeding programs (Table 1).

Table 1. Selected genotypes from the Germplasm Bank of Minas Gerais, in Patrocínio.

Nº	Genotype	Cross
1	MG 0579	*Dilla & Alghe* x H. Timor UFV 400-01
2	MG 0580	*Dilla & Alghe* x H. Timor UFV 400-03
3	MG 0581	*Dilla & Alghe* x H. Timor UFV 400-06
4	MG 0582	*Dilla & Alghe* x H. Timor UFV 400-07
5	MG 0583	*Dilla & Alghe* x H. Timor UFV 400-09
6	MG 0584	*Dilla & Alghe* x H. Timor UFV 400-12
7	MG 0585	*Dilla & Alghe* x H. Timor UFV 400-18
8	MG 0586	*Dilla & Alghe* x H. Timor UFV 400-19
9	MG 0587	*Dilla & Alghe* x H. Timor UFV 400-25
10	MG 0588	*Dilla & Alghe* x H. Timor UFV 400-30
11	MG 0589	*Dilla & Alghe* x H. Timor UFV 400-46
12	MG 0590	*Dilla & Alghe* x H. Timor UFV 400-47
13	MG 0591	*Dilla & Alghe* x H. Timor UFV 400-48
14	MG 0592	*Dilla & Alghe* x H. Timor UFV 400-52
15	MG 0593	*Dilla & Alghe* x H. Timor UFV 400-61

Two leaf samplings were conducted to compare features between seasons: the first occurred in the rainy season (February) and the second in the dry season (September). In each season, the same collection pattern was used. The leaves were collected from the third node of plagiotropic branches in the middle third of the plants and were immediately conserved in 70% ethanol (v. v^{-1}) (JOHANSEN 1940).

The anatomical analyses were performed using a completely randomized design with three replications, each leaf being considered a repetition. The cross sections were obtained with a table microtome, of LPC type, the para-dermal free hand cuts were using steel blade. Both underwent clarification with sodium hypochlorite (1.25% active chlorine), triple washing in distilled water, staining with safrablau solution (0.1% astra blue and 1% safranin, at a ratio of 7:3) for the cross sections and safranin 1% for the para-dermal sections, and the semi-permanent slides were mounted with 50% glycerol (v. v^{-1}) (KRAUS & ARDUIN 1997). The slides were observed and photographed with an Olympus BX 60 optical microscope coupled to a Canon A630 digital camera. The images were analyzed in UTHSCSA-Imagetool image analysis software.

The following characteristics in the cross sections of the leaf limb were evaluated: adaxial cuticle thickness (ACT); adaxial epidermal thickness (ADE); abaxial epidermal thickness (ABE); thickness of palisade parenchyma (PPA); thickness of the spongy parenchyma (SPA) and mesophilic thickness (MES). For the leaf vein, the following were evaluated: number of xylem vessels (NXV); diameter of xylem vessels (DXV), medullary parenchyma (MEP) and phloem thickness (PHT). The stomata were analyzed in the para-dermal sections, evaluating: stomatal functionality (polar diameter/equatorial diameter) and stomatal density (number of stomata per mm²).

To evaluate the existence of genetic variability among genotypes, the data were subjected to analysis of variance. Then, multivariate analyses were used to group the most similar genotypes and identify the main variables that would be used to determine genetic divergence among the genotypes.

The Tocher’s method, using Mahalanobis generalized distance (D2ii ') as a measure of dissimilarity and the UPGMA (Unweighted Pair-Group Method Using Arithmetic Averages) was used to calculate average values. The same weight for the two elements being integrated was used in all cases. The relative importance of characteristics was calculated using the method proposed by (SINGH 1981), which is based on the partitioning of total estimates for distances D2ii ', considering all possible pairs of individuals, for each part according to each characteristic. All analyses were performed using genetic and statistical computational application the application "GENES" (CRUZ, 2013).

RESULTS AND DISCUSSION

There was significant difference between averages only for ACT, ABE, PPA, SPA, MES and SD (Tables 2 and 3). Thus, there is genetic variability between genotypes for characteristics assessed. In the evaluation of the cuticle thickness in rainy season, MG 0579, MG 0580, MG 0581, MG 0582, MG 0584, MG 0585, MG 0586 and MG 0591 (8/15 accessions) were considered thicker (Table 2). Although differences in cuticle thickness may be more evident in plants under stress conditions such as high radiation and water deficit (RIBEIRO et al. 2012), no accesses were superior to the cuticle thickness in the dry season.

Table 2. Anatomical characteristics of 15 accessions of coffee from *Dilla & Alghe* x “Hibrido de Timor” from the Germplasm Bank of Minas Gerais, Patrocínio.

	Cuticule		Abaxial Epidermis		Stomatal Density	
Genotypes	Rainy	Dry	Rainy	Dry	Rainy	Dry
MG 0579	4.79 a A	4.70 a A	15.33 b A	17.05 a A	164.42 b A	106.39 a B
MG 0580	5.91 a A	4.12 a A	17.91 a A	16.40 a A	154.75 b A	154.75 a B
MG 0581	4.67 a A	3.92 a A	18.60 a A	14.33 a B	158.90 b A	113.30 a A
MG 0582	4.45 a A	3.76 a A	15.79 b A	14.53 a A	185.15 a A	160.28 a A
MG 0583	3.45 b A	4.19 a A	19.60 a A	15.57 a A	139.55 b A	121.59 a A
MG 0584	4.16 a A	4.17 a A	14.14 c A	14.09 a A	147.84 b A	132.64 a A
MG 0585	3.80 a A	4.22 a A	12.20 c A	14.07 a A	161.66 b A	125.74 a A
MG 0586	3.99 a A	4.19 a A	14.33 c A	12.98 a A	164.42 b A	150.61 a A
MG 0587	3.52 b A	4.65 a A	17.36 a A	16.33 a A	205.87 a A	146.46 a B
MG 0588	2.74 b B	4.42 a A	15.87 b A	14.94 a A	135.41 b A	136.79 a A
MG 0589	2.33 b B	4.43 a A	16.34 b A	14.96 a A	187.91 b A	145.08 a A
MG 0590	3.02 b A	4.53 a A	13.89 c A	15.25 a A	193.44 a A	132.64 a B
MG 0591	4.06 a A	4.44 a A	11.78 c B	14.32 a A	193.44 a A	139.55 a B
MG 0592	3.03 b A	3.81 a A	12.97 c B	14.70 a A	161.66 b A	116.06 a B
MG 0593	3.08 b A	4.24 a A	13.29 c B	15.24 a A	163.04 b A	142.32 a A
Average	3.80	4.25	15.29	14.98	167.83	134.95
CV (%)	23.51	11.40	8.70	8.58	15.23	17.13

Averages followed by the same lowercase letter in the column do not differ significantly from each other by the Scott- Knott averages grouping test, at 5% probability level and in the different capital letter in lines are significantly different from each other by the F test ($p < 0.05$).

By analyzing the variations between seasons, only accesses MG 0588 and MG 0589 presented different behaviors in the rainy season and in the dry season, having higher values of cuticle thickness in the dry season. Larger cuticle thickness is commonly considered for the prevention of transpiration, which consequently reduces water loss, usually found in sunny or xeric environments (BALIZA et al. 2012). Regarding abaxial epidermis, accesses MG 0580, MG 0581, MG 0583 and MG 0587 were considered superior in the rainy season. In the dry season, there was no significant difference between accessions. MG 0581 had superior abaxial epidermis in the rainy season and MG 0591, MG 0592 and MG 0593 had superior results in the dry season (Table 2). OLIVEIRA (2014) also obtained differences regarding the thickness of the abaxial epidermis when studying coffee plants subjected to treatment with hydro-retentor polymer.

Regarding stomatal density, accesses MG 0582, MG 0587, MG 0590 and MG 0591 were superior when compared to the others in the rainy season, and in the dry season, there was no difference between accessions. Regarding the evaluations between seasons, accesses MG 0579, MG 0580, MG 0587, MG 0590, MG 0591, MG 0592 were superior in the rainy season (Table 2). A higher stomatal density allows for stomatal opening in a shorter period, making them more efficient in capturing CO_2 and consequently decreasing its transpiration (RIBEIRO et al. 2012).

The thickness of the palisade parenchyma showed differences for the accesses evaluated in the rainy season (Table 3). The palisade parenchyma is closely related to photosynthesis, and further development of this tissue may allow greater fixation of CO_2 (QUEIROZ-VOLTAN et al. 2014). The accesses MG 0580, MG 0582, MG 0583, MG 0586 and MG 0590 were superior to the other accesses (Table 3). Regarding the evaluation seasons, accesses MG 0591 and MG 0592 behaved differently, and in the rainy season, access MG 0592 was superior, whereas access MG 0591 had higher thickness of palisade parenchyma in the dry season (Table 3).

Table 3. Anatomical characteristics of 15 accessions of coffee from *Dilla & Alghe* x "Hibrido de Timor" from the Germplasm Bank of Minas Gerais, Patrocínio.

	Palisade Parenchyma		Spongy Parenchyma		Mesophile	
Genotypes	Rainy	Dry	Rainy	Dry	Rainy	Dry
MG 0579	51.72 b A	49.51 a A	165.35 b A	176.65 a A	212.23 b A	229.69 a A
MG 0580	60.64 a A	50.70 a A	203.23 a A	185.73 a A	263.02 a A	238.04 a A
MG 0581	47.04 b A	54.40 a A	153.74 b A	162.32 a A	199.84 b A	219.45 a A
MG 0582	67.02 a A	57.06 a A	186.36 a A	193.97 a A	254.89 a A	253.81 a A
MG 0583	68.50 a A	59.80 a A	207.09 a A	166.72 a A	277.33 a A	227.17 a B
MG 0584	49.68 b A	47.79 a A	166.69 b A	168.78 a A	216.67 b A	216.68 a A
MG 0585	52.00 b A	47.88 a A	164.44 b A	184.33 a A	215.45 b A	238.01 a A
MG 0586	61.83 a A	51.03 a A	164.92 b A	151.80 a A	227.32 b A	205.23 a A
MG 0587	48.70 b A	49.75 a A	196.22 a A	175.45 a A	245.58 a A	228.17 a A
MG 0588	48.14 b A	49.07 a A	167.99 b B	187.24 a A	217.22 b A	238.70 a A
MG 0589	52.84 b A	41.44 a A	172.77 b A	167.87 a A	222.56 b A	211.14 a A
MG 0590	59.57 a A	42.27 a A	176.76 b A	167.12 a A	239.19 a A	214.57 a A
MG 0591	39.32 b B	54.86 a A	164.25 b A	178.29 a A	208.53 b A	234.37 a A
MG 0592	53.49 b A	38.42 a B	167.75 b A	156.82 a A	226.62 b A	197.98 a A
MG 0593	51.78 b A	47.72 a A	153.44 b A	164.40 a A	206.59 b A	216.24 a A
Average	54.15	49.45	174.07	172.50	228.87	224.62
CV (%)	15.43	17.68	9.07	10.32	8.21	9.16

Averages followed by the same lowercase letter in the column do not differ significantly from each other by the Scott-Knott averages grouping test at a 5% probability level, and different capital letters in lines are significantly different from each other by the F test ($p<0.05$).

Accesses MG 0580, MG 0582, MG 0583 and MG 0587 presented superior characteristics regarding the thickness of the spongy parenchyma in the rainy season (Table 3). By studying anatomically different genotypes of cassava, it could be observed that higher values of palisade parenchyma and spongy parenchyma may confer higher photosynthetic capacity to the genotypes that present them (RIBEIRO et al. 2012). Therefore, a higher photosynthetic capacity may play an important role in resistance against pathogens. Analyzing both seasons, only MG 0588 had a greater thickness of spongy parenchyma in the dry season than in the rainy season, the remaining accesses had no significant difference between seasons (Table 3).

Regarding the thickness of the mesophyll, accesses MG 0580, MG 0582, MG 0583, MG 0587 and MG 0590 were higher than the others in the rainy season. There was significant difference between seasons only for the access MG 0583, which had the highest mesophyll thickness in the rainy season (Table 3). Studies with *Coffea arabica* L. cultivar Oeiras also showed significant differences in the thickness of the leaf mesophyll when in full sun and in a shaded environment. These authors state that a larger mesophyll thickness, which was observed in the leaves of the cultivars planted in full sunlight, is a mechanical protection mechanism of the plant (GOMES et al. 2008).

The genetic variability of a population depends on the genetic divergence and the parents involved in the cross. However, when working with crossbreeding it is recommended to choose accesses with good performance for the characteristics of interest, that were more divergent from each other, or that complement some characteristic of one of the parents.

The genetic dissimilarity measures, estimated by the generalized distance of Mahalanobis (D2ii '), among the pairs of genotypes from the 15 genotypes studied (Table 2) had high magnitude (0.87 to 21.89), indicating a broad genetic variability among genotypes. Likewise, FONSECA et al. (2006) found a high variation (9.72 to 49.19) in conilon coffee clones. GUEDES et al. (2013b), evaluating 42 characteristics of the external morphology from the same genotypes used in this study, also found a high variation in genetic dissimilarity measures (0.44 to 59.62) (Table 4).

Table 4. Dissimilarity measures of the five most dissimilar genotypes and the five most similar genotypes based on the generalized distance of Mahalanobis (D2ii '), Patrocínio.

Similar Genotypes	Genetic Distance	Dissimilar Genotypes	Genetic Distance
MG 0593 – MG 0590	0.87	MG 0583 – MG 0591	21.89
MG 0593 – MG 0592	1.13	MG 0580 – MG 0592	17.30
MG 0591 – MG 0585	1.15	MG 0585 – MG 0583	16.86
MG 0581 – MG 0579	1.28	MG 0581 – MG 0591	14.49
MG 0593 – MG 0584	1.62	MG 0581 – MG 0582	13.15

The genotypes MG 0591 - MG 0583 were considered the most dissimilar between each other, with a genetic distance of 21.89 based on the generalized distance of Mahalanobis, and genotypes MG 0590 - MG 0593 were the most similar, due to having the smallest average distance between the pairs of estimated distances (0.87) (Table 4). In addition to being considered dissimilar, genotype MG 0591 presented superiority in six characteristics, at least in one of the evaluation seasons. The genotype MG 0583 was considered superior in all evaluated characteristics in both seasons.

It is worth mentioning that GUEDES et al. (2013a) also verified a high genetic dissimilarity between genotypes MG 0591 and MG 0583 for morpho-agronomic characteristics. Thus, these results evidenced the possibility of using these genotypes in breeding programs, however for these genotypes to be used it is necessary that they present superior anatomical characteristics and of interest to the breeding program in particular.

Grouping with use of the Tocher method while using as a genetic dissimilarity measure the Generalized Distance of Mahalanobis (Table 5), characterized the formation of five groups of genotypes. Most of the genotypes were allocated in group one, and this group had seven of the 15 studied genotypes. Genotype MG 0582 was considered the most divergent since it remained in an isolated group besides having superior characteristics to other genotypes such as cuticle and abaxial epidermis. GUEDES et al. (2013a), evaluating 42 morpho-agronomic descriptors of the same genotypes, observed that genotype MG 0582 also remained in an isolated group, which increases the possibility of obtaining a promising genotype associating external and internal morphological characteristics.

Table 5. Groups according to the Tocher method based on the distance (D^2) of Mahalanobis, from the 15 genotypes of *Dilla & Alghe* x “Hibrido de Timor” evaluated from the Germplasm Active Bank of Minas Gerais, Patrocínio.

Groups	Genotypes
1	MG 0590 MG 0593 MG 0592 MG 0585 MG 0584 MG 0586 MG 0591
2	MG 0579 MG 0581 MG 0588
3	MG 0587 MG 0589
4	MG 0580 MG 0583
5	MG 0582

The number of groups formed through the Tocher method demonstrates the wide variability between the genotypes studied. These results show that the genotypes present variability in leaf anatomy characteristics, showing that the leaves have foliar alterations with variations in their structure. These groups represent valuable information in the choice of parents within the breeding programs, since the development of new hybrids must be based on the magnitude of their distances and on the potential of the parents alone, besides presenting variability for the characters that are being improved (AZEVEDO et al. 2013).

In order to obtain a higher degree of reliability in the grouping of genotypes by genetic dissimilarity, the UPGMA method was also applied (Figure 1).

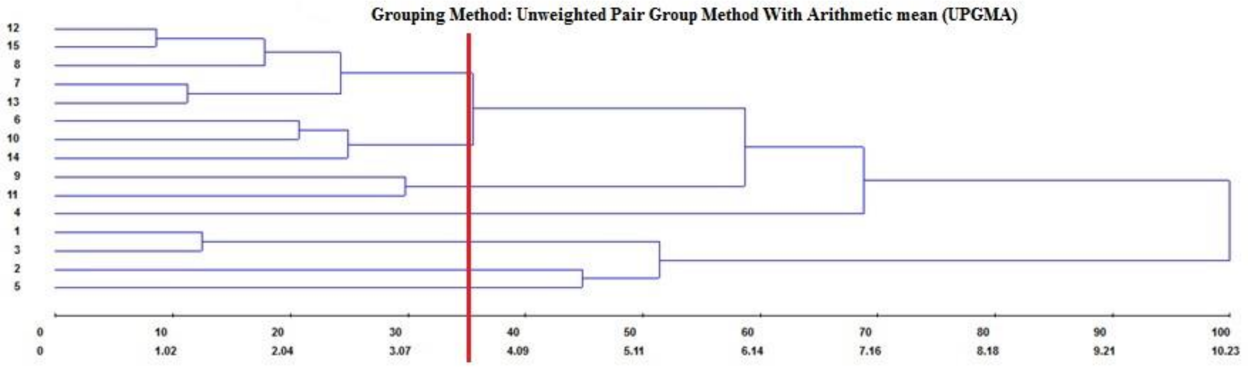

Figure 1. Dendrogram construction of 15 coffee genotypes of the “Hibrido de Timor” x *Dilla & Alghe* using the UPGMA method, obtained from the generalized distance of Mahalanobis, estimated based on 12 foliar anatomical characteristics, Patrocínio. 1-Access MG 0579; 2-Access MG 0580; 3-Access MG 0581; 4- Access MG 0582; 5-Access MG 0583; 6-Access MG 0584; 7-Access MG 0585; 8-Access MG 0586; 9-Access MG 0587; 10-Access MG 0588; 11-Access MG 0589; 12-Access MG 0590; 13-Access MG 0591; 14-Access MG 0592; 15-Access MG 0593.

The UPGMA diagram showed a high degree of branching due to the wide diversity observed between genotypes of the germplasm collection studied. This grouping method allowed the formation of seven distinct groups, the first group with five genotypes, the second group with three genotypes and the third group with two genotypes. Genotypes MG 0580, MG 0582 and MG 0583 were the most divergent among them, remaining in isolated groups.

The results were similar to those observed by the Tocher method, especially when a minimum limit of 35% was determined. Likewise, IVOGLO et al. (2008), in an experiment of genetic divergence of robusta coffee, found the same division of groups for the two groupings, and GUEDES et al. (2013b) in an experiment with Maragogipe coffee accesses also found similarity between grouping methods. The genotypes considered more similar by the generalized distance of Mahalanobis were gathered in the same group, both in the Tocher and in the UPGMA grouping. This shows the high similarity between these methods. It is important to note that genotype MG 0582, which was considered one of the most dissimilar, remained in an isolated group in both analyses.

For the study of the relative importance of characteristics, the analysis was done with use of the Tocher method, using all characteristics, and estimating its relative contribution. The characteristics that contributed most to genetic divergence were abaxial epidermis thickness (44.42%), spongy parenchyma thickness (16.60%) and stomatal density (14.33%), and the characteristic mesophyll thickness was the one that less contributed to genetic divergence, with only 0.56% (Table 6).

The study of anatomical variations among existing coffee cultivars may favor genetic improvement programs, reducing the selection time of promising materials. The knowledge of plant anatomy may be useful in the control of pathogens, since certain anatomical characteristics may confer resistance to pests and diseases to the genetic materials that have them, eliminating or reducing the need to use chemical products. Thus, studies on structural resistance are showing interesting aspects that may help minimize the use of pesticides besides helping to understand the response of vegetables when in different handlings (SILVA et al. 2005).

Table 6. Relative contribution of six anatomical characteristics to the divergence of the 15 accessions of "Hibrido de Timor" x *Dilla & Alghe* by the Singh method (1981), Patrocínio.

Variable	S.j	Value (%)
ABE	323.96	44.42
SPA	121.05	16.60
SD	104.47	14.33
ACT	94.34	12.94
PPA	81.43	11.17
MES	4.05	0.56

ABE: abaxial epidermal thickness; SPA: spongy parenchyma; ACT: adaxial cuticle thickness; SD: stomatal density; PPA: palisade parenchyma; MES: mesophile.

Thus, it is possible to use anatomical characteristics as tools to support the genetic improvement of coffee, seeking to identify superior genotypes (CASTANHEIRA et al. 2016), thus dynamizing the process of obtaining new cultivars and in a shorter period, offering responses to the main limitations that are new challenges on a day-to-day basis.

CONCLUSION

There is high genetic variability for the characteristics of foliar anatomy among the 15 genotypes evaluated, enabling the selection of superior genotypes based on these characteristics.

ACKNOWLEDGEMENTS

We thank Empresa de Pesquisa Agropecuária do Estado de Minas Gerais (EPAMIG), Agência de Inovação do Café (InovaCafé), CNPq for the scholarship and CAPES for financially assisting the Project.

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