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Coffee genotypes morphophysiological adaptation under coffee leaf rust biotic stress

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ABSTRACT

The identification of morphophysiological traits responsible for a better plant behavior when infected is useful for cultivar selection, and become crucial for breeding. We investigated the morphophysiological behavior of coffee genotypes before and after inoculation with the pathogen *Hemileia vastatrix*, causal agent of coffee rust. With multivariate techniques we identified the characteristics that most contribute to total genetic divergence of the genotypes. Ten genotypes of *Coffea arabica* from the Germplasm Bank of Coffee from Minas Gerais were sown in a nursery and then take to a greenhouse with controlled temperature and humidity. After one month of acclimatization, the artificial inoculation with the fungus *H. vastatrix* was carried out. The anatomical and physiological evaluations were performed 1 day before inoculation and 160 days after inoculation. When the first symptom emerged, plants were evaluated according to a descriptive scale for coffee rust. We observed significant differences in rust severity and ostiole opening between genotypes. Different groups were formed by the K-means method, based on morphophysiological characteristics. This shows that genetic variability exists between the coffee genotypes evaluated before and after inoculation with the pathogen. The most important characteristics that contributed to the total genetic divergence were xylem vessel diameter and stomatal conductance. In conclusion, inoculation with *H. vastatrix* caused a change in coffee genotypes based on morphophysiological characteristics.

Key words: *Coffea arabica*; canonical variables; *Hemileia vastatrix*.

1 INTRODUCTION

The coffee rust is caused by the biotrophic fungus *Hemileia vastatrix* Berk. and Br. which is the main pathogen responsible for the reduction of yield in *Coffea arabica*, under favorable conditions, cause losses from 30% to 50% of yield (Talhinhas et al., 2016; Zambolim, 2016). Fungus-resistant cultivars already exist on the market, however a continuous selection of resistant progenies is required (Carvalho et al., 2017), because the durability of resistance is difficult to predict, and new physiological races of the pathogen can break the resistance (Capucho et al., 2009).

The biotic stress in plants consists of physiological, structural and biochemical changes in response to attack of pathogens (Imran; Yun, 2020). These responses can be rapid acclimations or particular long-term adaptations, such as changes in leaf size, thickness, and stomatal density (Chattopadhyay et al., 2011). The plant resistance to pathogens is determined by genetic factors and, in some of their expressions, is influenced by the environment and pathogen-host interactions (Nelson et al., 2018).

It is important to understand the nature of pathogen-host interactions to recognize the impact of biotic stress on plants, considering the variability of anatomical and physiological characteristics in different cultivars within the species (Pandey et al., 2017). The combination of structural characteristics and biochemical reactions related to process of plant defense is essential for its maintenance in the ecosystem (Imran; Yun, 2020). Studies have used these characteristics to select resistant genotypes to main diseases, especially in early stages of breeding programs (Gortari et al., 2018; Patole et al., 2017).

Preformed defense mechanisms, physical and chemical barriers are often considered the first line of defense in plants against a pathogen (Gull; Lone; Islam Wani, 2019). Infection of yellow sigatoka pathogen in banana leaves promoted smaller stomatal densities and greater thicknesses of palisade parenchyma and epidermis (Araújo et al., 2014). Density and stomatal length are related to the development and sporulation of mildew (*Peronospora belbahrii*) in *Ocimum spp* (Homa et al., 2016). The net photosynthetic rate, leaf transpiration and stomatal conductance were negatively correlated with the number of anthracnose lesions in açaí plants (Castro et al., 2017). The study of preformed anatomical structures, as well as the physiological characteristics, is important for a better understanding of the mechanisms involved in resistance to pathogens (Mahajan; Dhillon; Brardwaj, 2017).

The success of a breeding program depends on the populations that present genetic variability for the characteristics under selection (Bailey-Serres et al., 2019). Morphological, agronomic and physiological characteristics have been used to select genotypes for future crosses, using

multivariate techniques (Azevedo et al., 2015; Guedes et al., 2013). However, the effect that the biotic stress cause in genotypes has not been studied since, although some mechanisms of response and defense have been elucidated, the physiological behavior of plants exposed to biotic stress is still a great challenge (Gull; Lone; Islam Wani, 2019). Thus, the objective of this paper is to study the morphophysiological behavior of coffee genotypes before and after inoculation with *Hemileia vastatrix* and to identify the characteristics that most contribute to the total genetic divergence of the genotypes.

2 MATERIAL AND METHODS

2.1 Plant material and fungal isolate

Eight genotypes of *Coffea arabica* from the Germplasm Bank of Coffee from Epamig, in Patrocínio, Minas Gerais state were used on inoculations. In addition, two checks were used: one resistant, Catiguá MG 3 and another susceptible to coffee rust, Topázio MG 1190 (Carvalho et al., 2017) (Table 1).

The *Hemileia vastatrix* isolate were obtained from naturally infected leaves of coffee (*Coffea arabica* L.) plants cultivated in Lavras, city of Minas Gerais, Brazil. It is located at approximately 920m altitude, with geographic coordinates 21º13'40"S and 44º57'50"W. The average temperature is 19.9 °C and the average annual rainfall is 1486 mm. The climate classification is Cwa according to Koppen (1948). The pustules from lesions were scraped and spores were packed in microtubes, protected from light.

2.2 Experimental design

The seeds were sown in bags of polyethylene containing substrate with soil and organic matter. The management of fertilization was performed in accordance with the technical recommendations for the crop (Guimarães et al., 1999).

The most vigorous and uniform seedlings, with three pairs of true leaves, were taken to greenhouse, to the acclimatization with control of temperature (23 °C) and relative humidity (70%). The experiment was carried out in randomized blocks, with three replicates, each plot consisting of three plants.

2.3 Inoculation of *Hemileia vastatrix*

A spore suspension was prepared with the concentration of 1 x 10^5 uredyinospores mL^{-1} agar-water (0.2% w v^{-1}) with Tween 20 (0.05% v v^{-1}). After one month of plants acclimatization the artificial inoculation was carried out. The suspension was sprayed throughout the abaxial face of the leaves with a hand sprayer. All the seedlings were kept in a humid chamber for 72 hours in darkness (Monteiro et al., 2016).

2.4 Rust severity assessment

On observing the initial symptoms of the disease, the severity was visually evaluated at 7-day intervals using the diagrammatic scale from 1 to 6, where 1: the lesions esquals to 0 to 3% severity; 2 equals 3 to 6% severity; 3 equals 6 to 12% severity; 4 equals 12 to 25% severity; 5 equals 25 to 50% severity; and 6 equals above 50% severity. Then, the area under disease of progress curve (AUDPC) was calculated according to Shaner and Finney (1977).

$$AUDPC = \sum_{i}^{n-1}\left[\frac{(x_i + x_{i+1})}{2}\right] * t \quad (1)$$

where n is the number of evaluations, x is the disease severity, i is the number of evaluations and t is the time interval between two consecutive evaluations.

Table 1: Genotypes from the Germplasm Bank of Coffee from Epamig.

ID*	Genotype	Genealogy[1]
1	MG 0579	*Dilla & Alghe* × Híbrido de Timor UFV 400-01
2	MG 0580	*Dilla & Alghe* × Híbrido de Timor UFV 400-03
3	MG 0581	*Dilla & Alghe* × Híbrido de Timor UFV 400-06
4	MG 0582	*Dilla & Alghe* × Híbrido de Timor UFV 400-07
5	MG 0583	*Dilla & Alghe* × Híbrido de Timor UFV 400-09
6	MG 0587	*Dilla & Alghe* × Híbrido de Timor UFV 400-25
7	MG 0588	*Dilla & Alghe* × Híbrido de Timor UFV 400-30
8	MG 0591	*Dilla & Alghe* × Híbrido de Timor UFV 400-48
9	Catiguá MG3	Catuaí Amarelo IAC 86 × Híbrido de Timor UFV 440-10
10	Topázio MG1190	Catuaí × Mundo Novo

* ID: identification number. [1] *Dilla & Alghe*: Ethiopian origin.

2.5 Ostiole opening

For evaluation of ostiole opening a scanning electron microscope (SEM) was used. Before inoculation a leaf of each genotype in each block was collected in the morning, probably when the stomata were open. The samples were cut into pieces with a maximum of 2 cm in width and a maximum height of 3 cm. Leaf samples were prepared by fixing for 24 h in modified Karnovsky solution [2.5% glutaraldehyde and 2.5% formaldehyde in 0.05 m sodium cacodylate buffer (pH 7.2) containing 0.001 m calcium chloride], washing with 0.05 m sodium cacodylate buffer (pH 7.2).

Following fixation, leaf samples were dehydrated in water: acetone step gradient with acetone concentrations of 25, 50, 75, 90 and 100%. Exposure to each step was for 10 min, and the 100% step was repeated three times. Acetone was removed using a critical point drier Bal-Tec CPD 030 (Balzers, Liechtenstein). Samples were fixed to aluminum stubs with double-sided adhesive carbon tape, and coated under vacuum with a thin film of metallic gold using a Bal-Tec SCD 050 evaporator. A Nano Technology Systems (Carl Zeiss, Oberkochen, Germany) model LEO EVO 40 XVP SEM was used to take photographs that were processed using UTHSCSA-Imagetool software (UTHSCSA, San Antonio, USA).

2.6 Physiological evaluations

The physiological analyzes were performed 1 day before inoculation (1 DBI), third leaves completely expanded before pathogen inoculation and 160 days after inoculation (160 DAI) in the first fully expanded leaf after inoculation stress.

Leaf gas exchange rates, including net photosynthetic rate (A: μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs: mol H_2O m^{-2} s^{-1}), transpiration rate (E: mmol m^{-2} s^{-1}), internal carbon (Ci: μmol CO_2 mol^{-1}), carboxylation ratio (Ci/Ca), instantaneous carboxylation efficiency (A/Ci) (Silva et al., 2010) and intrinsic efficiency of water use (iWUE) (A / gs) (Silva et al., 2013) were measured on upper canopy fully expanded leaves between 9:00 to 11:00 h with a portable photosynthetic system LiCor-6400XT (LI-Cor Biosciences, Lincoln, USA). Measurements were carried out at 25 °C chamber temperature and 1000 μmol m^{-2} s^{-1} photon flux density.

Falker chlorophyll indexes for chlorophyll a and b were measured on leaf fully exposed to the sun, mature and healthy in the median region of the branches in three replicates in each plant canopy. The measurements were obtained from the portable chlorophyll meter ClorofiLOG CFL1030 (Falker, Brazil), the reading is obtained from photodiodes after light emission at lengths 635, 660 and 880 nm (Barbieri Júnior et al., 2012).

2.7 Anatomical evaluations

Two leafs were collected at 1 DBI (leaves of the third pair completely expanded) and 160 DAI (the first leaf completely expanded). The leaves were fixed in 70% alcohol (Johansen, 1940) and, after 72 hours, placed in a new 70% alcohol solution.

Paradermal sections were obtained by free hand method and cleared with sodium hypochlorite solution 1.2% active chlorine basis, rinsed in distilled water tree times, stained with 1% safranin solution, and mounted on slides with coverslips with 50% glycerol (Kraus; Arduin, 1997). In a microscope Red 200 (Kasvi, São José do Pinhais, Brazil), we evaluated the following characteristics: the number of stomata (NST); the number of epidermal cells (NEC); polar diameter of the stomata (POL) in μm; and equatorial diameter of the stomata (EQA) in μm. With these estimates it was obtained the stomatal frequency ((FRQ = nst/ nec) * 100); stomatal density (DEN = nst / mm ^{2}); stomatal index (SIN = (nst/ (nec + nst)* 100); and the relation between the polar diameter and the equatorial diameter (POL / EQA).

For the transversal sections the plant material was submitted to concentrations of ethanol (70%, 80%, 90% and 100%), at intervals of 2 hours. The samples were incorporated into historesin according to the manual instructions (Leica Microsystems, Wetzlar, Germany). Sections (8 μm) were obtained with a semi-automated rotating microtome MRP 2015 (Lupe Laboratory Equipment Technology Industry, Brazil). The sections were stained with 1% toluidine blue (m v^{-1}) and mounted on slides with verniz (Paiva et al., 2006). In a microscope Red 200 (Kasvi, São José do Pinhais, Brazil), we evaluated: thickness of the abaxial face cuticle (TBC) in μm; thickness of the epidermis of abaxial face (TEB) in μm; thickness of epidermis of adaxial face (TED) in μm; thickness of palisade parenchyma (PPA) in μm; thickness of the spongy parenchyma (SPP) in μm; thickness of the leaf blade (LBL) in μm; mesophyll thickness (MES) in μm; number of xylem vessels (NXV); diameter of xylem vessels (DXV) in μm; and phloem thickness (PHL) in μm.

2.8 Statistical analyses

Initially, a Lilliefors test was performed to verify compliance with data normality assumptions. Rust severity and ostiole opening data were submitted to analysis of variance and Scott-Knott test ($P < 0.05$) were performed using GENES software (Cruz, 2016). For rust severity, the mathematical model used was a split-plot randomized block, design with 3 replications, and 5 times of evaluation. For the ostiole opening, was randomized block mathematical model, with 3 replications.

The multivariate method based on canonical variables was used to identify and group the genotypes based on the

morphophysiological characteristics evaluated 1 DBI and 160 DAI. A numerical value (score) of each variable is calculated for each genotype. With these scores, a scatter plot was created to the visualization of the genetic divergence, with the groups obtained by non-hierarchical K-means method. The relative contribution of each variable to the genetic divergence was calculated based on Singh (1981). All analyzes were performed in the GENES software (Cruz, 2016).

3 RESULTS

At 1 DBI and 160 DAI, the average air temperatures were approximately 17 °C and 25 °C, with air relative humidity of 80% and 60%, respectively.

3.1 Ostiole opening and rust severity

Data transformation was necessary after the verification of non-normality according to the Lilliefors test, so it was transformed to $\sqrt{x+1}$. The genotypes were statistically different considering the ostiole opening. They were grouped into three distinct groups, the genotype MG 0582, MG 0579, MG 0580, MG 0587 and Topázio MG 1190 presented the highest mean (Figure 1A). And for rust severity, the genotypes were different statistically (P<0.01) and means were divided into four distinct groups. The genotype Topázio MG 1190, considered susceptible, presented the highest severity, followed by MG 0582 and Catiguá MG 3 genotypes. The other genotypes were grouped together and considered rust resistant (Figure 1B).

3.2 Canonical variables

3.2.1 Anatomical evaluations

Before inoculation (1 DBI), three groups of genotypes were formed when the K-means method was applied. Group I was formed by genotypes MG 0579, MG 0580 and Topázio MG 1190, group II formed by genotypes MG 0581, MG 0582 and Catiguá MG 3 and group III formed by genotypes MG 0583, MG 0587, MG 0588 and MG 0591 (Figure 2).

After inoculation (160 DAI), the genotypes were grouped by the K-means method, and three groups were formed. Group I was composed by genotypes MG 0579 and MG 0591, group II by genotypes MG 0580, MG 0581, MG 0583, MG 0587, MG 0588, Catiguá MG 3 and Topázio MG 1190; genotype MG 0582 was isolated in group III. In general, 70% of the genotypes were allocated to the same group (Figure 3).

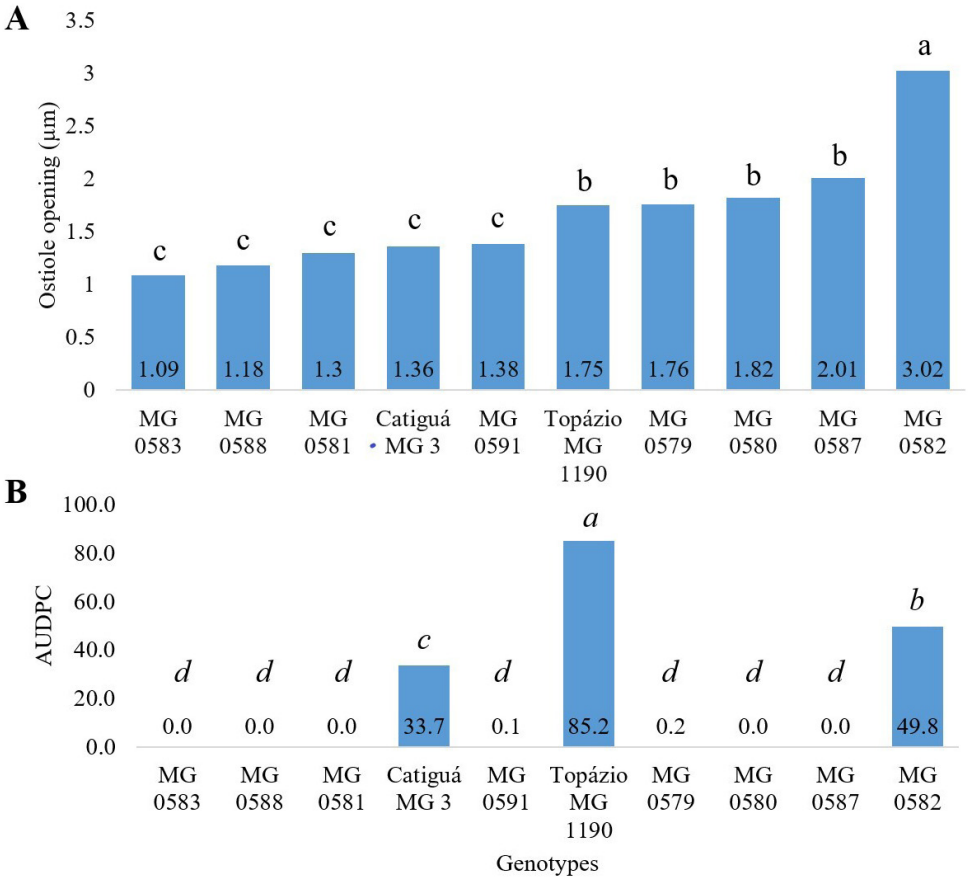

Figure 1: Ostiole opening and rust severity (AUDPC) of coffee genotypes. Mean values with the same letter are not significantly different according to Scott-Knott test 5%.

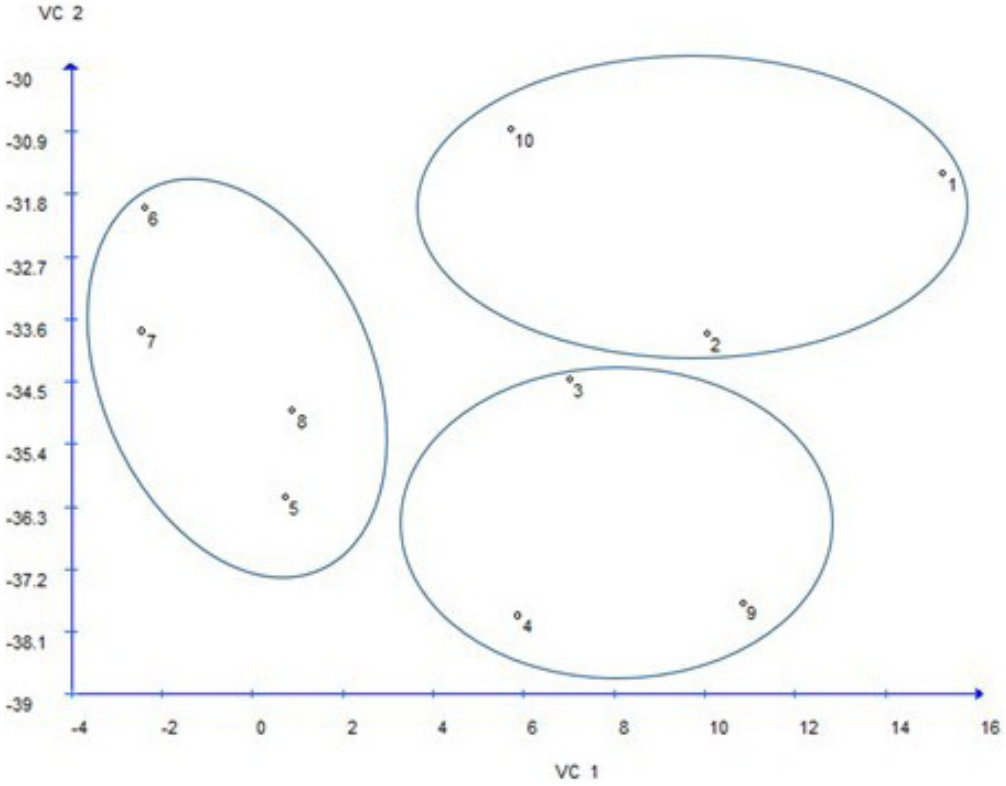

Figure 2: Biplot of 10 genotypes for anatomical characteristics one day before inoculation with the *Hemileia vastatrix* (1 DBI) (VC1: 71.02% and VC2: 83.13%). The genotypes were grouped according to the K-means method. 1- MG 0579; 2- MG 0580; 3- MG 0581; 4- MG 0582; 5- MG 0583; 6- MG 0587; 7- MG 0588; 8- MG 0591; 9- Catiguá MG3; 10- Topázio MG 1190.

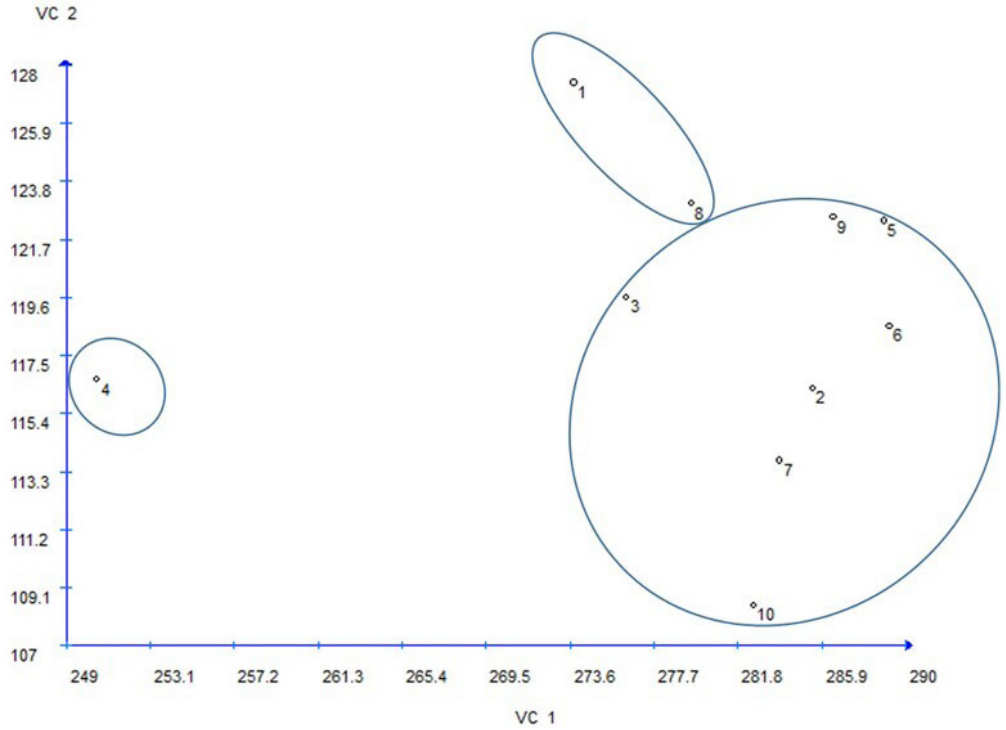

Figure 3: Biplot of 10 genotypes for anatomical characteristics after inoculation with the *Hemileia vastatrix* (160 DAI) (VC1:71.02% and VC2:83.13%). The genotypes were grouped according to the K-means method. 1- MG 0579; 2- MG 0580; 3- MG 0581; 4- MG 0582; 5- MG 0583; 6- MG 0587; 7- MG 0588; 8- MG 0591; 9- Catiguá MG3; 10- Topázio MG 1190.

3.2.2 Physiological evaluations

Before inoculation (1 DBI), three groups were formed. Group I with genotypes MG 0581, MG 0582 and Catiguá MG 3; group II composed of MG 0579 and MG 0580 and group III composed of genotypes MG 0583, MG 0587, MG 0588, MG 0591 and Topázio MG 1190 (Figure 4). Similar with the anatomical grouping in the same conditions. Only the genotype Topázio MG 1190 had grouped differently. Anatomically, Topázio MG 1190 was grouped with MG 0579 and MG 0580 (Figure 2) and physiologically grouped with MG 0583, MG 0587, MG 0588 and MG 0591 (Figure 4).

After inoculation, three groups were formed. Group I: the genotypes MG 0582, Catiguá MG 3 and Topázio MG 1190, and the group II formed by the genotypes MG 0579, MG 0580, MG 0581, MG 0583, MG 0588 and MG 0591. Genotype MG 0587 was in-group III (Figure 5). It is noted that 60% of the genotypes were allocated in the same group.

3.2.3 Importance of characters

The anatomical characteristics that most contributed to the divergence of the genotypes before inoculation (1 DBI) were LBL (28, 41%), PPA (20, 44%) and DXV (11, 44%), with more than 60% of accumulated variation. After inoculation, PHL (19, 08%), DXV (16, 52%) and POL/EQA (14, 38%) are the most important characteristics for variation (Figure 6).

The physiological characteristics that most contributed to the genetic divergence of the genotypes before inoculation (1 DBI) were gs (45, 78%), E (29,19%) e A (11, 56%). For the period after inoculation (160 DAI), gs (58, 45%), A/Ci (18, 26%) e E (16, 74%) were the most important (Figure 7).

4 DISCUSSION

In the present study, the Catiguá MG 3, resistant to rust, was tolerant to the pathogen, presenting intermediate level of disease severity (Petek; Sera; Fonseca, 2008), when compared to the other genotypes. Similar results were found in studies with evaluation of rust severity in resistant and susceptible coffee cultivars in regions of Minas Gerais (Carvalho et al., 2017). The Topázio MG 1190 showed high rust severity, confirming the high susceptibility to the pathogen (Cardoso et al., 2016).

In the late 1950s, Coffee Rusts Research Center (CIFC) plant breeding programme for obtaining resistant varieties discovery "Híbrido de Timor" (HDT) genotypes, most of them offering resistance to all known rust races. HDT-derived varieties, that join the resistance of HDT and the good agronomic traits of commercial varieties, has been used in the crop fields (Diniz et al., 2012). The MG 0582, derived from the Timor Hybrid, which contain resistance genes to rust (Silva et al., 2018), also presented intermediate level of disease severity. Incidence and severity of disease at intermediate levels may indicate horizontal resistance and under natural epidemic conditions, severity is the component that best discriminates levels of horizontal resistance (Botelho et al., 2010).

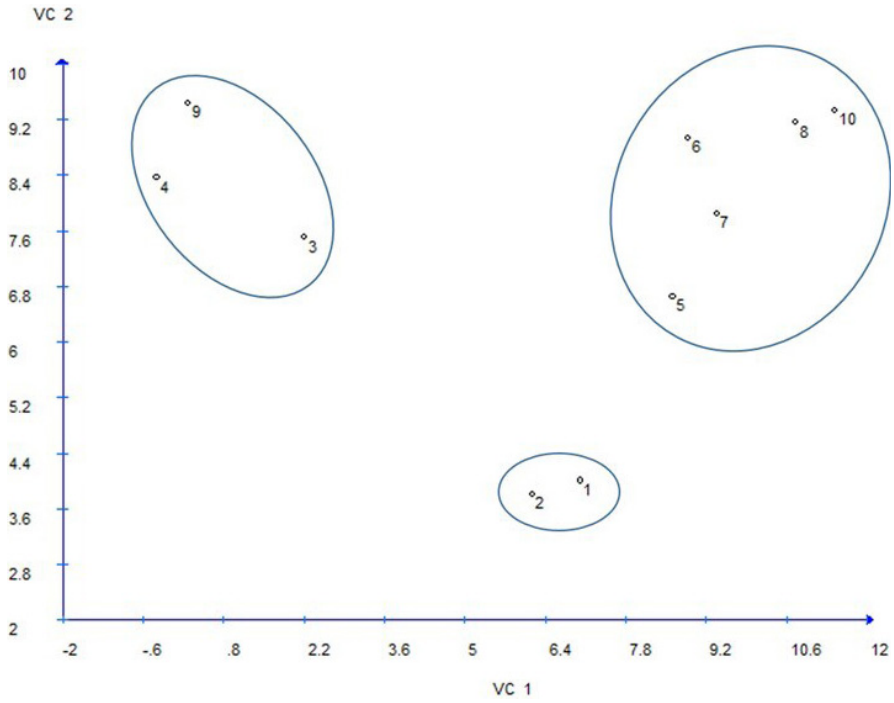

Figure 4: Biplot of 10 genotypes for physiological characteristics before inoculation with the *Hemileia vastatrix* (1 DBI) (VC1- 71.02% and VC2 -83.13%). The genotypes were grouped according to the K-means method. 1- MG 0579; 2- MG 0580; 3- MG 0581; 4- MG 0582; 5- MG 0583; 6- MG 0587; 7- MG 0588; 8- MG 0591; 9- Catiguá MG3; 10- Topázio MG 1190.

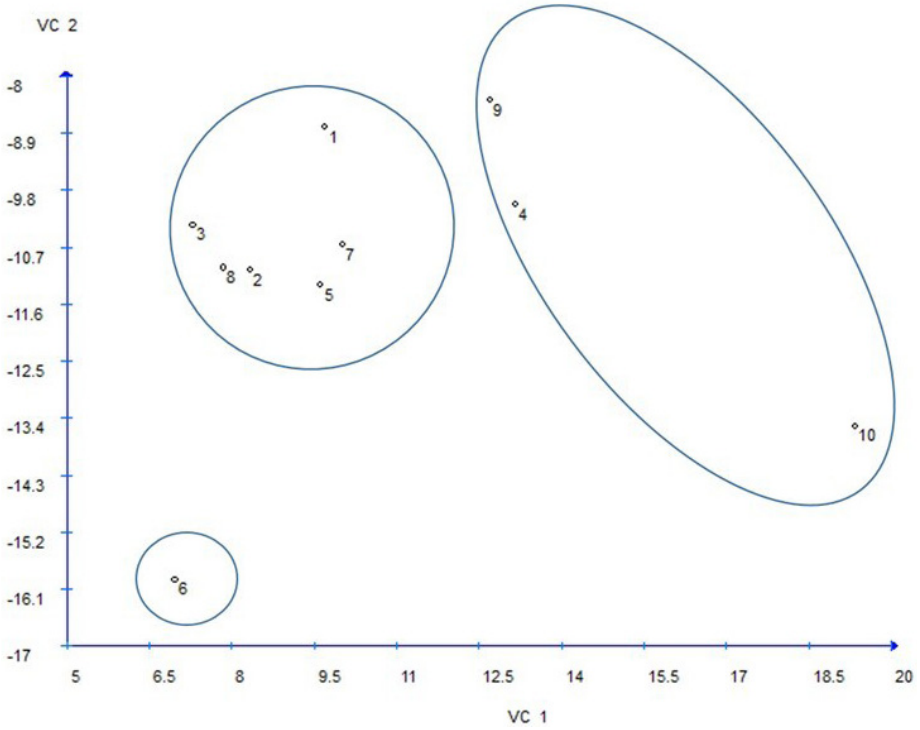

Figure 5: Biplot of 10 genotypes for physiological characteristics after inoculation with the *Hemileia vastatrix* (160 DAI) (VC1: 71.02% and VC2: 83.13%). The genotypes were grouped according to the K-means method. 1- MG 0579; 2- MG 0580; 3- MG 0581; 4- MG 0582; 5- MG 0583; 6- MG 0587; 7- MG 0588; 8- MG 0591; 9- Catiguá MG3; 10- Topázio MG 1190.

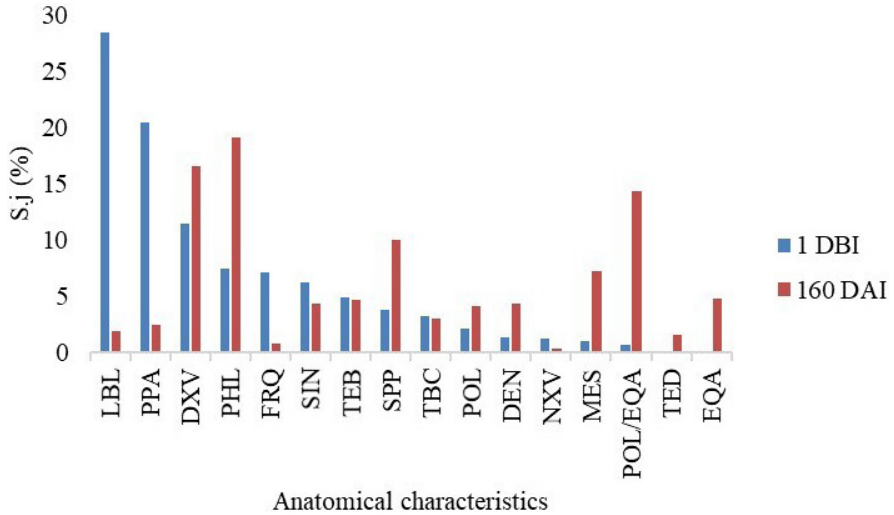

Figure 6: Importance of anatomical characteristics before (1 DBI) and after (160 DAI) inoculation with *Hemileia vastatrix*. [1]S, j: contribution of the variableto the value of Mahalanobis distance between genotypes i and i'.

The greater ostiolar opening and the second greater severity of disease was observed in genotype MG 0582. Thus, the susceptibility may be associated with structural factors of the host, since the fungal hyphae penetrates the ostiolar opening. In addition, the fungus *Hemileia vastatrix* initiates infection by the stomata (Diniz et al., 2012). Peanut genotypes with higher ostiole openings are susceptible to the biotrophic fungus of the angular spot (*Phaeoisariopsis personata*) (Jyosthna et al., 2004) and cacao susceptible to the fungus *Ceratobasidum theobromae* (Susilo et al., 2017). For the other genotypes, according to the results of the present study, no relation between disease severity and ostiolar opening was found. This result corroborates the Lima, Lopes and Café Filho (2010) studies that no correlation between characteristics of ostiolar opening in *Capsicum* ssp plants and resistance to powdery mildew was observed.

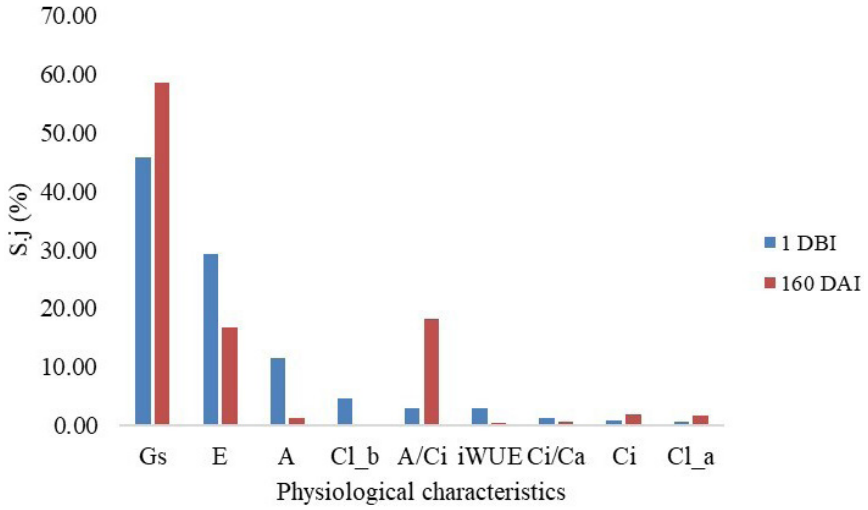

Figure 7: Importance of physiological characteristics before (1 DBI) and after (160 DAI) inoculation with *Hemileia vastatrix*. *[1]S, j: contribution of variable x to the value of Mahalanobis distance between genotypes i and i'.*

In the present work, the first two canonical variables explain more than 70% of the total variation and, therefore, explain in a satisfactory way the manifested variability among the genotypes, allowing the graphical representation of genotypes dispersion (Guedes et al., 2013).

The K-means method is a procedure to study the genetic divergence between accessions and cultivars that has been widely used, including the analysis of large amounts of coffee genotypes in order to form less heterogeneous groups (Sobreira et al., 2016). Although the coffee has a narrow genetic base (Sousa et al., 2017), the different groups formed by the K-means method based on morphophysiological characteristics showed genetic variability among the coffee genotypes (Figures 2, 3, 4 and 5). When the population presents a narrow genetic base, it is important to identify genetically divergent groups of progenies to indicate genotypes to controlled crosses in order to increase the genetic variation (Viana et al., 2018).

The genotypes MG 0579 and MG 0591 and the genotypes MG 0588 and MG 0581 were allocated to the same group, based on morphophysiological characteristics at 160 DAI (Figures 2 and 4). Guedes et al. (2013), evaluating the morphological and agronomic characteristics of these genotypes in the field, observed that they were grouped in the same way, thus reaffirming their similarity.

Although the divergence between the evaluated genotypes was observed after stress caused by inoculation, most of them presented the same behavior based on anatomical (70%) and physiological (60%) characteristics. The MG 0582 genotype was isolated in a group after inoculation, according to the anatomical characteristics, differing from the other genotypes that presented disease severity (Figure 3). It is possible that the MG 0582 has developed structural mechanisms against the pathogen, but it is unknown the mechanisms to reestablish after a biotic stress (Crisp et al., 2016).

At 160 DAI, the genotypes that presented disease symptoms (MG 0582, Catiguá MG 3 and Topázio MG 1190) were in the same group based on the physiological characteristics. Biotrophic fungi colonize only living tissues and thus cause damage and modifications in host physiology, directly, either via chemical secretion, or indirectly, through induced responses in plants (Debona et al., 2014). The decrease in photosynthetic efficiency and limitations of stomatal and mesophilic conductance, as well as biochemical changes, are the main effects caused by foliar pathogens (Bermúdez-Cardona; Wordell Filho; Rodrigues, 2015).

The diameter of xylem vessels is among the three characteristics that most contributed to the divergence of the genotypes, in 1 DBI and 160 DAI. The ability of a plant to absorb and transport water and nutrients depends on anatomical structures such as xylem (Lucas et al., 2013). In addition, xylem is one of the main components of the vascular system related to the plant vigor (Santarosa et al., 2016). Therefore, less vigorous plants may be more susceptible to pathogens attack. Besides that, changes in the vascular system of leaves can significantly influence photosynthesis, plant growth and development (Figueiredo et al., 2013).

Stomatal conductance is the physiological characteristic that most contributed to the divergence of the genotypes in the two evaluation times. The characteristic is related to the stomata opening, proportional to the number, size and opening diameter of the stomata. Bailey-Serres et al. (2019) affirms that the dynamic control of stomatal opening is one of the characteristics that provide greater resilience to plants subjected to variable environments. In addition, the stomatal profile depends on genetic and environmental factors (Galon et al., 2013), which may influence the entry and maintenance of pathogens after penetration (Castro et al., 2017; Jiang et al., 2016). Thus, the diameter of xylem vessels

and stomatal conductance may be important characteristics in genetic divergence in order to select rust resistant genotypes that have the capacity to reestablish after the stress caused by the *Hemileia vastatrix*.

The adaxial epidermal thickness and the carboxylation ratio was the lesse important for the genotypes divergence in the two evaluations, wich suggests that these morphophysiological characteristics may be dispensable in future works, reducing labor, costs and time (Azevedo et al., 2015).

In general, the coffee genotypes respond differently to the inoculation with the fungus *Hemileia vastatrix*. Before inoculation, the genotypes were grouped in a similar way, for physiological and anatomical characteristics. However, after inoculation, the genotypes were grouped differently between physiological and anatomical characteristics. There is evidence that plants tend to alter their physiology and metabolism in response to stress (Bruce et al., 2007; Pandey et al., 2017). These changes may be genetic, biochemical or structural, promoting different responses to future stresses, giving plants adaptive capacity and acclimative benefits over a lifetime or for future generations (Crisp et al., 2016).

5 CONCLUSIONS

The analyzed coffee plants were grouped differently, both for anatomical and physiological characteristics, before and after inoculation. This suggests different adaptations of the plants to stress caused by the *Hemileia vastatrix* fungus.

The characteristics that most contributed to the total genetic divergence of the analyzed coffee genotypes before and after fungus inoculation were xylem vessel diameter and stomatal conductance.

6 ACKNOWLEDGMENTS

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7 AUTHORS' CONTRIBUTION

MTRV wrote the manuscript and performed the experiment, HPAA assisted in the experiment and co-work the manuscript, FACP conducted statistical analyses and co-work the manuscript, MAFC review and approved the final version of the work, RJG review and approved the final version of the work.

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