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Treasured exceptions: Association of morphoanatomical leaf traits with cup quality of *Coffea arabica* L. cv. "Catuaí"

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ABSTRACT

The morphoanatomical characteristics of leaves were associated with altitude, hillside, and the cup quality of coffee produced in the *Matas de Minas* region. Although the small magnitude, there are correlations between cup quality with altitude and morpho-anatomical traits. Despite facing the differences of management inherent to 363 sampling sites, Northwestern hillside had significant lower cup quality, whereas only stomata density (SD) and thickness of the leaf epidermis in the adaxial face (AdET) showed significant differences between hillsides. Altitude, leaf mass per area (LMA) and SD, and to a lesser extent the thickness of the leaf epidermis in the abaxial face (AbET), were correlated (Spearman's correlation) with cup quality. Interestingly, AbET correlations were negative. Mantel's test significant correlations were found between coffee cup quality *vs.* altitude, LMA and petiole phloem area (PPhA). The spatial autocorrelation was significant only with LMA. Also, SD, to a lesser extent, was associated with cup quality. Despite the complexity of the association among the environment, plant growth and development, this is the first report to associate morpho-anatomical features of the leaf with the coffee cup traits. Even with the expectation of genotype/species *vs* environment interactions, and the influence of other parameters associated with post-harvest, roasting and brewing, the evaluation of LMA, SD, AdET, AbET and the thickness of the palisade parenchyma (PPT) allow a novel approach to access coffee cup quality.

Abbreviations: ACQ, acidity of coffee cup quality; BCQ, body of coffee cup quality; CCQ, clean of coffee cup quality; ECQ, equilibrium of coffee cup quality; GSCQ, general score of coffee cup quality; TCQC, total cup quality of coffee; ATQ, after taste of coffee cup quality; SCQ, sweetness of coffee cup quality; TCQ, taste of coffee cup quality; LBT, thickness of the leaf blade, cross section (μm); AbET, thickness of the leaf epidermis, abaxial face (μm); SPT, thickness of the spongy parenchyma (μm); PPT, thickness of the palisade parenchyma (μm); AdET, thickness of the leaf epidermis, adaxial face (μm); PPhA, petiole phloem area in a cross section (mm^{-2}); PPA, petiole parenchyma area in a cross section (mm^{-2}); PTA, petiole cross area in a cross section (mm^{-2}); PVA, petiole vascular tissue area in a cross section (mm^{-2}); PXA, petiole xylem area in a cross section (mm^{-2}); SD, stomata density (*number per* mm^{-2}); VD, venation density (*mm of leave veins per* mm^{-2}); LMA, leaf mass per area ($g\ m^{-2}$); CGA, chlorogenic acid.

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1. Introduction

The environment influences the productivity and nuances of coffee cup quality (Avelino et al., 2005; Bertrand et al., 2006; Bosselmann et al., 2009; Cargnelutti Filho, Maluf, Matzenauer, & Stolz, 2006; Carr, 2011; Decazy et al., 2003; Ferreira, Queiroz, Silvac, Tomaz, & Corrêa, 2016; Muschler, 2001). Altitude is mentioned as an indicator of cup quality (Avelino et al., 2005; Bertrand et al., 2006; de Silveira et al., 2016; Decazy et al., 2003; Ferreira et al., 2016) as well as mean air temperature during seed development is also reported to influence the sensory profile and coffee cup quality (Bertrand et al., 2012). It was observed that location profoundly impacts the levels of gene expression in coffee samples (Pallavicini, Devasia, Paolo Edomi, & Lorenzo Del Terra, 2019). Positive attributes of acidity and fruity character were associated with coffee produced at cool climates, where volatile compounds, such as ethanol and acetone, were indicators of these cool temperatures. Alcohols, aldehydes, hydrocarbons and ketones were positively associated to elevated temperatures and high solar radiation, while the sensory profiles displayed major defects as green and earthy flavour (Bertrand et al., 2012).

The demand for better-quality products stimulates the adoption of tools that allow the improvement, selection, maintenance and assessment of the cup quality. The reports of the effects of physiological or morphoanatomical traits focus on light influence on growth development of coffee seedlings (Pompelli et al., 2012; Rodríguez-López et al., 2014) and plants (Ronchi et al., 2016), but not on the concurrent effects of environmental variables on these features' association with cup quality.

Despite the cup quality is the main criteria for coffee, quality alternative approaches such as the study of the contribution of plant structure, metabolism and development, are relevant. Nevertheless, other approaches to evaluate coffee taste properties are available providing accurate discrimination of coffee samples (Dong et al., 2017, 2019). Thought morphoanatomical structure of the vegetative organs are pre-existent and give support to the development of the coffee beans, its prospect association with cup quality is self-evident. Shading significantly increased fruit number and length of fruiting branches of *C. canephora* (Assis, Gross, Pereira, Mielke, & Júnior, 2019), whereas contrasting results of shading on *C. arabica* (Rodríguez-López et al., 2014; Vaast, Bertrand, Perriot, Guyot, & Génard, 2006; Worku, de Meulenaer, Duchateau, & Boeckx, 2018) support that other features can influence vegetative, reproductive as well as cup quality traits. Additionally, Assis et al. (2019) observed an interaction between varieties and shade treatments, although the mesophyll thickness decreased with shading treatments. Nonetheless, no relation to productivity features was associated with morphoanatomical characteristics.

Plant responses to environmental stimuli are experienced throughout their lifetime (Gratani, Covone, & Larcher, 2006) and allow plants to cope with favourable or either harsh conditions expressing phenotypic plasticity that extends from vegetative, reproductive and, possibly to cup quality traits. The expression of selected genes were associated with the quality of the beverage and location (Pallavicini et al., 2019) while morphoanatomical and physiological adjustments contributed to plant adaptation to the environment (Lusk, Reich, Montgomery, Ackerly, & Cavenderbares, 2008).

This is the first report of leaf morpho-anatomical features being associated with the coffee cup quality. This information aims to contribute to the production and commercialization of coffee beans and to increase the added value to the income of producers in the *Matas de Minas* region. The objective of this research was to evaluate the relationship between coffee cup quality with morphoanatomical leaf traits from plants grown at four hillsides at different altitudes in Minas Gerais, Brazil.

2. Materials and methods

2.1. Study area

The experiment was carried out from January 2015 through July 2015 in sampled *Coffea arabica* L. 'Catuaí' plantation areas at different hillside positions with altitude ranging from 528 to 1321 m. There was a total of 363 sampling sites distributed across 29 cities in the *Matas de Minas* region in Minas Gerais State, Brazil, ranging from −19.666842 to −20.981625 latitude and −41.450655 to −43.022330 longitude (Fig. 1). The farms/sampling sites were chosen to fulfil the sampling of only one coffee variety to minimize the possible influence of different varieties on cup quality.

The annual averages and standard deviation of maximum (28.0 ± 2.5 °C) and minimum (16.3 ± 2.7 °C) average temperatures, relative humidity (73.8 ± 7.7%), total insolation (204.3 ± 43.9 h and decimals) and sum of monthly rainfall average (940.2 mm) were based on the information of three meteorological stations. Meteorological data of monthly weighted averages from January to December/2015 is available in Fig. S1. Averages of maximum and minimum average temperatures, relative humidity, total insolation and rainfall (Source: Rede do INMET) were compiled based on three meteorological stations: Caparaó (reference 83639), Caratinga (reference 83592) and Viçosa (reference 83642). Additional information on the climatological data of *Matas de Minas* region is provided in Fig. S1.

Sample locations were classified according to altitude and topography (hillsides) based on the influence of the apparent movement of the sun throughout the year in these regions and were georeferenced (Garmin Etrex 30 GPS) for altitude, latitude and longitude. The hillsides are differentiated and nominated as Northeastern ("*Soalheira Fria*"), Northwestern ("*Soalheira quente*"), Southeastern ("*Noruega Fria*") and Southwestern ("*Noruega Quente*") sides (Ferreira et al., 2018). Distance between plants inside the rows and between rows, management of fertilization, disease and pest control practices were not controlled and might differ as were carried out according each farmeŕs option, so variation among these practices is acknowledged.

Leaf samples for morphoanatomical and coffee for roasting and quality evaluation were collected from the same plants but in different expeditions depending on the fruit set and maturation. Sampling and sample processing were standardized to avoid further undesirable variation. Leaf samples were collected from January to February 2015, while fruit samples were collected from June to July 2015. Fruits were collected manually at the cherry (mature) stage, after which they had their mucilage removed and shade-dried. After drying, the following variables were analysed as coffee cup quality traits (Table 1) Clean (CCQ), Sweetness (SCQ), Acidity (ACQ), Body (BCQ), Taste (TCQ), After taste (ATQ), Equilibrium (ECQ), Overall (GSCQ) and Total quality (TCQC). The following morphoanatomical leaf features were analysed as traits (Table 1 and Fig. 2): Stomata density (SD, $n\ mm^{-2}$), Leaf mass per area (LMA, $g\ m^{-2}$), Venation density area (VD, $mm\ mm^{-2}$), Petiole area, cross section (PTA, mm^{-2}), Petiole vascular tissue area, cross section (PVA, mm^{-2}), Petiole parenchyma area, cross section (PPA, mm^{-2}), Petiole xylem area, cross section (PXA, mm^{-2}), Petiole phloem area, cross section (PPhA, mm^{-2}), Leaf blade thickness (LBT, μm), Epidermis, adaxial face (AdET, μm), Palisade parenchyma (PPT, μm), Lacunar (spongy) parenchyma (SPT, μm) and Epidermis, abaxial face (AbET, μm).

2.2. Coffee cup quality traits

2.2.1. Coffee fruit sampling

Georeferenced sites were located in field and samples collected when the maturation was achieved. Fruit sampling was carried out on 10 coffee trees in the same row from both sides at the medium third of coffee tree top. The sample collection position for leaves and fruit samples was standardized and based on the recommendation procedures

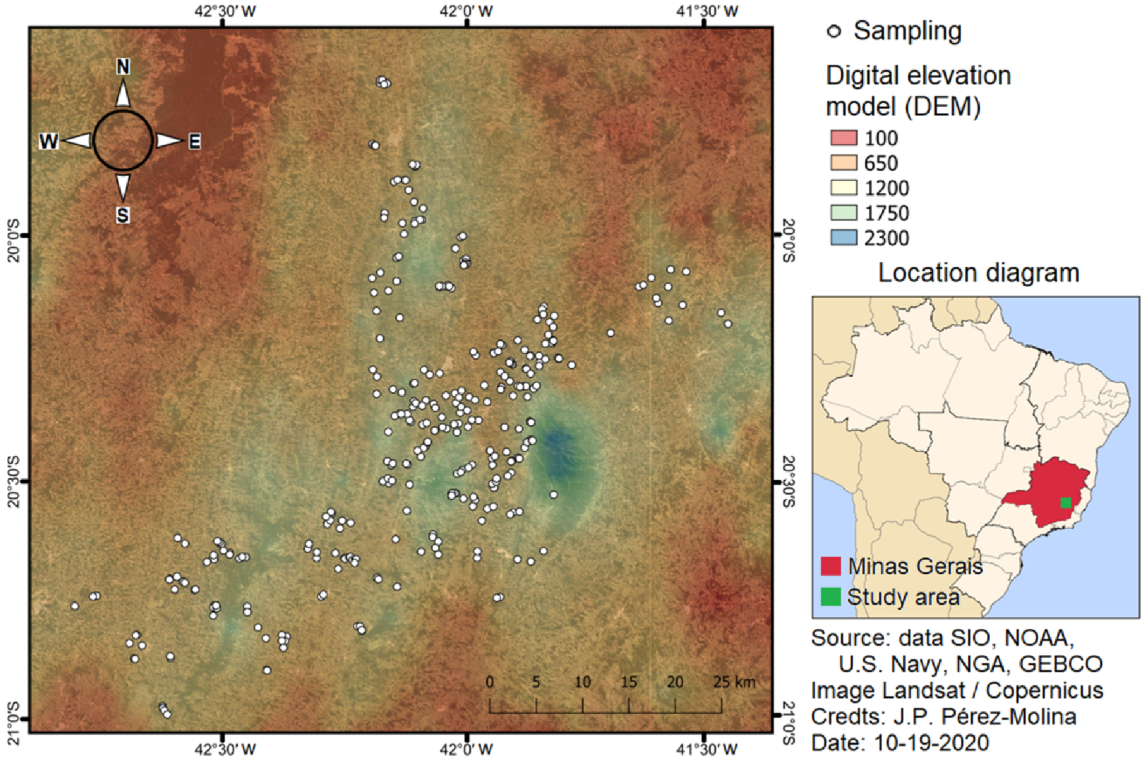

Fig. 1. Collection areas of the sampling *C. arabica* "Catuaí" plants in the *Matas de Minas* region, Minas Gerais, Brazil. Samplings sites $n = 363$.

Table 1
Comparison of the averages of cup quality and morphoanatomical leaf trait from *C. arabica* "Catuaí" plants cultivated in four hillside positions of plantation areas in *Matas de Minas* region, Minas Gerais, Brazil.

Traits	Variable (Unit)	Abbr.	Hillside position				*KW*	*P*
			Noruega fria (SE hillside)	*Noruega quente* (SW hillside)	*Soalheira fria* (NE hillside)	*Soalheira quente* (NW hillside)		
Coffee cup quality traits	*Clean*	CCQ	**5.87 ± 0.03b**	**6.03 ± 0.04 a**	**6.03 ± 0.05 a**	**6.03 ± 0.03 a**	**17.6**	**
	Sweetness	SCQ	**5.86 ± 0.04b**	**5.97 ± 0.08 ab**	**6.07 ± 0.08 a**	**6.13 ± 0.05 a**	**15.9**	**
	Acidity	ACQ	**6.01 ± 0.04b**	**6.13 ± 0.07 ab**	**6.23 ± 0.07 a**	**6.22 ± 0.04 a**	**14.9**	**
	Body	BCQ	**6.03 ± 0.03b**	**6.14 ± 0.06 a**	**6.18 ± 0.06 a**	**6.21 ± 0.04 a**	**16.0**	**
	Taste	TCQ	**5.90 ± 0.05b**	**6.11 ± 0.08 a**	**6.10 ± 0.08 ab**	**6.16 ± 0.05 a**	**12.4**	**
	After taste	ATQ	**5.90 ± 0.05b**	**6.01 ± 0.07 ab**	**6.08 ± 0.08 ab**	**6.16 ± 0.05 a**	**13.2**	**
	Equilibrium	ECQ	**5.93 ± 0.03b**	**6.12 ± 0.06 a**	**6.07 ± 0.06 a**	**6.07 ± 0.03 a**	**12.9**	**
	Overall	GSCQ	**5.93 ± 0.03b**	**6.08 ± 0.06 a**	**6.13 ± 0.05 a**	**6.14 ± 0.03 a**	**23.4**	***
	Total quality	TCQC	**83.4 ± 0.26b**	**84.6 ± 0.45 a**	**84.9 ± 0.47 a**	**85.1 ± 0.30 a**	**19.4**	***
Morpho-anatomical leaf features	*Stomata density* ($n\ mm^{-2}$)	SD	**158.0 ± 3.00b**	**175.2 ± 6.24 a**	**158.9 ± 6.43 ab**	**174.2 ± 3.90 a**	**14.2**	**
	Leaf mass per area ($g\ m^{-2}$)	LMA	60.7 ± 0.65	63.6 ± 1.27	63.3 ± 1.16	61.3 ± 0.94	6.2	*n.s.*
	Venation density ($mm\ mm^{-2}$)	VD	7.43 ± 0.17	N.D.	N.D.	7.57 ± 0.16	381^{U}	*n.s.*
	Petiole area, cross section (mm^{-2})	PTA	5.32 ± 0.17	N.D.	N.D.	4.96 ± 0.18	222^{U}	*n.s.*
	Petiole vascular tissue area, cross section (mm^{-2})	PVA	0.67 ± 0.03	N.D.	N.D.	0.63 ± 0.04	237^{U}	*n.s.*
	Petiole parenchyma area, cross section (mm^{-2})	PPA	4.65 ± 0.15	N.D.	N.D.	4.33 ± 0.16	219^{U}	*n.s.*
	Petiole xylem area, cross section (mm^{-2})	PXA	0.34 ± 0.02	N.D.	N.D.	0.36 ± 0.03	217^{U}	*n.s.*
	Petiole phloem area, cross section (mm^{-2})	PPhA	0.31 ± 0.01	N.D.	N.D.	0.28 ± 0.01	258^{U}	*n.s.*
	Leaf blade thickness (μm)	LBT	0.27 ± 0.01	0.27 ± 0.01	0.29 ± 0.01	0.27 ± 0.01	6.4	*n.s.*
	Epidermis, adaxial face (μm)	AdET	**0.028 ± <0.01 a**	**0.025 ± <0.001b**	**0.026 ± <0.001b**	**0.029 ± 0.001 a**	**37.1**	***
	Palisade parenchyma (μm)	PPT	0.062 ± 0.002	0.061 ± 0.002	0.062 ± 0.002	0.061 ± 0.002	0.3	*n.s.*
	Lacunar (spongy) parenchyma (μm)	SPT	0.173 ± 0.004	0.166 ± 0.004	0.180 ± 0.005	0.171 ± 0.005	5.0	*n.s.*
	Epidermis, abaxial face (μm)	AbET	0.019 ± 0.001	0.018 ± <0.001	0.020 ± <0.001	0.019 ± <0.001	5.1	*n.s.*

Mean ± Standard Error. Equal letters indicate no statistically significant difference between the hillside positions (*LSD*, $P > 0.05$). *KW*: Kruskal-Wallis test ($\alpha = 0.05$); *d.f.* = 4, 360; *n.s.*: not significant; N.D.: no data; **: $P < 0.01$; ***: $P < 0.001$; *P*: probability model. ^{U}: Mann–Whitney *U* test ($\alpha = 0.05$).

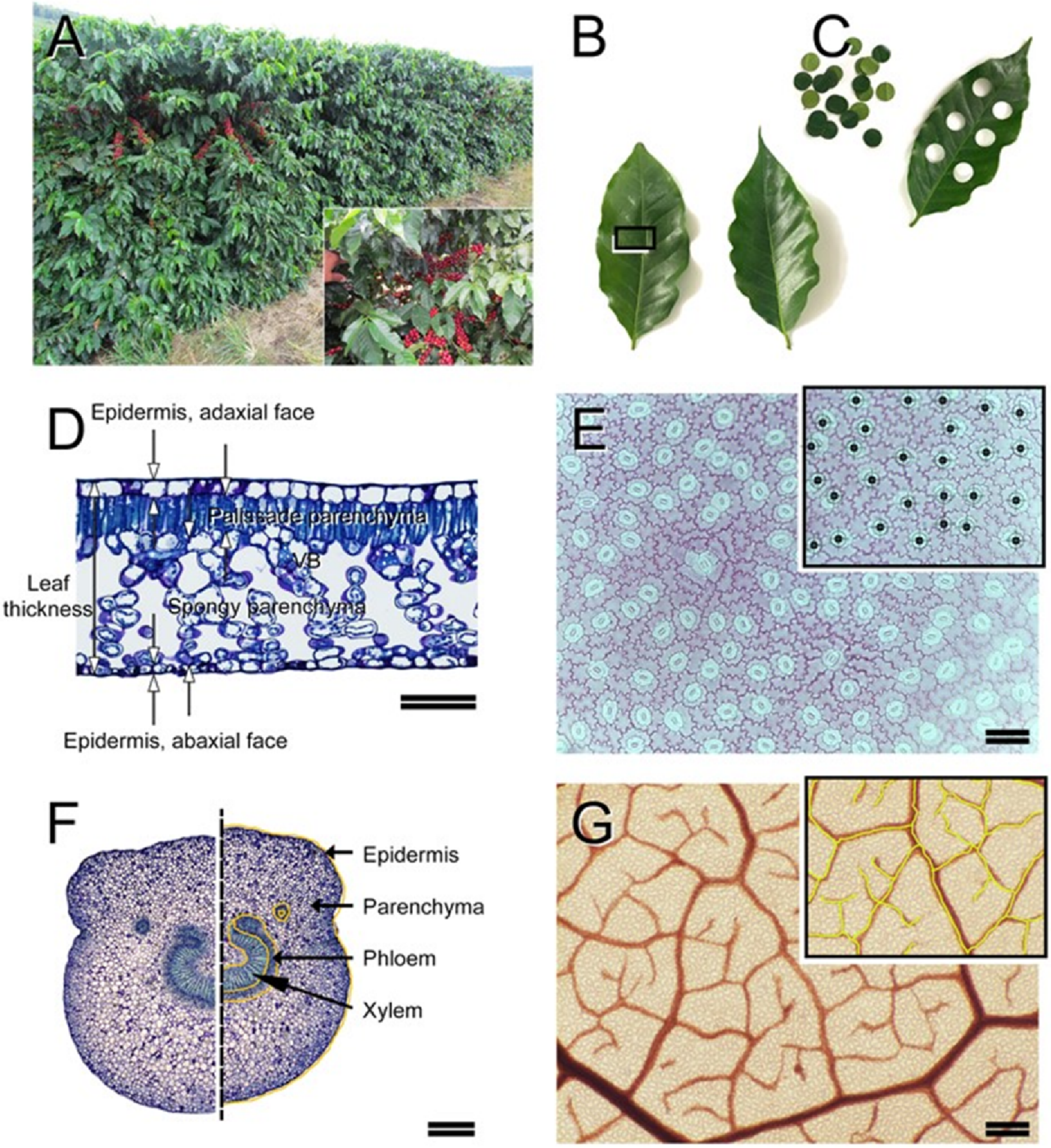

Fig. 2. Representative coffee plants conducted in field conditions and descriptive illustration of leaf sampled for leaf mass per area and leaf anatomy traits. A – View of productive plants of *Coffea arabica* L. "Catuaí vermelho IAC 144" experimental field and detail of mature coffee cherries and leaves sampled for anatomical analysis; B – leaf sampling, mid third of leaf surface, for anatomical analysis; C – leaf samples for leaf mass per area; D – leaf cross section and tissues evaluated; E – abaxial epidermal surface highlighting stomata count for stomata density (SD); F – petiole cross section and tissues evaluated and G – leaf bleached sample for venation density (VD) evaluation. VB – vascular bundle. Barrs D – 100 μm; E – 80 μm; F – 500 μm and G – 200 μm.

for the chemical/nutritional analysis of coffee trees (Ribeiro, Guimarães, & Alvarez, 1999). Equal amount of fruits of each plant were collected to compose one sample of 10 L. At least one fruit sampling was performed according to a minimum of one or a maximum of four samples at each farm. These samplings were representative of at least one to the four hillsides evaluated, respectively.

2.2.2. *Coffee processing*

All samples were transported to the processing center at the same day they were harvested. The samples were peeled in manual pulper with continuous water flow, artificially dried under drying air temperature from 35 °C to 40 °C for approximately one week until moisture content was 11 to 11.5%. The monitoring of the water content in the fruits was performed with a digital moisture meter for cereals (Gehaka, model G800). There were two processing units to easy the collection of the samples, one at the *Sindicato dos Produtores Rurais*, Manhumirim City, and another at the Seed Processing Sector at Federal University of Viçosa.

After drying, the parchment coffee samples were stored in mesh bags kept in a storage room, with 60% humidity at 20 °C, from three to four months. A portable peeler model (DRC-1 No. 830) was used for dehusking the samples. The samples were packed in plastic packaging and stored for a period of approximately two months until the seed physical quality tests (sieve and number of defects) and cup quality sensory test were performed.

2.2.3. *Sensorial analysis*

The standardized processing resulted in sample units that were representative of the coffee produced at each site. The physical quality test was carried out according to the normative instruction of the

Brazilian competent government agency (*Instrução Normativa número 8 do Ministério de Estado da Agricultura, Pecuária e Abastecimento – MAPA*) published at July 11th, 2003. This normative instruction rules the Brazilian Standards for technical identity and quality for the classification of raw and processed coffee. All the routine standards for the physical analysis of commercial parameter for coffee were acknowledged.

Physical classification was performed before sensory analysis in a way that the coffee to be tasted went through a process of picking up defects, such as green beans, black and broken beans, etc., which constitute the intrinsic defects. Given this procedure in collecting and preparing the samples, the presence of extrinsic defects, such as stone, sticks, etc., was unlikely, although it was observed and removed if existent.

Cup quality analysis was carried out by three Q-certified cuppers (Ferreira et al., 2018) from the Brazilian Monte Alegre Enterprise in November 2015, following the preliminary quality assessment procedure. Each sample was composed by five cups and drink quality evaluation carried out by the Q-grader. This analysis was carried out according to the criteria stablished by Brazil Specialty Coffee Association (BSCA) according to the standardized procedures in use to estimate the superior quality in most of the coffee producing countries. The following attributes were assessed: Overall perception, Clean Cup, Balance, Aftertaste, Sweetness, Body, Flavor and Final Score. All organoleptic standards were according the Brazil Specialty Coffee Association (BSCA) following the national and international methodology of Cup of Excellence (CoE) adapted from BSCA. The tasters' sensory opinions of each sample were registered during the coffee cupping sessions and genotypes that have presented a Final Score greater than 80 were classified as Specialty Coffees based on BSCA methodology.

2.3. *Morphoanatomical leaf features*

2.3.1. *Study of leaf surface*

Leaves were sampled from coffee trees in the same row at the medium third of coffee tree top, based on the recommendation procedures for the chemical/nutritional analysis of coffee trees (Ribeiro et al., 1999) as described previously. The leaf sampling followed the selection of healthy and expanded leaves. Injured or unhealthy leaves were not considered for this sampling. Leaf samples with approximately 0.5 cm^2 were bleached with chloral hydrate until the tissues were completely translucent. The samples were washed with distilled water, stained for 30 min with Safranin 1% solution and the slide-mounted with glycerinated gelatine. The stomata density per area (mm^2) was determined in samples from five expanded and healthy leaves from an average of 5 fields per leaf. Each leaf was collected from a different plant in the sampling site.

2.3.2. *Histological analysis*

Samples of the middle of the blade and half of the petiole length of *C. arabica L.* leaf were fixed in FAA_{50} (formalin, acetic acid, ethanol 50%; 5: 5: 90 v/v), for 48 h. The samples were dehydrated in ethylic series and included in methacrylate (Historesin, Leica) following the manufacturer's recommendations. The leaves were cross sectioned (5 μm thick) in an automatic advance microtome (RM 2155, Leica Microsystems Inc, Deerfield, USA). The sections were stained with Toluidine Blue pH 4.7 (O'Brien & Mccully, 1981) for 5 min. and mounted with synthetic resin (Permount, Fisher).

Images for histological and leaf surface analyses were obtained in a light microscope (model AX-70 TRF, Olympus Optical, Tokyo, Japan) coupled with a digital camera (model AxioCam HRc, Zeiss, Göttinger, Germany) and a microcomputer equipped with the image capture software Axio Vision. Measurements were carried out from 5 distinct fields of each sample, using software Image-Pro® Plus (version 4.1, Media Cybernetics, Inc, Silver Spring, USA).

The evaluated traits were the stomata density (SD), venation density (VD), leaf mass per area (LMA), and the thickness of the leaf blade (LBT); of the abaxial (AbET) and adaxial epidermis (AdET) faces of leaves; lacunar (spongy) parenchyma (SPT); and palisade parenchyma (SPT). The petiole total area (PTA) and the areas attributed to the phloem (PPhA); parenchyma (PPA); vascular tissue (PVA) and xylem (PXA) were measured.

LBT, AbET, SPT, SPT and AdET were measured as the thickness (μm) of each item in each section. PPhA, PPA, PTA, PVA and PXA were measured as the area (μm^2) occupied by each tissue in petiole cross sections. SD was estimated based on the number of stomata at the abaxial surface of leaves (*number of stomata* μm^{-2}), and LMA was estimated using leaf discs (Cavatte et al., 2012). Coffee plant leaves were sampled in field and 20 discs of 1.86 cm^2 were collected from healthy and expanded leaves. Leaf discs were oven-dried at 65 °C to reach a constant weight and LMA was calculated as the ratio of dry mass to leaf discs area ($g\ m^{-2}$). VD was approached as the linear length covered by vascular tissue in leaf samples ($mm\ mm^{-2}$).

2.4. *Plasticity index*

The plasticity index associated with the coffee cup quality traits and morpho-anatomical leaf features, ranging from 0 (no plasticity) to 1 (maximal plasticity), were calculated as the difference between the minimum and the maximum mean values among the sites, divided by the maximum mean value (Valladares, Sanchez-Gomez, & Zavala, 2006).

2.5. *Data analysis*

All variables were evaluated using Kruskal-Wallis and Mann Whitney *U* test for the factor hillside position. Fisher's Least Significant Difference (*LSD*) tests were applied to compare the means between hillside positions. All statistical assumptions were checked. Spearman correlation coefficients (r_s) between all pairs of variables were made. Mantel's test correlations (r) were estimated based on the similarities of cup quality traits *vs.* the similarities of distance, altitude and morphoanatomical leaf features of all sampling's sites.

These similarities were calculated by the difference of the variable between two sampling sites in a comparison matrix, only spatial distance was calculated by Euclidean distance. Mantel test measures the correlation between two matrices typically containing measures of distance or similitude, and this is one way of testing for spatial autocorrelation (Giraldo, Caballero, & Camacho-Tamayo, 2018). Mantel test is based on linear correlation and hence subject to the same assumptions of Pearson correlation, because of this limitation, the permutations of Monte-Carlo test method ($n = 2000$ interactions) was used to satisfy the assumptions not met, *e.g.*, the normal distribution (Crabot, Clappe, Dray, & Datry, 2019).

The means between the plasticity index of the cup quality and morpho-anatomical leaf features were compared using Mann – Whitney *U* test; and Kruskal-Wallis for the factor hillside position (*LSD* tests were applied to compare the means between hillside positions).

Multivariate associations among cup quality and morpho-anatomical leaf features were analysed using a principal component analysis (PCA). It was performed using the *R* package "factorextra" (Kassambara & Mundt, 2020). All of the statistical analyses were performed using *R* programming language version 3.6.1 (RCoreTeam, 2020) and with a significance level of $\alpha = 0.05$.

3. Results

All coffee cup quality variables showed at least one significant difference between hillside positions (Table 1). "*Noruega fria*" proved to be the only site to show small but significant difference with one or more sites. All other sites showed no significant differences from each other but associated with a better cup quality. Among the morphoanatomical leaf features, only SD and AdET showed significant differences between the sites. These variables showed different patterns, SD was lower in "*Noruega fria*", while the AdET was lower in "*Noruega fria*" and "*Soalheira quente*" compared to the other hillsides.

A total of twenty-three morphoanatomical leaf and cup quality traits were evaluated, from which 36.2% of the Spearman's correlations were significant. All coffee cup quality variables were correlated with each other ($P < 0.05$; r_s from 0.63 to 0.91; Fig. 3). The altitude, LMA and SD, and to a lesser extent AbET (not correlated with CCQ, SCQ, and TCQ), were significantly correlated with cup quality variables (Fig. 3A). These were small to moderate positive correlations, r_s ranging from 0.14 to 0.32, except for AbET correlations that were, interestingly, all negative.

PPT and VD were only correlated with ECQ and GSCQ ($r_s = 0.18$ and 0.15, respectively; $P < 0.05$). All other morphoanatomical leaf features were not correlated with coffee cup quality; however, they were correlated with each other. Mesophyll traits (Fig. 3B) were correlated with morphological leaf traits (Fig. 3C), and petiole traits were only correlated with each other (Fig. 3D) and, as expected, correlated with VD and petiole anatomical traits (Fig. 3C, D).

A moderate influence of altitude is observed in leaf blade attributes as correlations r_s with LMA, SD and PPT ranged from 0.14 to 0.32. Interestingly, correlations of altitude with both epidermal faces were negative. LMA increased with SD and VD, where palisade parenchyma contributed more to the mesophyll thickness compared to spongy parenchyma as their observed correlations of 0.92 and 0.48, respectively. The palisade parenchyma showed a small, although significant, increase with the altitude, LMA and SD ($P < 0.05$; r_s from 0.2 to 0.22), while the spongy parenchyma did not.

VD decreased with all petiole characteristics, except for PPhA, which r_s was non-significant. On the other hand, it increased with LMA and SD. Petiole anatomy characteristics were highly and positively correlated among themselves, while the phloem cross section area increased with the altitude and LMA.

Mantel's test correlations (Table 2) were found only between the similarities of coffee cup quality traits *vs.* the similarities of altitude, LMA and PPhA of all sampling's sites; and the spatial autocorrelation was significant only with LMA. SD, to a lesser extent, was associated with GSCQ. The Mantel correlations of cup quality with altitude ranged from 0.09 to 0.20, while with leaf morphoanatomical features ranged from 0.06 to 0.14.

All the hillside positions presented significant differences between the means of the plasticity index of the coffee cup quality *vs* morpho-anatomical leaf features ($P < 0.05$). Plasticity index of the morpho-anatomical leaf features differed between the hillside positions, but no differences were found between the hillside positions for the plasticity index of the cup quality (Table 3).

The variability explained by multivariate associations among cup quality and morpho-anatomical leaf features was less than 52% (PC1 and PC2; see Fig. S2). PCA described the positive relationships of TCOG with altitude, LMA, and SD, and negative with the thickness of the leaf epidermis (AdET and AbET); results similar to previously mentioned Spearman correlations.

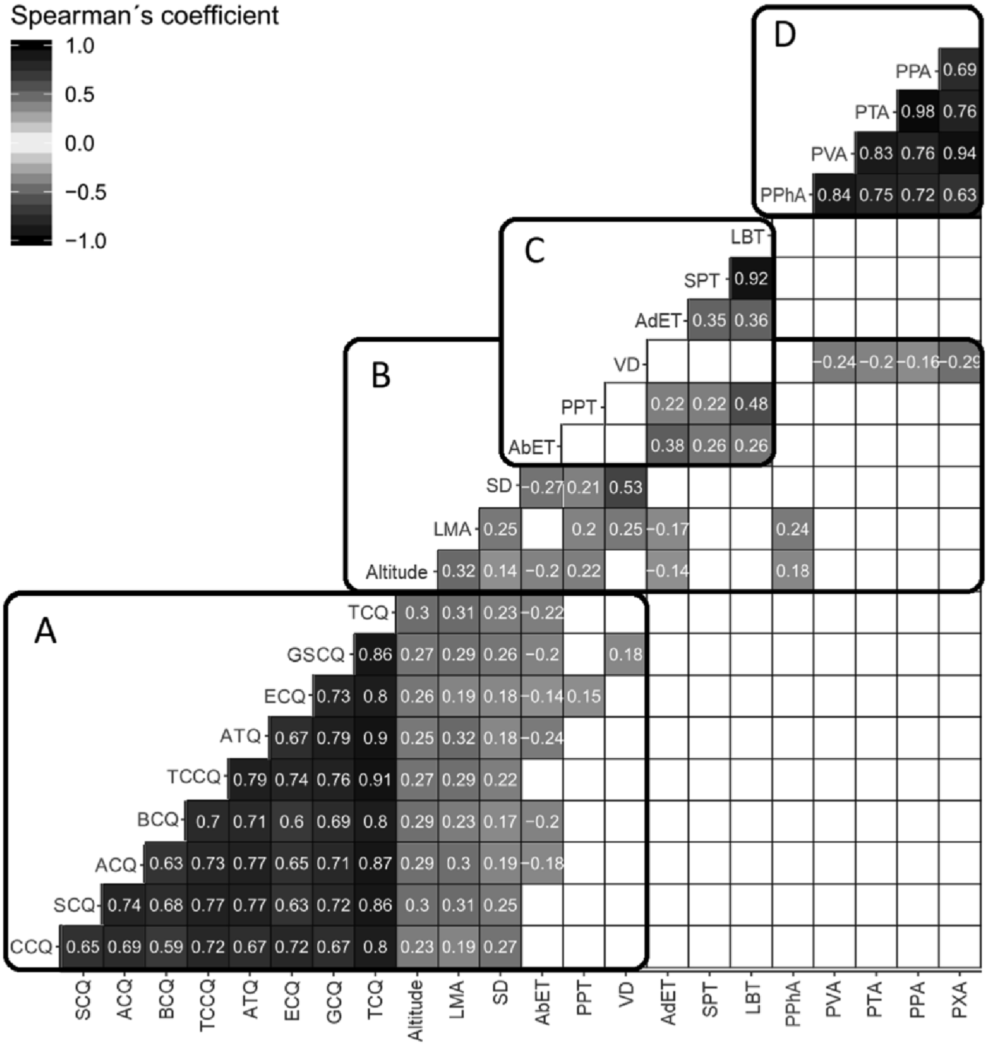

Fig. 3. Spearman correlation coefficients (r_s) between all pairs of variables of coffee cup quality traits and morphoanatomical leaf features for all hillside positions ("*Soalheira Fria*", "*Soalheira quente*", "*Noruega Fria*" and "*Noruega Quente*"). $P < 0.05$ for boxes with grey to the black gradient (from low to high Spearmańs coefficient, respectively), and no significant for a white box ($P > 0.05$). Letters from A to D show the groups formed *posteriori of most relevant correlations* between variables pairs. Coffee cup quality traits: Clean (CCQ), Sweetness (SCQ), Acidity (ACQ), Body (BCQ), Taste (TCQ), After taste (ATQ), Equilibrium (ECQ), Overall (GSCQ), and Total quality (TCQC), and morphoanatomical leaf traits: Stomata density (SD, *n* mm^{-2}), Leaf mass per area (LMA, *g* m^{-2}), Venation density area (VD, *mm* mm^{-2}), Petiole area, cross section (PTA, mm^{-2}), Petiole vascular tissue area, cross section (PVA, mm^{-2}), Petiole parenchyma area, cross section (PPA, mm^{-2}), Petiole xylem area, cross section (PXA, mm^{-2}), Petiole phloem area, cross section (PPhA, mm^{-2}), Leaf blade thickness (LBT, *μm*), Epidermis, adaxial face (AdET, *μm*), Palisade parenchyma (PPT, *μm*), Lacunar (spongy) parenchyma (SPT, *μm*), and Epidermis, abaxial face (AbET, *μm*).

Table 2
Mantel's test correlations (*r*) between the similarities of coffee cup quality traits *vs.* the similarities of distance, altitude, and morphoanatomical leaf features of samples collected from 363 sites cultivated with *C. arabica* "Catuaí" plants in four hillside positions of plantation areas in *Matas de Minas* region, Minas Gerais, Brazil.

Variable (Unit)	Abbr.	Distance	Altitude	Coffee cup quality								
				CCQ	SCQ	ACQ	BCQ	TCQ	ATQ	ECQ	GSCQ	TCQC
Altitude (m)	—	*n.s.*	—	**0.09** **	**0.20** ***	**0.16** ***	**0.16** **	**0.11** ***	**0.16** ***	**0.11** ***	**0.16** ***	**0.19** ***
Leaf mass per area (g m^{-2})	LMA	**0.06** *	**0.14** ***	*n.s.*	**0.13** ***	**0.12** ***	**0.11** **	**0.08** **	**0.13** ***	**0.08** **	**0.13** **	**0.14** ***
Stomata density (n mm^{-2})	SD	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	**0.08** *	*n.s.*
Venation density (mm mm^{-2})	VD	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Petiole transversal sectional area (mm^{-2})	PTA	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Vascular tissue area, cross section (mm^{-2})	PVA	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Petiole, parenchyma area, cross section (mm^{-2})	PPA	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Petiole xylem area, cross section (mm^{-2})	PXA	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Petiole phloem area, cross section (mm^{-2})	PPhA	*n.s.*	*n.s.*	**0.10** *	**0.13** **	*n.s.*	*n.s.*	**0.11** **	*n.s.*	*n.s.*	*n.s.*	**0.09** *
Leaf blade thickness (μm)	LBT	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Epidermis, adaxial face (μm)	AdET	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Palisade parenchyma (μm)	PPT	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Lacunar (spongy) parenchyma (μm)	SPT	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Epidermis, abaxial face (μm)	AbET	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

n.s.: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. The number of permutations based on Monte-Carlo test were of $n = 2000$ interactions. Coffee cup quality traits: Clean (CCQ), Sweetness (SCQ), Acidity (ACQ), Body (BCQ), Taste (TCQ), After taste (ATQ), Equilibrium (ECQ), Overall (GSCQ), and Total quality (TCQC).

Table 3
Plasticity index of the coffee cup quality and morpho-anatomical leaf features from *C. arabica* "Catuaí" plants cultivated in four hillside positions of plantation areas in *Matas de Minas* region, Minas Gerais, Brazil.

Traits	Abbr.	Hillside position				Mean ± EE
		Noruega fria (SE hillside)	*Noruega quente* (SW hillside)	*Soalheira fria* (NE hillside)	*Soalheira quente* (NW hillside)	
Coffee cup quality traits	CCQ	0.29	0.30	0.26	0.29	0.28 ± 0.01
	SCQ	0.38	0.43	0.36	0.29	0.36 ± 0.03
	ACQ	0.32	0.36	0.29	0.29	0.32 ± 0.02
	BCQ	0.27	0.36	0.29	0.29	0.30 ± 0.02
	TCQ	0.33	0.41	0.35	0.35	0.36 ± 0.02
	ATQ	0.36	0.35	0.33	0.35	0.35 ± 0.01
	ECQ	0.30	0.29	0.29	0.24	0.28 ± 0.02
	GSCQ	0.33	0.35	0.23	0.26	0.29 ± 0.03
	TCQC	0.19	0.21	0.18	0.18	0.19 ± 0.01
	Mean ± EE	**0.31 ± 0.02**	**0.34 ± 0.02**	**0.29 ± 0.02**	**0.28 ± 0.02**	**0.31 ± 0.02**
Morpho-anatomical leaf features	SD	0.56	0.47	0.49	0.62	0.54 ± 0.04
	LMA	0.65	0.46	0.42	0.47	0.50 ± 0.06
	VD	0.57	N.D.	N.D.	0.39	0.48 ± 0.13
	PTA	0.60	N.D.	N.D.	0.69	0.65 ± 0.06
	PVA	0.64	N.D.	N.D.	0.85	0.74 ± 0.15
	PPA	0.62	N.D.	N.D.	0.69	0.66 ± 0.05
	PXA	0.74	N.D.	N.D.	0.92	0.83 ± 0.13
	PPhA	0.55	N.D.	N.D.	0.64	0.59 ± 0.06
	LBT	0.61	0.38	0.34	0.61	0.48 ± 0.08
	AdET	0.61	0.28	0.40	0.78	0.52 ± 0.13
	PPT	0.70	0.59	0.59	0.57	0.61 ± 0.03
	SPT	0.65	0.46	0.47	0.69	0.57 ± 0.07
	AbET	0.81	0.35	0.34	0.52	0.51 ± 0.13
	Mean ± EE	**0.66 ± 0.03* b**	**0.49 ± 0.03* a**	**0.50 ± 0.08* a**	**0.67 ± 0.05* b**	**0.58 ± 0.06***

Asterisks (*) show statistically significant difference between the mean plasticity index of the coffee cup quality and morpho-anatomical leaf features (Mann–Whitney *U* test, *: $P < 0.05$). Kruskal-Wallis's tests; equal letters indicate no statistically significant difference between the mean of hillside positions (*LSD*, $P > 0.05$). N.D.: no data. Coffee cup quality traits: Clean (CCQ), Sweetness (SCQ), Acidity (ACQ), Body (BCQ), Taste (TCQ), After taste (ATQ), Equilibrium (ECQ), Overall (GSCQ), and Total quality (TCQC), and morphoanatomical leaf traits: Stomata density (SD, *n mm^{-2}*), Leaf mass per area (LMA, *g m^{-2}*), Venation density area (VD, *mm mm^{-2}*), Petiole area, cross section (PTA, *mm^{-2}*), Petiole vascular tissue area, cross section (PVA, *mm^{-2}*), Petiole parenchyma area, cross section (PPA, *mm^{-2}*), Petiole xylem area, cross section (PXA, *mm^{-2}*), Petiole phloem area, cross section (PPhA, *mm^{-2}*), Leaf blade thickness (LBT, *μm*), Epidermis, adaxial face (AdET, *μm*), Palisade parenchyma (PPT, *μm*), Lacunar (spongy) parenchyma (SPT, *μm*), and Epidermis, abaxial face (AbET, *μm*).

4. Discussion

This research is an approach of relationships of morphological and anatomical variables of coffee leaves with the cup quality. Originally, the datasheet was designed for other approaches, although also allowed morphoanatomical and cup quality traits association. It was possible to gather and analyse information from samples of the same plants of 363 sampling sites comprising the altitude, geographical coordinates, cup quality, leaf morphology and anatomy traits. The cup quality and morphoanatomical variables on this datasheet were submitted to Kruskal-Wallis test and to Spearmańs and Mantelś correlation test in the seek for relations that contribute to or can foresee, the potential value of the coffee beans grown for a beverage.

Despite the precise definitions in the literature, there are non-scientific expressions used by producers to identify the hillside in coffee grown in mountain regions. Due to specific characteristics of the topography and the influence of the apparent movement of the sun throughout the year in these regions, these hillsides will have different thermal characteristics. The Southeastern side ("*Noruega*") is colder, the Northwestern side ("*Soalheira*") is the warmer, while the others, Southwestern and Northeastern sides, exhibit temperate temperatures (Ferreira, Ribeiro, Fernandes Filho, Souza, & Castro, 2012).

Sampling and roasting procedures were standardized prior to cup quality evaluation for all samples. Good to excellent overall cup quality scores, ranging from 70.0 to 95.7 points, were observed for 99.2% of the sampled coffee. Contrary to the expected (Bertrand et al., 2012), coffee samples from *Noruega fria*, resulted the lowest overall cup quality at the *Matas de Minas*. As altitude was previously associated to coffee cup quality (Avelino et al., 2005; Bertrand et al., 2006; Decazy et al., 2003), it is worthy to note that samples studied by Bertrand et al. (2012) were collected from an altitude of 150–1032 m, whereas samples in the present study were collected from sites with altitudes ranging from 528 to 1321 m.

The importance of biochemical composition for sensory quality (Dong et al., 2019) must be considered to the complexity of cup quality. In general, sucrose, acidity, flavor and proportion of higher grades coffee increased with altitude (Worku et al., 2018). Shade affected positively bean size as well cup quality, although increased sucrose and chlorogenic acid (CGA) was observed in sun-grown coffee beans and further associated incomplete maturation to higher bitterness and astringency (Vaast et al., 2006). Despite evaluating altitudes ranging from 1150 to 1820, neither altitude, shade, processing and two-way interactions among these factors affected preliminary coffee quality score that varied from 5.9% grades from 47 to 62 at lower altitudes and shade, to 59.4% grades ≥85 at higher altitudes and no shade (Worku et al., 2018).

Increasing altitude is associated with decreased caffeine and CGA, linked with improved sucrose content and showed significant interaction with post-harvest processing and a significant effect for wet-processed, but not for dry-processed coffee (Worku et al., 2018). The increase on sucrose content with altitude was greater for plants grown without shade, while acidity increased with altitude when coffee was grown under shade although it was not significant when it was grown without shade. Again, the environmental effects on the phytochemical production/conversion contribute to the complexity of the several traits involved with cup quality. The above reports agree with the hypothesis that environmental elements have a significant influence on vegetative growth of coffee plants and cup quality.

The positive effect of altitude on cup quality (Avelino et al., 2005; Bertrand et al., 2006; Decazy et al., 2003) must be considered as there is an average difference of elevation of 196.5 m between the sampled sites by Bertrand et al. (2012) and the present approach. Apparently, environmental characteristics (temperature, rainfall, soil, hillside, altitude, etc.) are important to coffee cup quality.

There is an estimated drop in temperature of 0.7 °C for each 100 m altitude (Chalfoun & de Carvalho, 2001), what accounts for 1.4 °C less in the average of the sampling sites and benefits the cup quality from more elevated areas. Considering that altitude has a strong influence on changes in air temperatures, it affects the coffee cup quality (Cargnelutti Filho et al., 2006). The lower temperatures contribute to an extended ripening of the fruits, consequently, more chemical transformations occur in coffee beans, which in turn contributes to a greater accumulation of sugars, some acids and amino acids, favoring a high-quality beverage (Matiello, Santinato, Garcia, Almeida, & Fernandes, 2005; Muschler, 2001; Vaast et al., 2006).

Data on the effects of altitude and its interaction with cultivar and hillside are discussed, where cup quality features are influenced differently among hillsides, despite an overall similar cup quality (Silveira, 2015). Reasoning why *Noruega fria* exhibited not as good quality as *Soalheira quente* hillsides requires further analysis. Based on empirical information, it is hypothesized that moister conditions favour microorganisms contributing to undesirable fermentations or increased mould beans that hampers the quality of the final product (data not shown).

There are reports of the influence of environmental variables such as light and nitrogen availability on the vegetative growth of coffee seedlings, where most of leaf morphoanatomical traits responded to light with a classic full sunlight/ shade dichotomy (Pompelli et al., 2012). Similarly, the concomitant evaluation of cup quality, morphological and anatomical traits from the same plants and season in field conditions subsidized this first report of an exploratory association of leaf anatomical features to coffee cup quality.

Among 14 morphoanatomical variables, stomata density (SD) and the thickness of the adaxial face of leaf epidermis (AdET) showed statistical differences among the hillside positions. Dryer (Metcalfe, Davies, & Pereira, 1990; Xu & Zhou, 2008) and greater light intensity (Maiti, Satya, Rajkumar, & Ramaswamy, 2012; Pompelli, Martins, Celin, Ventrella, & DaMatta, 2010) environments are expected to stimulate plants to differentiate higher stomata density and smaller leaves. Higher SD is in accordance with the accumulated effect of the higher incidence of solar radiation on air temperature on the "*Soalheira Quente*" (NW) slope throughout the days and the year (Ferreira et al., 2012), what results in a dryer environment. This SD statistical differences and significant correlations with other traits support that the increase of stomatal density and the corresponding increase in the stomatal conductance observed in geological scale (Franks & Beerling, 2009) may play an important role in plant plasticity responses to environmental triggers.

The environmental stimuli resulting in the slightly thicker upper epidermis in NW as well as SE hillsides, respectively, is intriguing. The possibility of epidermal cells functions as lenses to focus higher photon flux densities and increased photosynthesis rates (Bone, Lee, & Norman, 1985; Martin, Josserand, Bornman, & Vogelmann, 1989) can be associated with different levels of solar radiation on these hillsides. Despite further leaf structural traits, such as palisade cells, and petiole tissue composition are also highlighted as influencing leaf function (Li, Ma, Niinemets, & Guo, 2017), coffee cup quality was correlated only to AdET (Table 1) and PPhA (Table 2). Petiole histological composition could not be approached as was not measured for all hillsides, but will be further addressed as histological slides and data are being revisited. Leaves are organs with determined growth and continuously being produced, plants may perceive environmental stimuli that result in small morphoanatomical changes that contribute to optimized photosynthesis rates. Significant, although negative, correlations of altitude and epidermal thickness reinforce the hypothesis of this possible attributes of epidermal cells.

There are contradictory associations of stomatal density to environmental stress such as drought (Maiti et al., 2012). Stomata density is correlated with stomatal conductance and thus productivity, contributing to the maintenance of photosynthetic activity under drought-stress, while there are crop drought tolerant genotypes that exhibit lower stomata density as a mechanism for reducing transpiration under stress condition. This is an open field to genotype interaction with environmental features that are critical either for stress tolerance as for productivity and quality traits aptitude depending on the response of crops challenged with environmental stresses. Despite no difference was observed among coffee genotypes approached by Kufa and Burkhardt (2011), the environmental influence on anatomical traits is illustrated as stomata density varied according to wet/dry seasons and interaction with shading levels.

Shaded coffee leaves or coffee leaves under the shade are expected to have lower stomatal index (Pompelli et al., 2010) and density (Kufa & Burkhardt, 2011; Pompelli et al., 2010) as compared to unshaded leaves. Despite conducted in open production areas and with no controlled shading, this tendency is captured as leaves of coffee plants grown at *Noruega fria* exhibited decreased SD compared to *Soalheira quente* hillside. This is in accordance with the less amount of solar radiation expected for *Noruega fria* hillside (Ferreira et al., 2012).

Observing the higher Spearman correlation coefficients within each of the cup quality and petiole anatomical traits, it is reasonable to expect that they will have different triggers that will northern the concurrent responses within each group of traits. On the other hand, altitude, LMA, SD and AbET are at an interface that will have a milder, although significant, influence or triggered or responses that are interconnected with cup quality traits. Considering the standardized procedures for sampling and roasting, it seems that cup quality ought to be impacted by both the standard of coffee variety and post-harvest procedures until cup grading, whereas petiole anatomical traits would be related only to the Catuaí variety in the present experimental design.

Altitude was positively linked to LMA, SD and with all the sampled features of the coffee cup quality. LMA and SD were positively related up to 32% with cup quality characteristics, which indicate an important coincidence that can be linked in evaluation and improvement of cup quality. Considering that LMA is an estimate of photosynthetic activity (de la Riva, Olmo, Poorter, Ubera, & Villar, 2016) and higher SD associated with greater CO_2 input (Maiti et al., 2012), the primary end products of the plant metabolism can provide more raw material to secondary metabolism that will contribute to cup quality.

Significant correlations between VD and LMA and SD are expected under the assumption that more cells, or cells with thicker cell walls of vascular and mechanical tissue cells will increase LMA, while SD will support a more efficient water vapor exchange as increased sap volume is provided. Similarly, to PTA, PVA, PPA, PXA and PPhA, no significant difference was observed between the hillsides evaluated, where the lack of VD data for *Noruega quente* and *Soalheira fria* hindered further exploring these associations. A significant and negative correlation of LMA with SD suggests that the balance between leaf area and its mass may be associated with the guard cell number (Xu & Zhou, 2008).

AbET had a negative relationship with some of the characteristics of cup quality and related to altitude and SD. All previous patterns had altitude as a common variable. Altitude can be the cause of morphoanatomic changes at the leaf level and, at the same time, be intertwined with coffee cup quality. LMA can be an indicator of a lower thickness and greater photosynthetic leaf area. Respectively, a higher SD and lower AbET, can be an indicator of greater CO_2 input capacity and a lower resistance of the entry of CO_2 through the lower epidermis.

Li et al. (2017) highlighted in their reviewed that despite similar LMA, leaves could differ in traits such as hydraulics, stomatal conductance, shade and drought tolerance, and that plant species could vary along several dimensions of trait trade-offs. Leaf anatomy is one driver of this variability where whole leaf analysis might hide linkages of structural components and their functions (Li et al., 2017).

This is supported as leaf mass per area (LMA) and palisade mesophyll thickness increased, whereas abaxial and adaxial epidermis thickness reduced, with an increase in photosynthetically active radiation (PAR) (Pompelli et al., 2012). This can reflect a greater photosynthetic efficiency in field conditions that would lead to a rapid and complete development of fruits before harvest and favouring the cup quality. Aside interactions between variables, there are significant correlations indicating that the leaf structure for carbon input, reduced carbon translocation and one of the variables that estimate the partition of the carbon fixed by photosynthesis process, approached by SD, PPhA and LMA, respectively, are interlinked. As proposed by Li et al. (2017) leaves can be divided into three key functional modules, although not necessarily independent from each other, where each module is composed by a set of correlated leaf traits. The correlations among SD, PPhA and LMA are in accordance with at least two of the foreseen leaf functional modules of light capture, the water-nutrient flow and the gas exchange.

Among the petiole anatomical characteristics, PPhA was the only variable positively correlated with some of the coffee cup quality traits, despite the similarity between the variables for all sampling points (Manteĺs test, Table 2). We hypothesize that a greater area of the phloem of the petiole could have a potential increase in the capacity of transport of foliar photo-assimilates in the direction of the fruits having consequences on growth and development and this could improve the quality of the fruits. This balance is an important feature as phloem tissue is responsible for reduced carbon transportation to other plant organs as well as developing fruits and seeds.

It is possible to reason the convergence of cup quality and morphoanatomical traits as increase in palisade will respond for light capture (Li et al., 2017) and there was a significant correlation of PPT and altitude added to the observation of PPhA was the only petiole anatomy variable that VD did not decrease significantly with. PPhA and LMA increased with altitude, being LMA an estimate of photosynthetic activity (de la Riva et al., 2016), and higher SD associated with greater CO_2 input (Maiti et al., 2012) although varying with season and shading levels (Kufa & Burkhardt, 2011), there was significant correlations of LMA, SD and PPT with altitude. Considering that means of plasticity index of the coffee cup quality *vs* morpho-anatomical leaf features varied with hillside, but no differences of plasticity index of the cup quality were found between the hillside positions corroborate to the idea that plant morphology and plant anatomy adaptation or plasticity to environmental triggers can contribute to coffee cup quality.

Spatial autocorrelation of the Mantel test highlighted the importance of LMA as a biomarker that can be further associated with coffee cup quality. It was significantly associated with altitude, a known environmental feature that contributes to cup quality (Avelino et al., 2005; Bertrand et al., 2006; Cargnelutti Filho et al., 2006; Decazy et al., 2003; Silveira, 2015) and to most cup quality traits, except for CCQ. The Mantel correlations of leaf morphoanatomical traits represented about 72.98% of the range covered by altitude correlations. This indicates that morphoanatomical characteristics as LMA, stomata density and the phloem tissue can be used as informative traits to access cup quality.

Light was an important environmental variable influencing leaf anatomy as well as morphological, growth and physiological aspects of the development of coffee plants. Shaded coffee leaves had thinner palisade and lower LMA, compared to seedlings conducted under full light (Pompelli et al., 2012). Nevertheless, this influence of light in the differentiation of coffee plant leaves grown in *Noruega Fria* and *Soalheira quente* hillsides was not observed as, among other morphoanatomical traits, there were no statistical differences for LMA and PPT averages. Despite the lack of experimental control in coffee production fields, there were significant differences of SD and AdET and significant correlations of LMA, SD, PFL, PPT and VD with at least one cup quality trait.

Rodríguez-López et al. (2014) observed that *C. arabica* seedlings conducted under entirely under 100%, 40% or 10% sunlight, showed decreasing plant height, total leaf area, number of leaves and number of plagiotropic shoots following shade increase. It was argued that adjustments in leaf number and leaf area, coupled with whole-plant physiological adjustments, could largely account for the differences in the biomass amongst treatments. This parallelism suggested between total biomass and photosynthesis ratio is tempting to be extended to support associations of cup quality and morphoanatomical traits.

Significant correlations of anatomical, thickness of the palisade parenchyma (PPT) and venation density (VD), with coffee quality traits, equilibrium (ECQ) and general score of coffee cup quality (GSCQ) respectively, support this reasoning. These observations are interpreted as either chlorenchyma and vascular tissue distribution in leaves are influenced by the same environmental triggers that will affect cup quality, or themselves can be explored as an indirect evaluation of coffee cup quality. Additional relevance of the ratio of palisade/spongy parenchyma to photosynthesis and possibly, cup quality, is yet to be studied. All the morphoanatomical relationships were low but significant, where simple leaf traits were linked with complex characteristics of coffee cup quality that implies processes from the flower pollination to the preparation of the grains for cup quality test. Our findings open an applied perspective about how simple morphoanatomical features can be related to cup quality.

In accordance with our results, the differences as greater LMA *vs* SD correlations values observed by Pompelli et al. (2010), can be attributed to the contrast of seedling *vs* adult plants and field *vs* experimental conditions. There were significant correlations between LMA and SD, ordinary epidermal cell density and ordinary epidermal cell area, while non-significant between LMA and thickness of leaves (Pompelli et al., 2010). Even considering small magnitude correlations, the association of a complex trait as coffee cup quality with leaf morphoanatomical traits in commercial production fields, without the rigor for controlled experiment design, represents a great accomplishment.

Interactions of genotypes and environment cannot be discarded as shade treatments influenced differently most characteristics of *C. canephora* (Assis et al., 2019). In general, Assis et al. (2019) reported that all coffee characteristics evaluated, mainly fruit number and length of fruiting branches increased with shading, although varieties responded differently as stomatal density, palisade and spongy parenchyma thickness reduced with shading increase. Rodríguez-López et al. (2014) observed that *C. arabica* seedlings had biomass increased linearly with increasing light supply, although plants that received higher amounts of light in the morning showed greater growth compared to those that received higher amounts in the afternoon.

Post-harvest processing has potential effects on biochemical composition and coffee quality attributes (Worku et al., 2018). Although the post-harvest contributes to the quality that is inherent of a place of origin, it does not improve the quality of the coffee harvested (Alves, 2005). In other words, despite the inherent quality of the production site, which is influenced by environmental and plant biology characteristics, the post-harvest stage in no way improves the quality of the coffee and, at most, ensures the potential quality favoured by the region, which will be expressed in the cup.

Additional discussion urges the evaluation of other characteristics such as sugar content, physiological parameters to give support any further association with cup quality. Although variables, such as soil nutrients and water status, microbiome interaction and even small microclimatic variations in precipitation and temperature regimes, were not evaluated here, they could be related to a complex trait such coffee cup quality. The effects of shade on the coffee quality may depend on the altitude associated with several environmental and soil conditions (Bosselmann et al., 2009; Muschler, 2001). For instance, the production and quality of coffee, and leaf blade and spongy parenchyma thickness, were increased by the application of Zn (Neves, 2009). Aspects as morphophysiological plasticity of coffee plants according to plant spacing within rows (Ronchi et al., 2016) and fruit thinning (Vaast et al., 2006) were not covered, although have a potential influence on cup quality. Additional sampling sites with constant and well-defined soil nutritional, rainfall and temperature regime characteristics ought to allow further outcomes for novel environmental and plant traits influencing cup quality.

The fact is that we have unexpected and significant, although small to medium, correlations of morphoanatomical leaf traits and coffee cup quality. Our data do not support that morphoanatomical leaf traits affect coffee cup quality, although, being correlated, the same environmental and physiological triggers must be acting concurrently and linked to both cup quality and morphoanatomical traits.

Further, we isolated factors as coffee variety, post-harvest processing and roasting parameters as they were standardized for all samples. Due to our experimental design, we could not control, coffee tree spacing, fertilization, brewing, soil characteristics, etc., neither their respective interactions and, still, we were successful to find significant correlations with altitude and morphoanatomical, and so regarded as potential factors influencing or associated with coffee cup quality. Cup quality is a complex trait and making sense of all the possible factors and interactions among these factors, these are sound results. For instance, the drying process (Dong et al., 2019) and roasting (Dong, Zhao, Hu, Dong, & Tan, 2017) of coffee beans markedly affected pH, total titratable acidity, total solids and total soluble solids. So, standardization of the procedures for sampling, drying and roasting parameters were essential to our results since other factors and interactions could not be controlled.

The present results are relevant in coffee production of *Matas de Minas* region as these environmental conditions hold differences that are peculiar to the hillsides. In the same hand it is acknowledged that cup quality is a result of complex interactions of factors, our data support the arisen of unexpected features such as morphoanatomical traits that may influence, or be concomitantly influenced by the same triggers of, coffee cup quality. Indeed, extreme care is recommended when interpreting these results as cup quality is, axiomatically, the result of complex interactions of factors during post-harvest processing and roasting.

Despite the complexity of coffee cup quality and an experiment in field conditions, we were able to identify morphoanatomical traits associated with cup quality that, similarly to vegetative growth parameters, are influenced by environmental variables. The interest in traits such as LMA derives from the promptness they can be accessed. Even with the expectation that there will be genotype/species *vs* environment interactions, the evaluation of LMA, SD, AdET, AbET and PPT allow a novel approach to access cup quality.

5. Conclusion

The altitudinal gradient was confirmed as a key aspect in the higher cup quality, as was the type of selected cultivar (Arabica). The altitude was related to LMA, SD, and AbET, leaf morphoanatomical traits that were positively related to cup quality. Significant differences of cup quality, SD and AdET between hillsides; and Spearman's and Mantel's test correlation for altitude, LMA, SD, AdET, PPT and AbET, and for LMA and PPhA, respectively, with cup quality evidence interlinked characteristics that allow a novel approach to indirectly access coffee cup quality.

CRediT authorship contribution statement

Junior Pastor Pérez-Molina: Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Edgard Augusto Toledo Picoli:** Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Funding acquisition. **Leonardo Araújo Oliveira:** Investigation. **Bruno Tavares Silva:** Investigation. **Genaína Aparecida Souza:** Investigation. **José Luís Santos Rufino:** Resources, Supervision, Funding acquisition. **Antônio Alves Pereira:** Visualization. **Marcelo Freitas Ribeiro:** Investigation, Writing - original draft. **Gian Luca Malvicini:** Writing - review & editing. **Luca Turello:** Writing - review & editing. **Sérgio Contrim ĎAlessandro:** Writing - review & editing. **Ney Sussumu Sakiyama:** Writing - review & editing. **Williams Pinto Marques Ferreira:** Resources, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Authors GLM and LT was employed by company IllyCaffè; and SCDA is a Consultor from Experimental Agrícola do Brasil Ltda. The authors declare that this research received funding from Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Consórcio Pesquisa Café, Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), and the support from the Laboratory of Plant Anatomy at Federal University of Viçosa and from the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG). The funders had no role in study design, data collection, analysis and decision to publish. All the other authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2021.110118.

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