UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO
CENTRO DE CIÊNCIAS AGRÁRIAS E ENGENHARIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E MELHORAMENTO

LARÍCIA OLÁRIA EMERICK SILVA

MORPHO-ANATOMICAL LEAF AND MORPHOLOGICAL FLORAL TRAITS IN *Coffea* spp. GENOTYPES

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Thesis presented to the Universidade Federal do Espírito Santo, Centro de Ciências Agrárias e Engenharias, as part of the requirements of the Programa de Pós-Graduação em Genética e Melhoramento, for the degree of Doctor in Genetics and Breeding.

Advisor: Dr. Fábio Luiz Partelli

Co-advisor: Drª. Miroslava Rakocevic

ALEGRE, ES

2023

Ficha catalográfica disponibilizada pelo Sistema Integrado de
Bibliotecas - SIBI/UFES e elaborada pelo autor

S586m
Silva, Larícia Olária Emerick, 1996-
Morpho-anatomical leaf and morphological floral traits in Coffea spp. genotypes / Larícia Olária Emerick Silva. - 2023.
88 f. : il.

Orientador: Fábio Luiz Partelli.
Coorientadora: Miroslava Rakocevic.
Tese (Doutorado em Genética e Melhoramentos) - Universidade Federal do Espírito Santo, Centro de Ciências Agrárias e Engenharias.

1. Melhoramento genético. 2. Coffea arabica. 3. Coffea canephora. 4. Altitudes. 5. Anatomia vegetal. I. Partelli, Fábio Luiz. II. Rakocevic, Miroslava. III. Universidade Federal do Espírito Santo. Centro de Ciências Agrárias e Engenharias. IV. Título.

CDU: 631.523

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Tese apresentada ao Programa de Pós-Graduação em Genética e Melhoramento do Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo, como requisito para obtenção do título de Doutora em Genética e Melhoramento.

Aprovado em 31 de julho de 2023.

COMISSÃO EXAMINADORA

Dr. Fábio Luiz Partelli (Orientador)
Universidade Federal do Espírito Santo (UFES)

Drª. Ana Isabel Faria Ribeiro (Membro externo)
Instituto Superior de Agronomia (ISA), Universidade de Lisboa (ULisboa)

Dr. Rodrigo Barbosa Braga Feitoza (Membro externo)
Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF)

Dr. José Manuel Ferreira Nobre Semedo (Membro externo)
Instituto Nacional de Investigação Agrária e Veterinária (INIAV)

Dr. João Felipe Brites Senra (Membro interno)
Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper)
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ACKNOWLEDGEMENTS

I would like to express my gratitude to God for my life and for granting me health, wisdom, and strength to overcome each precious day.

To my husband, Rafael, for being a true companion, for understanding, supporting, teaching, and motivating me every day.

To my parents, Renilton and Marlene, who always told me "you are capable" and, along with my siblings, sisters-in-law, nephews, and parents-in-law, provide me with comfort.

To the Universidade Federal do Espírito Santo and the Programa de Pós-Graduação em Genética e Melhoramento for the opportunity to pursue my Ph.D. I would like to thank all the professors who contributed to many moments of learning and exchange of experiences.

To the secretaries of the Programa de Pós-Graduação em Genética e Melhoramento, Sabrina Furtado and Fernanda, for their attention and dedication.

To the funding and project support agencies: Fundação de Amparo à Pesquisa do Espírito Santo - FAPES (Process - 2022-WTZQP and Process - 2022-M465D); Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Process - 309535/2021-2). The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (Process - 88882.385374/2019-01), for the grant of the scholarship.

To my advisor, Prof. Dr. Fábio Luiz Partelli, for the teachings, support, respect, and trust. Thank you for being empathetic and humane!

To my co-advisor, Drª. Miroslava Rakocevic, for the valuable teachings.

To the examining committee, Drª. Ana Isabel Faria Ribeiro, Dr. José Manuel Ferreira Nobre Semedo, Dr. Rodrigo Barbosa Braga Feitoza, and Dr. João Felipe Brites Senra, for accepting the invitation to participate in my thesis defense.

To the Khas Café e Campo de Lavanda group, who opened the doors for data collection, especially to Júlio Aguilar and Edinelson Zambon.

To the Núcleo de Excelência de Pesquisas em Café Conilon for their support in the logistics of the experiment and the shared knowledge.

To my friends André, Ester, Maria Juliete, and Raquel, who made themselves available to help, advise, and listen to me. And to my neighbor Dona Maria for her affection and care in Alegre, ES.

Finally, I would like to express my gratitude to everyone who has contributed to my education and is part of my life in any way.

OVERVIEW

Coffee farming plays a crucial role in the global economy and society by promoting direct and indirect employment. Coffee breeding programs have been dedicated to developing cultivars capable of overcoming various challenges in coffee production. In this regard, the phenotypic characterization of individuals within a population is essential for selecting individuals with desirable traits. The objective of this work was to investigate morpho-anatomical leaf and morphological floral traits in *Coffea* spp. and explore the genetic and environmental effects on these traits. To attain this objective, this work consists of three chapters. In the first chapter, the objective was to evaluate the potential of leaf anatomical traits, along with agronomic traits, for studying genetic variability in genotypes of two registered cultivars of *C. canephora*. Eight agronomic traits and seven leaf anatomic traits were evaluated in ten genotypes of *C. canephora.* Significant differences among the genotypes were observed for 13 out of the 15 traits ($P < 0.05$). These results evidenced the heterogeneity among the studied cultivars. Leaf anatomic traits showed the highest variations compared to agronomic traits. Five out of the seven leaf anatomical traits presented heritability values higher than 80%, with emphasis on stomatal density (95.69%) and stomatal pore length (92.72%). Cluster analysis used the Mahalanobis general distance (D^2) as a measure of genetic dissimilarity and divided the genotypes into two distinct groups. The inclusion of leaf anatomic traits to characterize *C. canephora* genotypes may assist plant breeders with better genetic discrimination and with greater security in plant selection when composing cultivars. In the second chapter, the objective was to study morphological floral traits of four genotypes of *C. canephora* and one of *C. arabica* cultivated at two different altitudes (36 and 1100 m.a.s.l.). The experiment comprised coffee plantations of both species at two altitudes (and their different climatic conditions), cultivated under in full sun exposure management. The number of petals and stamens varied between five and six in *C. canephora*, but the number of petals and stamens was always five in *C. arabica*. At two altitudes, Beira Rio 8 (*C. canephora*) genotype stood out for presenting the highest averages for all size traits related to corolla, and stigmatic lobes. The variability in floral morphology among *C. canephora* genotypes was not greater at high- than at low-altitude. Overall, flower size of all studied *C. canephora* and *C. arabica* genotypes was larger at low- than at high-altitude, but the stigmatic lobes length and the ratio between stigmatic lobe length and style length and partly the ratio between anther length and stamen length were greater at high-altitude. *C. arabica* flowers were smaller and mostly irresponsive to altitude, in contrast with *C. canephora* flowers. Conducting studies on morphological traits of flowers at a greater number of altitudes can provide better insights into the potential use of these traits in studies of adaptability and stability of *Coffea* spp. genotypes to different environmental conditions, and thus contribute to genetic breeding efforts to achieve resilience of coffee cultivation in the current scenarios of climate change. In the third chapter, the objective was to identify key morphological floral traits to optimize studies of phenotypic variability and thus save time in the process of developing new cultivars. Nine floral traits were measured in 760 flowers. It was proven that the flower phenology can be used to differentiate *Coffea* spp. genotypes. Some *C. canephora* genotypes showed the largest floral structures (Beira Rio 8, Guarani, 8R, and Clementino), while the genotypes with the smallest floral structures were genotypes belonging to the same species (Z8, Verdim D, Ouro Negro 1, Tardio C, NV8, Ouro Negro 2, Bamburral, LB1, and Peneirão). Among the nine evaluated floral traits, the five - petal length, anther length, stamen length, style length, and stigmatic lobe length - were strongly correlated with the traits of the greatest weight for data variability. The correlated traits had little contribution to the data variability, permitting that their exclusion had not compromised the inference of morphological flower variability in *Coffea* spp. genotypes. This suggested that studies aiming at differing the *Coffea* spp. genotypes based on floral structures may consider the four studied traits, tube length, tube diameter, corolla diameter, and the number of floral appendages (petals or stamens). The reduction of nine to these four traits can optimize time and efficiency in data collection. In the future works, the association of selected flower morphological traits with traits related to *Coffea* spp. production could additionally move ahead the process of coffee breeding programs.

Keywords: Altitude, Arabica coffee, Conilon coffee, Floral traits, Phenotypic variability, Stomata.

RESUMO GERAL

A cafeicultura desempenha um papel importante na economia global e na sociedade em geral pela promoção de empregos diretos e indiretos. Programas de melhoramento genético de café tem se dedicado a desenvolver cultivares capazes de contornar os mais diversos desafios da cafeicultura. Neste sentido, a caracterização fenotípica dos indivíduos que constituem uma população é essencial para selecionar os indivíduos com características desejáveis. O objetivo deste trabalho foi estudar caracteres morfo-anatômicos foliares e morfológicos florais em *Coffea* spp. e investigar os efeitos genotípicos e ambientais atuantes sobre esses caracteres. Para atingir esse objetivo, este trabalho reúne três capítulos. No primeiro capítulo buscou-se avaliar o potencial de caracteres anatômicos foliares, juntamente com caracteres agronômicos, para estudo da variabilidade genética em genótipos de duas cultivares registradas de *C. canephora.* Foram avaliados oito caracteres agronômicos e sete anatômicos foliares em dez genótipos de *C. canephora.* Diferenças significativas entre os genótipos foram observadas para 13 dos 15 caracteres ($P < 0,05$). Esses resultados evidenciaram a heterogeneidade entre as cultivares estudadas. Os caracteres anatômicos foliares apresentaram as maiores variações no que os caracteres agronômicos. Cinco, dentre os sete caracteres anatômicos foliares, apresentaram herdabilidade superior a 80% com destaque para a densidade estomática (95,69%) e comprimento do poro estomático (92,72%). A análise de agrupamento, considerando a distância de Mahalanobis como medida de dissimilaridade (D^2), apontou a distinção de dois grupos de genótipos. A inclusão de caracteres anatômicos foliares na caracterização de genótipos de *C. canephora* pode auxiliar os melhoristas de plantas na melhor discriminação entre genótipos e maior segurança na seleção de genótipos para geração de novas cultivares. No segundo capítulo o objetivo foi estudar caracteres morfológicos florais de quatro genótipos de *C. canephora* e um de *C. arabica* cultivados em duas altitudes distintas (36 e 1100 metros). Para isso, o experimento compreendeu lavouras de café de ambas as espécies em duas altitudes (e suas diferentes condições climáticas), cultivadas sob manejo a pleno sol. O número de pétalas e estames variou entre cinco e seis em *C. canephora*, mas o número de pétalas e estames foi sempre cinco em *C. arabica*. Nas duas altitudes, o genótipo Beira Rio 8 (*C. canephora*) se destacou por apresentar as maiores médias para todas as características de tamanho relacionadas à corola e lóbulos estigmáticos. A variabilidade na morfologia floral entre os genótipos de *C. canephora* não foi maior em alta-altitude do que em baixa-altitude. No geral, o tamanho da flor de todos os genótipos de *C. canephora* e *C. arabica* estudados foi maior em baixa-altitude do que em alta-altitude, mas o comprimento dos lóbulos estigmáticos e a razão entre o comprimento do lóbulo estigmático e o comprimento do estilete e, em parte, a razão entre o comprimento da antera e o estame comprimento foram maiores em alta-altitude. As flores de *C. arabica* eram menores e, em sua maioria, insensíveis à altitude, em contraste com as flores de *C. canephora*. A realização de estudos sobre características morfológicas de flores em um número maior de altitudes pode fornecer melhores informações sobre o potencial uso dessas características em estudos de adaptabilidade e estabilidade de genótipos de *Coffea* spp. às diferentes condições ambientais, e assim contribuir com os esforços de melhoramento genético para alcançar a resiliência da cafeicultura nos atuais cenários de mudanças climáticas. No terceiro capítulo o objetivo foi identificar caracteres morfológicos florais chave para otimizar estudos de variabilidade fenotípica e assim economizar tempo no processo de desenvolvimento de novas cultivares. Um total de 36 genótipos de *C. canephora* e os dois de *C. arabica* foram estudados. Nove caracteres florais foram medidos em 760 flores. Foi comprovado que a fenologia da flor pode ser utilizada para diferenciar genótipos de *Coffea* spp. Alguns genótipos de *C. canephora* apresentaram as maiores estruturas florais (Beira Rio 8, Guarani, 8R e Clementino), enquanto os genótipos com as menores estruturas florais foram genótipos pertencentes à mesma espécie (Z8, Verdim D, Ouro Negro 1, Tardio C, NV8, Ouro Negro 2, Bamburral, LB1 e Peneirão). Dentre os nove caracteres florais avaliados, os cinco - comprimento da pétala, comprimento da antera, comprimento do estame, comprimento do estilete e comprimento do lóbulo estigmático - foram fortemente correlacionados com as características de maior peso para a variabilidade dos dados. Os caracteres correlacionados pouco contribuíram para a variabilidade dos dados, permitindo que sua exclusão não tenha comprometido a inferência da variabilidade morfológica das flores em genótipos de *Coffea* spp. Isso sugere que estudos visando diferenciar genótipos de *Coffea* spp. baseados em estruturas florais podem considerar os quatro caracteres estudados, comprimento do tubo, diâmetro do tubo, diâmetro da corola e o número de apêndices florais (pétalas ou estames). A redução de nove para esses quatro caracteres pode otimizar tempo e eficiência na coleta de dados. Em trabalhos futuros, a associação de caracteres morfológicos florais selecionados com caracteres relacionados a produção de *Coffea* spp. poderia, adicionalmente, avançar no processo de programas de melhoramento do café.

Palavras-chave: Altitude, Café arábica, Café conilon, Caracteres florais, Estômatos, Variabilidade fenotípica.

SUMMARY

GENERAL INTRODUCTION

Coffee farming plays a relevant role in both the global economy and society in general (Rhiney et al., 2021). As one of the world's most valuable agricultural commodities, coffee has a significant impact on international markets, driving the economies of various producing countries and providing livelihoods for millions of people (Giovannucci et al., 2004; Schroth et al., 2015; Suárez et al., 2021; ICO, 2022). Brazil is globally recognized as one of the leading producers and exporters of coffee, making a significant contribution to the global market (Conab, 2022).

World coffee production is mainly based on *Coffea arabica* L. (Arabica coffee) and *C. canephora* Pierre ex Froehner (Conilon/Robusta coffee) (Davis et al., 2021). Varieties of *C. arabica* are known for their high quality, smooth flavor, and pleasant aroma (Gebeyehu et al., 2015). These varieties are widely cultivated in higher altitude regions where climatic conditions, including moderate temperatures and well-distributed rainfall, contribute to their cultivation and final beverage quality (Avelino et al., 2005; Bertrand et al., 2012). Varieties of *C. canephora* are grown in regions with hotter and more humid climates, mainly in lower altitude areas. *C. canephora* has a stronger and more bitter taste compared to *C. arabica* (Tsegay et al., 2020), making it a popular choice for coffee blends, especially in instant coffee (Lima Filho et al., 2015). However, the cultivation of *C. canephora* at high-altitudes (720 m) has shown promise in obtaining higher-quality coffees (Pinheiro et al., 2019). Additionally, *C. canephora* is known for its disease resistance (Van Der Vossen, 2009; Alkimim et al., 2021), making it a viable option for producers in regions with historical phytosanitary problems.

Coffee farming faces a series of challenges that impact production and coffee quality worldwide (Bianco, 2020). These challenges include phytosanitary issues (Botelho et al., 2017; Talhinhas et al., 2017), climate change (DaMatta et al., 2018), economic pressures, and consumer demands (Montagnon et al., 2012; Worku, 2023). To overcome these challenges, plant breeding has played a crucial role in the pursuit of developing coffee varieties that are more productive (Alkimim et al., 2021), resistant to pests and diseases (Almeida et al., 2021; Nonato et al., 2021), adapted to different climatic conditions (Partelli et al., 2019; Silva et al., 2022), and exhibit desirable sensory quality (Sobreira et al., 2015; Ferreira et al., 2021). These varieties can be obtained through crosses between different coffee genotypes, followed by careful selection and field testing (Marie et al., 2020).

Genetic variability, which refers to the diversity of genes present in a species or population, is essential for the development of new cultivars (Allard, 1964; Ferrão et al., 2021). Plants possess a vast number of genes and alleles that can be combined in different ways, resulting in diversification of observable traits, or phenotype (Holland, 2007; Li et al., 2020). Understanding genetic variability through the phenotypic variability of individuals within study populations enables more informed decisions in proposing new combinations, identifying, and selecting the best individuals or varieties.

Despite the increasing use of molecular markers to support breeding programs, particularly in recent decades, the study of genetic variability based on phenotype is still predominant, especially in public institution breeding programs (Machado et al., 2017; Starling et al., 2019; Ferrão et al., 2021). Phenotypic traits related to above-ground and agronomic traits (Muvunyi et al., 2017; Akpertey et al., 2019), vigor and yield (Akpertey et al., 2022), root system development (Silva et al., 2020), sensory quality of the beverage (Sobreira et al., 2015), nutrient accumulation in fruits (Rodrigues et al., 2023), leaf anatomy (Giles et al., 2019; Dubberstein et al., 2021), flower morphology (Silva et al., 2021), among others, are used to infer the genetic variability of coffee genotypes. Although the search for new traits with potential use as phenotypic markers is interesting to assist breeding programs, it is necessary to understand the impact of environmental effects on these traits, as well as the relationship of responses among them, to optimize the evaluation process. Using highly correlated traits, in addition to being unnecessary effort, can compromise the accuracy of results during data analysis (Abdi and Williams, 2010).

The identification of minimal traits for inferring variability or the study of the relative importance of traits are tools capable of assisting in the selection of more efficient traits for genotype discrimination. Singh (1981) proposed estimating the relative importance of traits based on the variance associated with each trait for a specific group of genotypes. Jolliffe (1972) proposed selecting traits of interest based on the association (correlation) of each trait with principal components generated for a set of traits and genotypes. These two tools are used in animal and plant breeding programs for selecting minimal traits that optimize evaluations and ensure accuracy in genetic discrimination among individuals based on phenotype (Rêgo et al., 2011; Meira et al., 2013; Souza et al., 2023).

In addition to the relationship among traits, knowledge of environmental effects capable of altering the expression of these phenotypes is essential for understanding the genetic potential of coffee. For widely used traits such as productivity and grain quality in coffee, studies already show the possibility of phenotypic changes due to altitude, temperature, soil, water conditions, and management (Bote and Vos, 2017; Martins et al., 2019; Lourenço et al., 2022). However, for anatomical leaf traits and flower morphology, for example, there is still a gap in understanding these environmental factors and the potential challenges in using these markers in breeding programs. Therefore, studies on genetic parameters, correlations, and genotype x environment interactions are important to support coffee breeding programs in optimizing the evaluation and selection processes of genotypes.

GENERAL OBJECTIVE

To study morpho-anatomical leaf traits and floral morphology in *Coffea* spp. and investigate the genetic and environmental effects on these traits.

SPECIFIC OBJECTIVES

To evaluate the potential of leaf anatomic traits, along with agronomic traits, in the evaluation of genetic variability in two *C. canephora* registered cultivars.

Study floral morphological traits of four genotypes of *C. canephora* and one of *C. arabica* cultivated at two distinct altitudes (36 and 1100 m.a.s.l).

Identify key floral morphological traits for study a phenotypic variability in *Coffea* spp. genotypes with the aim of saving time and effort in the evaluation process for the development of new cultivars.

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CHAPTER 1: MORPHO-AGRONOMIC AND LEAF ANATOMICAL TRAITS IN *Coffea canephora* GENOTYPES

ABSTRACT

Genetic variability is the foundation for the advancement of coffee breeding programs. This study evaluated the potential of leaf anatomy and morpho-agronomic traits in studies of genetic variability in *C. canephora* cultivars. Ten genotypes were distributed in randomized block designs with three replicates. Significant differences among genotypes were detected by F-test ($P<0.05$) for 13 of 15 evaluated traits. These results evidenced the heterogeneity of the studied cultivars, which is essential in composition of genetic basis in breeding programs. The Scott-Knott clustering detected differences among the genotypes, separating them into four groups of means. Leaf anatomy traits presented the largest variations. Five out of seven leaf anatomy traits presented heritability higher than 80%, with emphasis on stomatal density (95.69%) and stomatal pore length (92.72%). Positive correlations were observed among morpho-agronomic and anatomic traits. The clustering analysis (UPGMA method), considering Mahalanobis distance (D^2), revealed the distinction of two groups of genotypes. The inclusion of leaf anatomic traits to characterize *C. canephora* genotypes may assist plant breeders with better genetic discrimination and with greater security in plant selection when composing cultivars.

Keywords: Conilon coffee, Electron Microscopy, Leaf Microscopy, Plant breeding.

CAPÍTULO 1: CARACTERES MORFO-AGRONÔMICOS E ANATÔMICOS FOLIARES EM GENÓTIPOS DE *Coffea canephora*

RESUMO

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

Palavras-chave: Café conilon, Microscopia eletrônica, Microscopia foliar, Melhoramento de plantas.

INTRODUCTION

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

One hundred and thirty species of coffee have been identified (Davis et al., 2021), however, *Coffea arabica* L. and *C. canephora* Pierre ex A. Froehner represent the total coffee cultivation in Brazil. Besides the diversity among coffee species, intraspecific variety is also reported (Alkimim et al., 2018; Silva et al., 2020). *C. canephora* is an allogamous species due the genetic self-incompatibility, and such mechanisms block flower fertilization by pollen from the plant itself. So, these features are fundamental for *C. canephora* reproductive success and ensures a wide genetic base in this crop (Moraes et al., 2018).

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

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

Among plant organs, microscopy studies with leaves have been conducted in several crops (Schollert et al., 2015; Faralli et al., 2019; Singh et al., 2020). Leaves are essential to plants, since they are responsible for supplying photoassimilates; and therefore, are one of the first organs responding to imbalances in plant metabolism (Taiz and Zeiger, 2017). Among the leaf structures studied, stomata are structures that arouse interest, as they are highly variable between species and within species (Gul et al., 2019). Such variations are considered as immediate response to environmental conditions (Castro, 2009; Matthews and Lawson, 2019) and to genetic components acting on stomata development (Casson and Gray, 2008; Chater et al., 2017; Zoulias et al., 2018) both contributing to changes in stomatal development.

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

MATERIALS AND METHODS

Plant material and experimental design

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

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

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

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

The genotypes were cultivated after vegetative propagation in June 2018 at the Experimental Farm of Universidade Federal of Espírito Santo (UFES), located at São Mateus, Espírito Santo, Brazil (coordinates 18º 42' 58" S / 39º 51' 32" W; altitude 36 m; temperature annual mean 24 °C; precipitation annual mean 1.240 mm). The region presents tropical climate characterized by warm wet summers and a short dry period, which is classified as Am by Köppen (Alvares et al. 2013). Spacing was 2 m between lines and 1 m between plants (5.000 plants.ha^{-1}). Crop formation was managed by conduction of two orthotropics branch per plant (10.000 rods.ha^{-1}), and a supplementary irrigation by dripping was adopted. The treatments were constituted by the distinct genotypes, and each experimental unit was composed by three plants. The genotypes were arranged in random blocks, with three repetitions.

Morpho-agronomic traits

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

Leaf anatomic traits

Six leaves per experimental unit of each coffee genotype were collected during morning from the third or fourth leaf pairs from the top of plagiotropic branches. Fragments of collected leaves (1 cm^2) were fixed with 50% FAA (formaldehyde, glacial acetic acid and 50% ethanol, 1:1:9) for 48h. The samples were preserved by fixation with 2.5% glutaraldehyde, 2% formaldehyde and 0.1 M sodium cacodylate buffer. Next, they were transferred to a recipient covered with laminated paper and submerged during 1h at room temperature in aqueous solution containing 2% OsO_4 (osmium tetroxide), 0.2 M sodium cacodylate buffer and 2.5% potassium ferrocyanide. After this period, the samples were washed with the same buffer and milliQ water, dehydrated with ethanolic series (30, 50, 70, 90 and 100%), and critical point dried. The dried material was mounted on stubs and covered in gold. Images from leaf surface were obtained in Scanning Electron Microscope (SEM), using a 50 µm scale, for measurements of stomata polar diameter (PD – µm), equatorial diameter (ED – µm), stomatal pore length (SPL – µm) and stomatal pore width (SPW – µm) (Figure 1b). These anatomic traits enabled estimations of stomatal functionality (SF), stomatal area index (SAI) and stomatal density (SD) (Castro et al., 2009). Image analyses were conducted with ImageJ software Papadopulos et al. 2007).

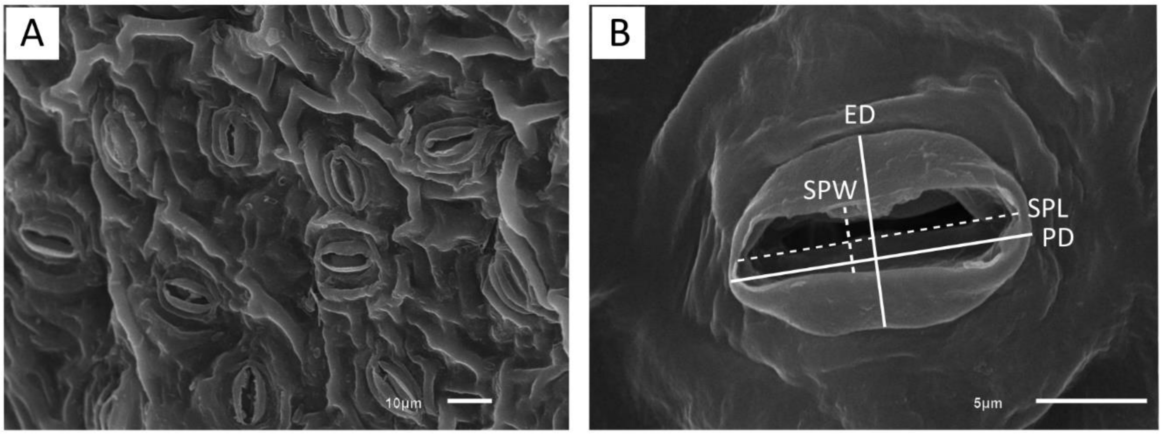


Figure 1 - Scanning electron nicroscopy (SEM) images of *Coffea canephora* ellipsoid stomata. Scale bar = 10µm (A) and 5µm (B). ED: stomata equatorial diameter (µm); PD: stomata polar diameter (µm); SPL: stomatal pore length (µm); SPW: stomatal pore width (µm).

Data analysis

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

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

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

RESULTS

Morpho-agronomic traits, leaf anatomic traits and genetic parameters

The F-test detected significant differences ($p<0.05$) among *C. canephora* cultivars in 13 of 15 traits (Table 2). The VCe for those traits varied between 3.31-24.08%. VCg, which quantifies the influence of genetic components in each trait, varied between 5.75-23.36%, and the highest VCg values were found in LA and SD. The trait LA exhibited the highest VCe and

VCg values (24.08 and 23.36%, respectively). Moreover, SD, PD and ED presented the highest IV (VCg/VCe) values, which were higher than one.

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

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

Variables	QM Genotype	Residue	Means	VCe(%)	VCg (%)	VI	h^2 (%)
LA	1810.61**	473.49	90.37	24.08	23.36	0.97	73.85
PL	0.289**	0.047	1.44	15.09	19.78	1.31	83.75
CV	10.26**	2.48	15.25	10.33	10.56	1.02	75.83
H	264.29^{ns}	168.65	80.71	16.09	6.99	0.43	36.18
LPB	200.85*	62.24	54.72	14.41	12.42	0.86	69.01
NROS	5.53^{ns}	4.18	10.32	19.8	6.50	0.32	24.45
D	210.78**	56.2	63.15	11.87	11.36	0.95	73.34
SDM	0.061**	0.010	1.07	9.37	12.14	1.30	83.43
PD	2.82**	0.30	13.88	3.96	6.61	1.67	89.33
ED	0.93**	0.10	9.09	3.55	5.75	1.62	88.67
SPL	3.42**	0.25	9.75	5.12	10.55	2.06	92.72
SPW	0.44**	0.089	2.31	12.93	14.85	1.14	79.84
SF	0.03**	0.006	1.53	5.31	6.11	1.15	79.85
SAI	661.32**	106.05	128.65	8.00	10.58	1.32	83.96
SD	0.025**	0.001	0.425	7.86	21.33	2.71	95.66

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

The Scott-Knott test grouped the genotypes in two, three or four distinct mean groups to depend on the trait (Table 3). Morpho-agronomic traits presented lower variations compared to leaf anatomic traits, as LA, PL, H, D, and SDM formed only two groups of means. The leaf anatomic traits exhibited higher variations, and up to four mean groups were distinguished. Such variations are easily spotted by SEM images (Figure 2).

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

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

Means followed by the same letter in columns belong to the same group, after Scott-Knott test with 5% probability.LA: leaf area (cm); PL: petiole length (cm); CV: leaf central vein length (cm); LPB: length of plagiotropic branches (cmD: plant diameter (cm); PD: stomata polar diameter (μm); ED: stomata equatorial diameter (μm); SPL: stomatal pore length (μm); SPW: stomatal pore width (μm); SF: stomatal functionality; SAI: stomatal area index; SD: stomatal density; SDM: specific dry mass per leaf square centimeter (g). G1: Pirata*; G2: Verdim R*; G3: Bamburral*; G4: A1* **; G5: Clementino*; G6: Beira Rio *; G7: P1**; G8: Verdim TA**; G9: NV2; G10: NV8**. * Tributum cultivar (Partelli et al., 2020); ** Andina cultivar (Partelli et al., 2019). Scale bar = 50 μm.

Four mean groups were found for SPL (Table 3). This trait was highly variable between NV2 and NV8. Moreover, SD and PD formed three mean groups, the first varied between

0.290-0.563 µm, whereas the latest varied between 12.79-15.85 µm, respectively. Two mean groups were formed for ED, SAI and SF. The highest means for ED and SAI were found in NV8 (10.44 µm and 157.87 µm, respectively). The genotypes with the highest and the lowest SF were, respectively, P1 and NV2, due to the smaller polar diameter found in NV2. High PD/ED ratios indicate greater stomata functionality, since stomata have an ellipsoid shape (Figure 1a, b). SPW values formed a single mean group; although, significant differences have been reported for this trait. Leaves from all genotypes are hipostomatous (Figure 1a).

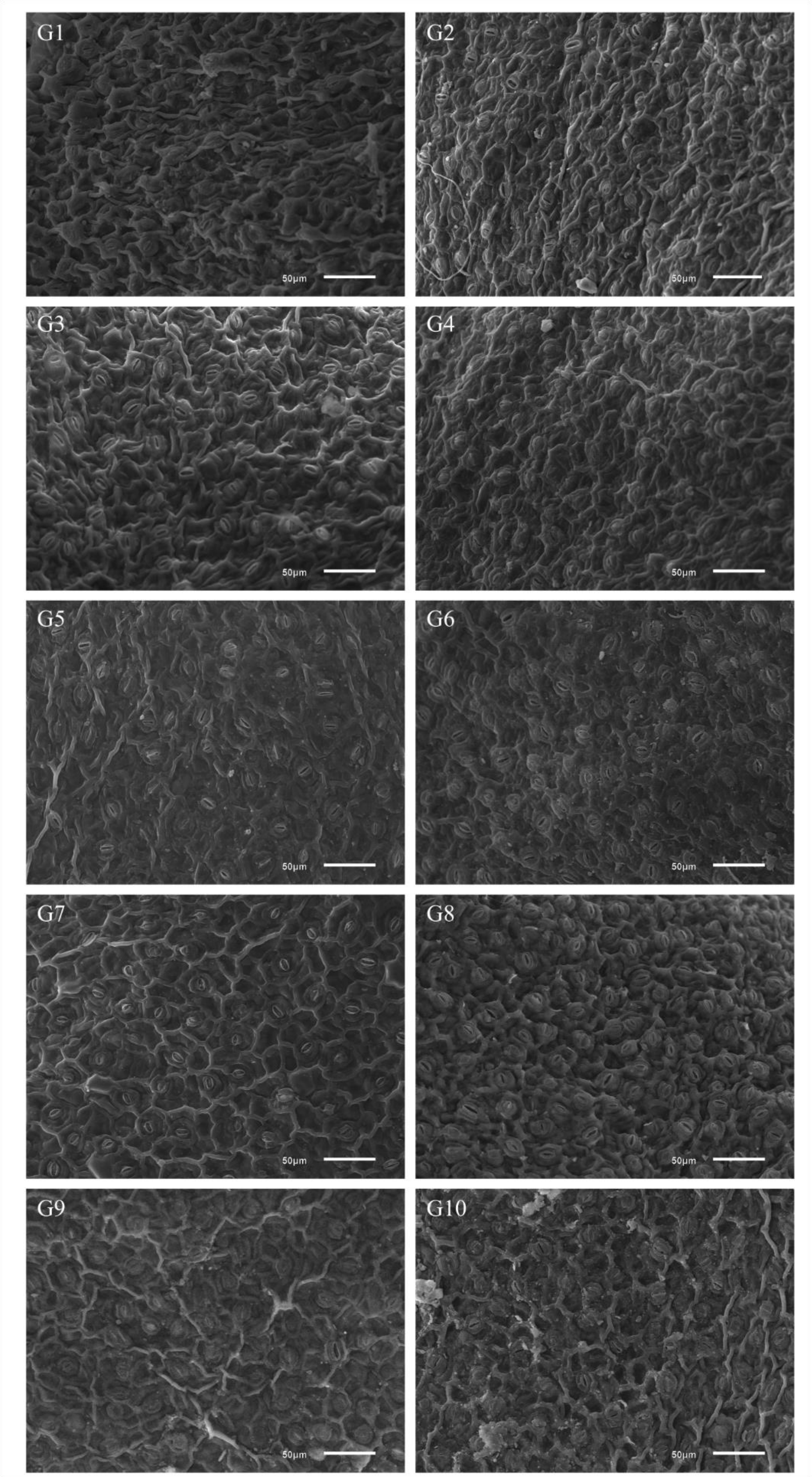


Figure 2 - Scanning Electron Microscopy (SEM) images of *Coffea canephora* leaf surfaces.

Cluster analysis

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

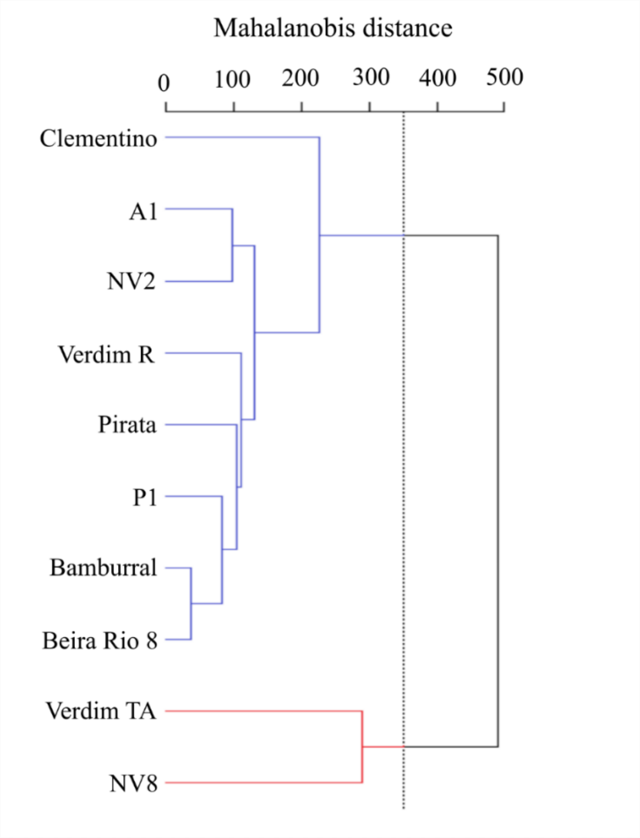


Figure 3 - Dendrogram of genetic dissimilarity among 10 *Coffea canephora* genotypes, obtained by UPGMA clustering method and Mahalanobis general distance. Fifteen morpho-agronomic and anatomic traits were considered.

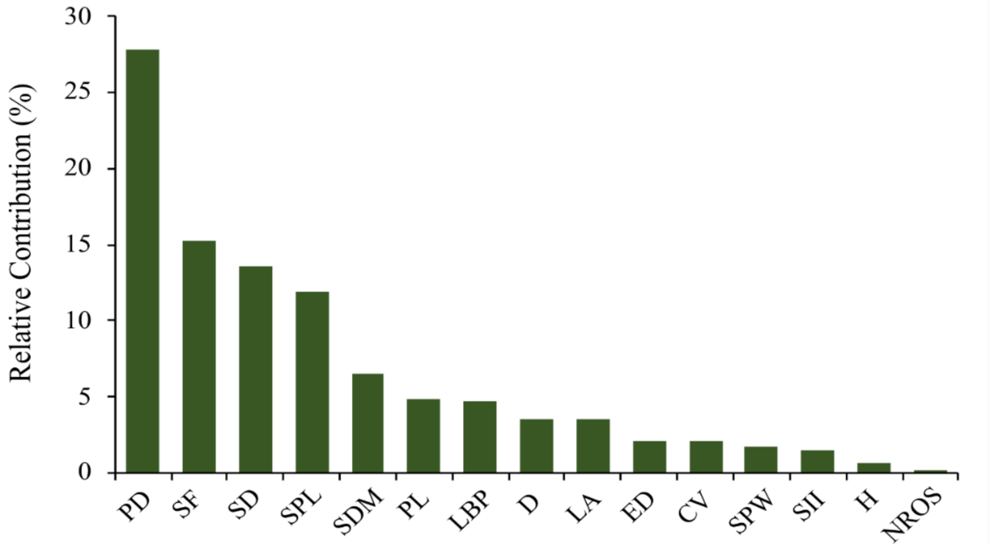
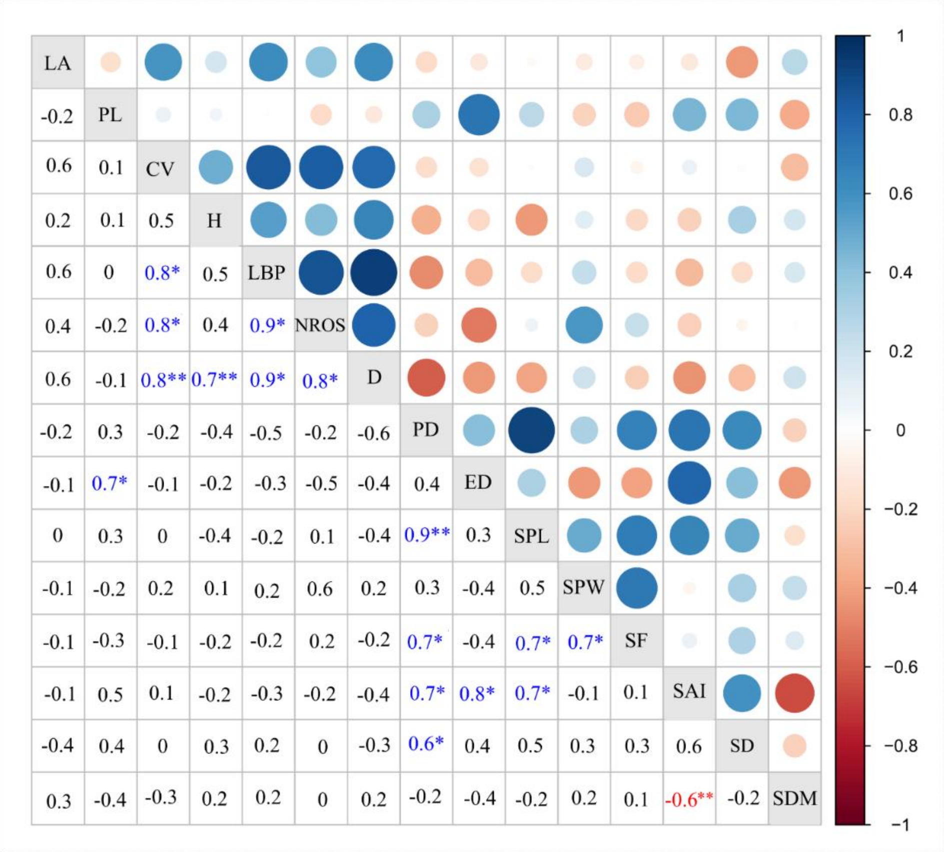


Figure 4 - Trait relative contribution for divergence by Singh (1981) method. LA: leaf area (cm); PL: petiole length (cm); CV: leaf central vein length (cm); H: plant height (cm); LPB: length of plagiotropic branches (cm); NROS: number of rosettes; D: plant diameter (cm); PD: stomata polar diameter (μm); ED: stomata equatorial diameter (μm); SPL: stomatal pore length (μm); SPW: stomatal pore width (μm); SF: stomatal functionality; SAI: stomatal area index; SD: stomatal density; SDM: specific dry mass per leaf square centimeter (g).

Correlations among morpho-agronomic and leaf anatomic traits

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

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



LA														
-0.2	PL													
0.6	0.1	CV												
0.2	0.1	0.5	H											
0.6	0	0.8*	0.5	LBP										
0.4	-0.2	0.8*	0.4	0.9*	NROS									
0.6	-0.1	0.8**	0.7**	0.9*	0.8*	D								
-0.2	0.3	-0.2	-0.4	-0.5	-0.2	-0.6	PD							
-0.1	0.7*	-0.1	-0.2	-0.3	-0.5	-0.4	0.4	ED						
0	0.3	0	-0.4	-0.2	0.1	-0.4	0.9**	0.3	SPL					
-0.1	-0.2	0.2	0.1	0.2	0.6	0.2	0.3	-0.4	0.5	SPW				
-0.1	-0.3	-0.1	-0.2	-0.2	0.2	-0.2	0.7*	-0.4	0.7*	0.7*	SF			
-0.1	0.5	0.1	-0.2	-0.3	-0.2	-0.4	0.7*	0.8*	0.7*	-0.1	0.1	SAI		
-0.4	0.4	0	0.3	0.2	0	-0.3	0.6*	0.4	0.5	0.3	0.3	0.6	SD	
0.3	-0.4	-0.3	0.2	0.2	0	0.2	-0.2	-0.4	-0.2	0.2	0.1	-0.6**	-0.2	SDM

Figure 5 - Pearson correlation and p-values significance (* and **corresponded to the significance of $p < 0.05$, 0.01, respectively) for: LA: leaf area (cm); PL: petiole length (cm); CV: leaf central vein length (cm); H: plant height (cm); LPB: length of plagiotropic branches (cm); NROS: number of rosettes; D: plant diameter (cm); PD: stomata polar diameter (µm); ED: stomata equatorial diameter (µm); SPL: stomatal pore length (µm); SPW: stomatal pore width (µm); SF: stomatal functionality; SAI: stomatal area index; SD: stomatal density; SDM: specific dry mass per leaf square centimeter (g).

DISCUSSION

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

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

The ANOVA results evidenced the heterogeneity of the studied clonal population, essential for composition of the genetic base in breeding programs. Similar results were reported by Gama et al.

(2017) and Giles et al. (2019) evaluating anatomical characteristics of coffee leaves, although these studies analyzed images with optical microscopy.

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

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

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

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

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

The knowledge on plants from measurements of stomata size and density is fundamental in plant biology, since they are related with stomatal conductance (Sack and Buckley, 2016). In this study, Pirata and Verdim TA presented the highest SD values, whereas NV2 had the lowest mean values, which is next to half than in Pirata and Verdim TA. According to Castro et al. (2009), leaves adapted to drought present higher stomatal density and smaller stomata, correlated to a more efficient stomatal conductance. The understanding of the development base of those traits allows the targeting of plant enhancing towards grain productivity (Sack and Buckley, 2016).

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

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

In this study, the estimated maximum distance between groups was 490.13 (Mahalanobis distance). Such a high value reinforces the potential of leaf anatomical traits, together with morpho-agronomic traits, to increase the level of precision in estimating genetic divergence between genotypes. Giles et al. (2019) evaluated the genetic divergence among 34 coffee genotypes by observing agronomic and leaf anatomic traits obtained by light microscopy and obtained 71.41 (Mahalanobis Distance) of distance maximum estimate among groups.

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

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

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

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

CONCLUSION

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

Acknowledgments

We thank to the Fundação de Amparo à Pesquisa e Inovação do Espírito Santo - FAPES (Proc. 2022-WTZQP for Fábio Luiz Partelli and Proc. 2022-M465D for Miroslava Rakocevic and Fábio Luiz Partelli), Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Proc. 309535/2021-2 for Fábio Luiz Partelli), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Proc. 88882.385374/2019-01 for Larícia Olária Emerick Silva).

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CHAPTER 2: MODIFICATIONS IN FLORAL MORPHOLOGY OF *Coffea* spp. GENOTYPES AT TWO DISTINCT ALTITUDES

ABSTRACT: Floral traits can be influenced by altitude in various species with implication on crop productivity, but no information is available for the *Coffea canephora* and *C. arabica*. It was hypothesized that the: 1) number of stamens and petals can variate related to flower size, which is dependent on species and genotype; 2) variations in the morphological traits of the coffee flower occur in response to the difference in altitude, 3) greater variability in flower morphology among *C. canephora* genotypes is expressed at higher than at lower altitudes, due to environmental pressure, and 4) variability in the morphological the flower traits between low- and high-altitude in *C. arabica* is smaller than in *C. canephora* genotypes. For this, the experiment comprised coffee plantations of both species at two altitudes (and their different climatic conditions), cultivated under in full sun exposure management. The number of petals and stamens varied between five and six in *C. canephora*, but the number of petals and stamens was always five in *C. arabica*. At two altitudes, Beira Rio 8 (*C. canephora*) genotype stood out for presenting the highest averages for all size traits related to corolla, and stigmatic lobes. The variability in floral morphology among *C. canephora* genotypes was not greater at high- than at low-altitude. Overall, flower size of all studied *C. canephora* and *C. arabica* genotypes was larger at low- than at high-altitude, but the stigmatic lobes length and the ratio between stigmatic lobe length and style length and partly the ratio between anther length and stamen length were greater at high-altitude. *C. arabica* flowers were smaller and mostly irresponsive to altitude, in contrast with *C. canephora* flowers. Conducting studies on morphological traits of flowers at a greater number of altitudes can provide better insights into the potential use of these traits in studies of adaptability and stability of *Coffea* spp. genotypes to different environmental conditions, and thus contribute to genetic breeding efforts to achieve resilience of coffee cultivation in the current scenarios of climate change.

Keywords: *Coffea arabica*, *Coffea canephora*, Corolla diameter, Floral traits, Genetic variability, Petal number.

CAPÍTULO 2: MODIFICAÇÕES NA MORFOLOGIA FLORAL DE GENÓTIPOS DE *Coffea* spp. EM DUAS ALTITUDES DISTINTAS

RESUMO: Os caracteres florais podem ser influenciados pela altitude em várias espécies com implicações na produtividade das culturas, mas não há informações disponíveis para *Coffea canephora* e *C. arabica*. Foi hipotetizado que: 1) o número de estames e pétalas pode variar em relação ao tamanho da flor, que é dependente da espécie e do genótipo; 2) variações nos caracteres morfológicos da flor do cafeeiro ocorrem em resposta à diferença de altitude, 3) maior variabilidade na morfologia da flor entre os genótipos de *C. canephora* é expressa em altitudes mais altas do que em altitudes mais baixas, devido à pressão ambiental, e 4) a variabilidade nos caracteres morfológicos das flores entre baixa e alta-altitude em *C. arabica* é menor do que em genótipos de *C. canephora*. Para isso, o experimento compreendeu lavouras de café de ambas as espécies em duas altitudes (e suas diferentes condições climáticas), cultivadas sob manejo a pleno sol. O número de pétalas e estames variou entre cinco e seis em *C. canephora*, mas o número de pétalas e estames foi sempre cinco em *C. arabica*. Entre as duas altitudes, o genótipo Beira Rio 8 (*C. canephora*) se destacou por apresentar as maiores médias para todos os caracteres relacionados ao tamanho da corola e lóbulos estigmáticos. A variabilidade na morfologia floral entre os genótipos de *C. canephora* não foi maior em alta do que em baixa-altitude. No geral, o tamanho da flor de todos os genótipos de *C. canephora* e *C. arabica* estudados foi maior em baixa-altitude do que em alta-altitude, mas o comprimento dos lóbulos estigmáticos e a razão entre o comprimento do lóbulo estigmático e o comprimento do estilete e, em parte, a razão entre o comprimento da antera e o comprimento do estame foram maiores em alta-altitude. As flores de *C. arabica* eram menores e, em sua maioria, insensíveis à altitude, em contraste com as flores de *C. canephora.* A realização de estudos sobre caracteres morfológicos de flores em um número maior de altitudes pode fornecer melhores informações sobre o potencial uso desses caracteres em estudos de adaptabilidade e estabilidade de genótipos de *Coffea* spp. às diferentes condições ambientais, e assim contribuir com os esforços de melhoramento genético para alcançar a resiliência da cafeicultura nos atuais cenários de mudanças climáticas.

Palavras-chave: *Coffea arabica*, *Coffea canephora*, Diâmetro da corola, Caracteres florais, Variabilidade genética, Número de pétalas.

INTRODUCTION

The two main cropped coffee species, *Coffea arabica* L. and *C. canephora* Pierre ex Froehner, support world coffee industry accounts for about US$433 billion in annual revenue, for a global production close to 10.2 million tons in the last years (ICO, 2022), with Brazil constituting the greatest producer, accounting for *ca*. 28% (Conab, 2022). One of the main components of technological packages to increase coffee yield and quality is the development of coffee germplasm with higher productive potential under different environmental conditions (Akpertey et al. 2022; Partelli et al. 2022; Zaidan et al. 2023). The self-fertilization inherent to *C. arabica* guarantees the reproduction (Wright et al. 2013). Cross fertilization and the mechanism of genetic self-incompatibility, inherent to *C. canephora*, increases genetic diversity and reduces the risk of inbreeding depression (Castro et al. 2008).

A successful and synchronized flower development is essential for this crop sustainability (Miranda et al., 2020). The floral morphology of *C. arabica* and *C. canephora* is mostly similar, with flowers gathered in inflorescences that develop from serial buds located in the leaf axils, in plagiotropic branches. Each flower is usually composed of five petals fused into an elongated and cylindrical corolla tube, with five stamens of long anthers and short filaments inserted into the corolla, a long thin style with a stigma of two branches, and a lower ovary of two chambers, each containing one ovule (Charrier and Berthaud, 1985). However, in *C. canephora* and *C. arabica* the flowers may have five or six petals, and flower size may vary with the cultivation system (full sun exposure or with shade) (Prado et al., 2019), whereas the size of the floral structures of *C. canephora* may vary according to the genotype of the plant (Silva et al., 2021). Overall, this points that both genotype and environment determine the morphological traits of the flower in *Coffea* spp, including the number of floral structural components.

The ability of plants to respond to environmental stimuli allows them to deal with unfavorable conditions (Molina et al., 2021). The difference in altitude (usually due to different air humidity and temperature conditions, as well as water availability) can alter coffee fruit development, quality, and yield (Avelino et al., 2005; Worku et al., 2018; Ginbo, 2021; Cassamo et al., 2022). Additionally, development of flowers is also impacted by altitude in other species, modifying the frequency of sexual dimorphism in biomass production in *Ilex paraguariensis* (Rakocevic et al., 2023), a reduction in the weight and size of *Bellidiastrum michelii* flowers (Kiełtyk, 2021), and an increase in corolla diameter, flower length and tube diameter in *Campanula rotundifolia* with increasing altitude (Maad et al., 2013). Higher altitudes may also influence the higher frequency of abnormal flowers in *Stylidium armeria* (Hoffmann et al., 2009). However, in *C. canephora* and *C. arabica*, there is still no report about the impacts of different altitudes on flower morphology.

Due to the genetic diversity of the two cultivated species of *Coffea* spp., as well as the different climate traits at their original evolution regions (Ramalho et al, 2014), some genotypes may be more stable or better adapted to altitude than others (Partelli et al., 2022). One way to infer the intensity of genotypic differences in relation to environmental responses of *C. canephora* is to evaluate the interaction of genotype x environment (G × E) (Kiwuka et al., 2021). The G x E interaction can be particularized when the different genotypes response are expected under different environmental conditions. Such interactions can be decomposed into simple and complex parts (Baye et al., 2011). The first are expressed by the difference in the variability of the genotypes, when no change in genotype ordering is expressed, even when they are grown in different environments. The second are assessed by the difference in the ordering of the superior genotypes in each environment, so that the best in one environment is not the best in another (Cruz et al., 2012). To our knowledge, this evaluation of the simple and complex G x E interactions regarding coffee flower morphological traits have never been explored under distinct environmental conditions.

This study aimed to evaluate and characterize the G × E interaction for morphological traits of coffee flowers, to estimate genetic parameters and investigate the potential use of these traits in breeding programs. In this context, our work was orientated according to the hypotheses that: **1**) the number of stamens and petals can variate related to flower size, which is dependent on species and genotype; **2**) the variations in the morphological traits of the coffee flower occur in response to the difference in altitude, **3**) a greater variability in flower morphology among *C. canephora* genotypes is expressed at higher than at lower altitudes, due to environmental pressure, and **4**) the variability in the morphological the flower traits between the two altitudes of *C. arabica* is smaller than in *C. canephora*.

MATERIAL AND METHODS

Environmental Conditions, Plant Material, and Experimental Design

The experiment was performed in two coffee plantations with different climatic conditions induced by two different altitudes, both cultivated under full sun system management, in the state of Espírito Santo (ES), Brazil. The experiment 1 was installed at 1100 m.a.s.l. (high-altitude) in June 2019 in the municipality of Venda Nova do Imigrante (20°26'08.21" S, 41°03'58.01" W). The climate of the region is type Cfb, corresponding to a temperate or subtropical oceanic climate of Köppen-Geiger's classification, with a mild summer, without a dry season and with uniformly distributed rainfall (Alvares et al., 2013). The mean annual temperature of the region is 19 °C and the mean annual precipitation is *ca*. 1420 mm (Figure 1, INMET data, 2022). The soil of the experiment at high-altitude site is classified as Dystrophic Red-Yellow Latosols (Santos et al., 2018). The experiment 2 was installed at 36 m.a.s.l. (low-altitude) in June 2018 in the municipality of São Mateus

(18º 42’ 58” S, 39º 51’ 32” W). The climate of the region is tropical Aw savanna type, with dry winter and rainy summer (Alvares et al., 2013). The region mean annual values of temperature and rainfall are c*a*. 24 °C and 1370 mm, respectively (Figure 1, INMET data, 2022). The soil of the experiment at low-altitude site is classified as Dystron Cohesive Yellow Argisol (Santos et al., 2018).

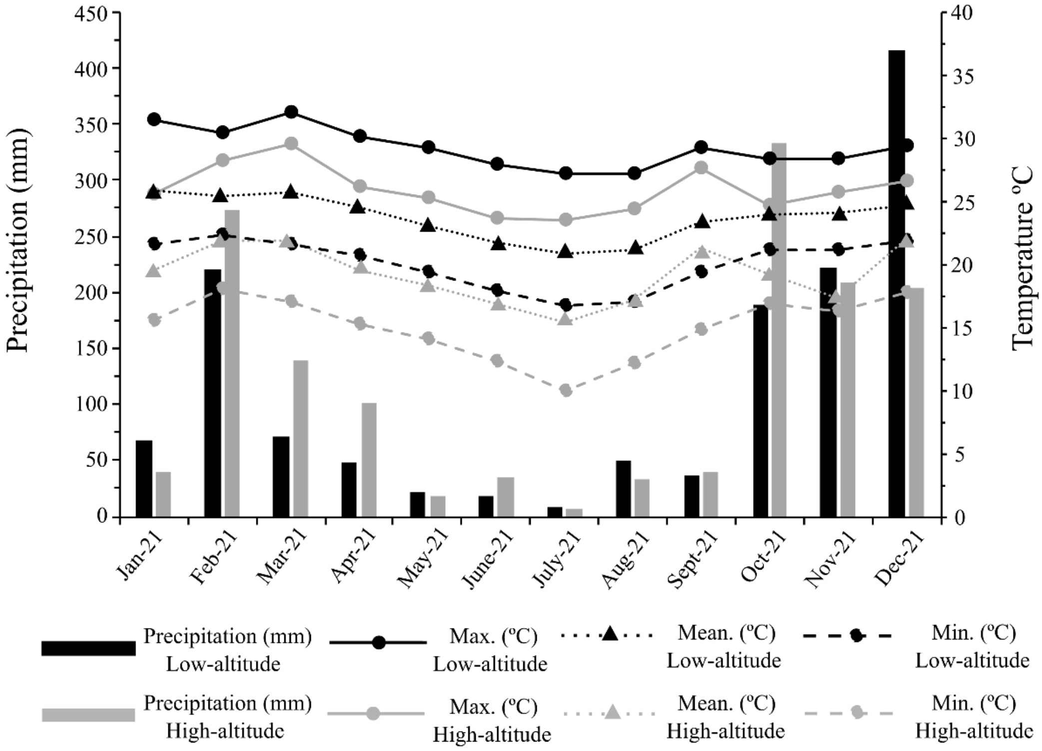

Figure 1- Weather conditions in São Mateus - ES, Brazil (low-altitude) and Venda Nova do Imigrante, ES, Brazil (high-altitude) in 2021.

Coffee plantation management practices were applied according to the crop-specific technical guidelines, consisting basically of weed control with herbicides and brush cutters, preventive phytosanitary management, Ca import, and fertilization (Partelli et al., 2021). It was applied around 1.5 of $CaCO_3$ t/ha^{-1} at each altitude. The fertilizations were carried out according to the soil and productivity analysis, according to Paye et al. (2019). Plant needs and phenological stages were also considered, applying around 300 of CH_4N_2O, 50 of P_2O_5 and 200 of K_2O_2 kg ha^{-1} at high-altitude and 500 of CH_4N_2O, 80 of P_2O_5 and 400 K_2O_2 kg ha^{-1}, divided in six times along the year (every 60 days).

Both experimental areas received drip irrigation. The irrigation hoses were located 5 cm away from the coffee trunk, and emitters were spaced every 0.5 m in the planting rows. The irrigation was executed, when necessary, based on soil water balance method, with use of drip irrigation, reference evapotranspiration was calculated according to Penman-Monteith (Allen et al., 1998).

Five genotypes were evaluated in each of the two altitudes: four *C. canephora* cv. Conilon genotypes: Beira Rio 8 and Clementino, belonging to cultivar Tributum (Partelli et al., 2020); L80 belonging to the cultivar Plena (Partelli et al., 2022); A1 belonging to the cultivars Andina and Tributum (Partelli et al., 2019; 2020). Additionally, one *C. arabica* cultivar (Arara) was also used. The experimental was conducted in completely randomized block design, with three blocks. Within each block, 10 biological replicates (flowers) were randomly collected. The treatments consisted of five genotypes (G) and two environments (E).

Flower morphology

The flower evaluations at low-altitude took place from July to August 2021, while the same evaluations at high-altitude took place from September to October 2021. This difference in measurement time is fully justified by the fact that a later anthesis occurs in plants at higher elevations, as compared with lower-altitude counterparts.

The flowers were collected from plagiotropic branches in the most productive, middle third of three plants per genotype. Field measurements were performed immediately after the flower blossom using a digital caliper (resolution=0.1 mm) with precision of 0.2 mm. The traits evaluated in each flower were: corolla diameter (CD), tube diameter (TD), tube length (TL), petal length (PL), stamen length (StaL), anther length (AntL), stigmatic lobes length (StgL), style length (StyL), all in mm, except for the number of floral appendages (NFA) - petals and stamens – that always was equal at one flower, five and five, six and six (Figure 2A), which was counted. The NFA was used to show the frequency of flowers with six petals and stamens among the randomly collected flowers of *Coffea* spp. The ratios between CD/NFA, AntL/StaL, and StgL/StyL were calculated, to relate the flower size or shape with female or male flower appendages.

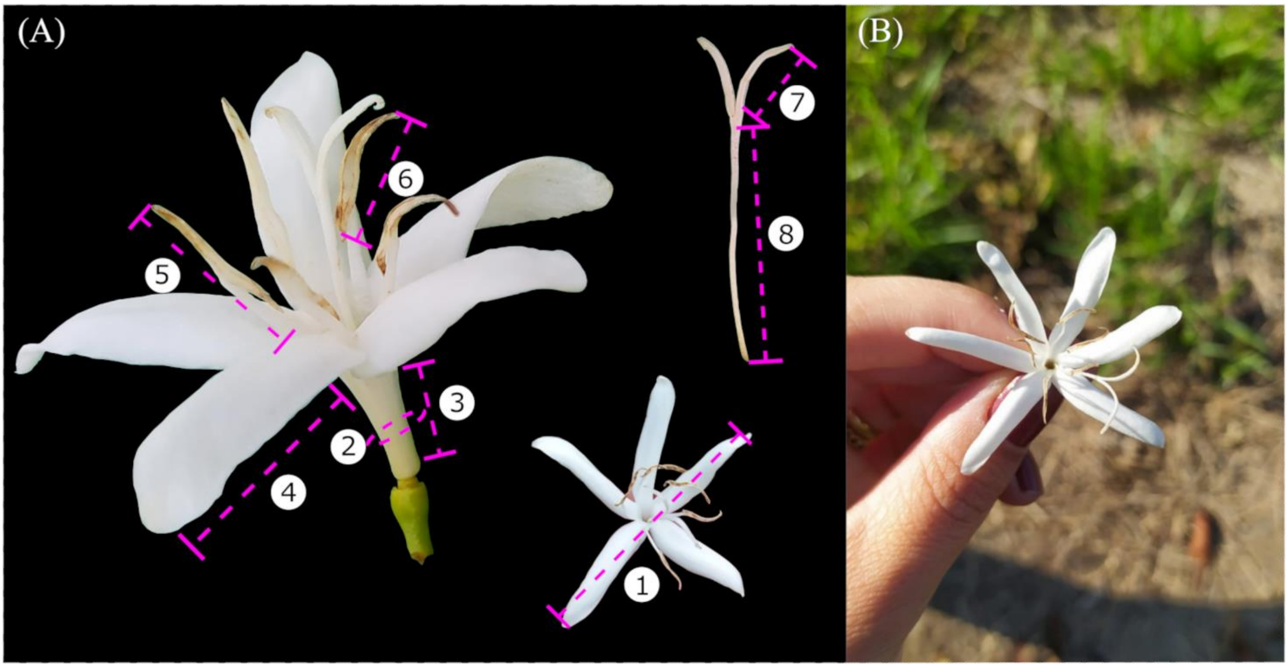

Figure 2- (A) Traits evaluated on pentamerous flowers of *C. canephora* and *C. arabica*: (1) diameter of corolla (mm), (2) diameter of corolla tube (mm), (3) length of corolla tube (mm), (4) length of petals(mm), (5) length of stamen (mm), (6) length of the anther (mm), (7) length of stigmatic lobes (mm), (8) length of style (mm), and number of floral appendages (petals and stamens); (B) Non pentamerous flowers of *C. canephora*.

Statistical analysis

Before the two-way ANOVA was performed, the data was subjected to Shapiro-Wilk normality tests and Bartlett homogeneity tests, followed by a two-way ANOVA. When observed the existence of significant differences between genotypes was identified by variance analysis, a mean comparison Tukey test was performed. All testes were performed at significance level of 5%.

Using the flower traits evaluations identified above (2.2), the following statistical parameters were evaluated using variance components for each flower trait: genetic coefficient of variation (CVg), environmental coefficient of variation (CVe), variation index (VI), and genotypic coefficient of determination (H^2). The G × E effects were expressed as simple and complex interactions, by presenting mean response graphs of genotypes in each environment (Cruz et al., 2012). Simple interaction occurs when there is a change in the magnitude of performance of genotypes, but their ordering in different environments remains unchanged. The complex interaction occurs when there is a differentiated response of the genotypes to different environments so that the ordering of the genotypes is altered (Cruz et al., 2012). All analyzes used the R software (R Core Team, 2021), packages 'stats', and 'ExpDes'. To verify the possible correlations among the morphological traits of coffee flowers, Pearson's linear correlation was performed, using the ‘corrplot’ package.

RESULTS

Floral morphological traits in Coffea spp.

The analysis of variance showed that genotype and environmental (associated with contrasting altitude sites) effects were significant for all studied flower traits. The G X E interactions were also significant for all flower traits except for AntL, StgL and StgL/StyL ratio (Tables 1,2).

The mean corolla diameter (CD) ranged from 28.33 to 43.37 mm at low-altitude and from 26.32 to 35.97 mm at high-altitude (Table 1). At low-altitude, the CD of Beira Rio 8 and Clementino was higher and significantly different from the others (Table 1, Figure 3). At high-altitude, Beira Rio 8, Clementino and L80 showed as well the highest CD, followed by A1 with an intermediate size, and Arara with the smallest value. This trait was consistently greater at low-than in high-altitude, with significant differences observed for Beira Rio 8, Clementino and A1.

Table 1- Comparison of estimated means (n = 30) for: corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), petal length (PL, mm), anther length (AntL, mm), stamen length (StaL, mm), stigmatic lobes length (StgL, mm), style length (StyL mm), number of floral appendages (NFA, petals or stamens), CD/NFA, AntL/StaL, StgL/StyL among genotypes of *Coffea canephora* and *C. arabica* (shaded), grown at low- and high-altitudes.

Flower parameters												
Low-altitude												
Genotypes	CD	TD	TL	PL	AntL	StaL	StgL	StyL	NFA	CD/NFA	AntL/StaL	StgL/StyL
Beira Rio 8	43.37 aA	1.87 aA	12.40 aA	21.95 aA	11.89 aA	15.79 aA	8.98 aB	22.88 aA	5.6 abA	7.74 aA	0.75 bA	0.39 abB
Clementino	39.89 aA	1.84 aA	11.15 aA	20.10 aA	10.64 aA	14.12 abA	7.39 bB	23.12 aA	5.8 aA	6.88 bA	0.75 bB	0.32 bcB
L80	33.18 bA	1.58 bA	10.93 aA	17.34 bA	11.50 aA	13.52 bA	7.34 bB	18.15 bA	5.9 aA	5.63 cB	0.85 abB	0.40 aB
A1	31.73 bcA	1.60 bA	11.62 aA	16.22 bA	10.97 aA	12.68 bA	5.54 cB	21.85 aA	5.4 bA	5.83 cA	0.86 abA	0.25 cB
Arara	28.33 cA	2.00 aA	7.23 bA	13.48 cA	9.05 bA	9.16 cA	4.29 cB	14.76 cA	5.0 cA	5.67 cA	0.98 aA	0.29 bcB
High-altitude												
Beira Rio 8	35.97 aB	1.51 aB	10.00 aB	18.94 aB	9.53 aB	12.54 aB	10.29 aA	16.32 abcB	5.2 bB	6.92 aB	0.76 cA	0.63 aA
Clementino	33.42 aB	1.47 aB	10.16 aA	15.66 bcB	9.71 aB	10.57 abB	7.75 bA	17.40 abB	5.6 aA	5.93 bcB	0.92 abA	0.45 bcA
L80	33.06 aA	1.45 aB	10.31 aA	16.46 bA	10.79 aB	10.83 abB	8.56 bA	18.66 aA	5.3 bB	6.24 abA	1.00 aA	0.46 abA
A1	26.73 bB	1.16 bB	9.00 aB	13.86 cdB	9.87 aB	12.13 aA	6.48 cA	15.39 bcB	5.2 bB	5.12 cB	0.83 bcA	0.42 cA
Arara	26.32 cA	1.52 aB	7.01 bA	12.56 dA	8.46 bB	9.46 bA	5.83 cA	13.80 cA	5.0 bA	5.27 cA	0.89 abcA	0.42 bcA

For each parameter, different low-case letters indicate statistical differences between genotypes, within each environment (altitudes), while different upper-case letters indicate statistical differences between the two environments for each genotype.

The mean tube diameter (TD) ranged from 1.58 to 2.00 mm at low-altitude and from 1.16 to 1.52 mm at high-altitude (Table 1). At low-altitude, Arara, Beira Rio 8 and Clementino had greater TD than the two genotypes (Table 1, Figure 3), while at high-altitude, only the A1 showed a smaller value than all the others. Significantly greater TD values were observed in low- than in high-altitude, for all genotypes.

The mean tube length (TL) ranged from 7.23 to 12.40 mm at low-altitude and from 7.01 to 10.31 mm at high-altitude (Table 1). Arara significantly differ from the other genotypes in both altitudes. Again, high-altitude values are always lower than in low-altitude, although significantly only for Beira Rio 8, and A1.

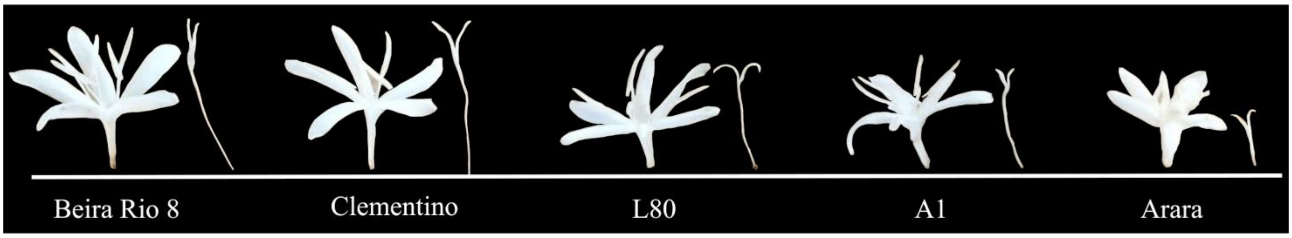

Figure 3- Flower morphology of five coffee genotypes from the low-altitude. The Beira Rio 8, Clementino, L80 and A1 genotypes belong to *C. canephora*, whilst the Arara genotype to *C. arabica.*

Among all morphological traits of flowers evaluated, the petal length (PL) was the one with greatest variability among genotypes, only in high-altitude. The mean petal length (PL) ranged from 13.48 to 21.95 mm at low-altitude and from 12.56 to 18.94 mm at high-altitude (Table 1). At low-altitude, Beira Rio 8 and Clementino showed as well the highest PL, followed by L80 and A1 with an intermediate size, and Arara with the smallest value. At high-altitude, Beira Rio 8 had higher PL and was significantly different from the others. Additionally, L80 differed from A1 and Arara, while Clementino differed only from Arara. As for the previous traits, PL demonstrated greater values under low-altitude, significantly for Beira Rio 8, Clementino and A1.

The mean anther length (AntL) ranged from 9.05 to 11.89 mm at low-altitude and from 8.46 to 10.79 mm at high-altitude (Table 1). Consistently, Arara showed the shortest AntL values in either altitude. No significant differences were observed among the *C. canephora* genotypes at either altitude. For all genotypes significantly greater values were observed under low- than high-altitude.

The mean stamen length (StaL) ranged from 9.16 to 15.79 mm at low-altitude and from 9.46 to 12.54 mm at high altitude (Table 1). At low-altitude, Beira Rio 8 and Clementino exhibited greater StaL values, while Clementino was also comparable to L80 and A1, which showed intermediate values. Arara displayed the smallest StaL value. At high-altitude, Beira Rio 8, Clementino, L80 and A1 exhibited greater StaL values, with Clementino and L80 being comparable to Arara. This trait consistently displayed greater values at low-altitude compared to high-altitude, with significant differences observed for Beira Rio 8, Clementino, and L80.

The mean lobes length (StgL) ranged from 4.29 to 8.98 mm at low-altitude and from 5.83 to 10.29 mm at high-altitude (Table 1). At both altitudes, Beira Rio 8 showed the greatest StgL values, followed by Clementino and L80 with intermediate values, and A1 and Arara with the smallest values. In contrast with all other traits, larger StgL values were obtained at high- than low-altitude.

The mean style length (StyL) ranged from 14.76 to 23.12 mm at low-altitude and from 13.80 to 18.66 mm at high-altitude (Table 1). At low-altitude, Beira Rio 8, Clementino and A1 showed greater StyL values, followed by L80 with intermediate value and Arara with the smallest value. At high-altitude, Beira Rio 8, Clementino and L80 showed greater StyL values, while A1 and Arara showed the smallest values. The values of StyL in low-altitude were greater than high-altitude, with significant differences observed for Beira Rio 8, Clementino and A1.

The mean number of floral appendages (NFA) ranged from 5.0 to 5.9 at low-altitude and from 5.0 to 5.6 at high-altitude (Table 1). At low-altitude, the Beira Rio 8, Clementino and L80 showed greater NFA values, but Beira Rio 8 did not different from A1, and Arara had the smallest value. At high-altitude, only the Clementino showed greater NFA value than all the others. The values of NFA in low-altitude were greater than high-altitude, with significant differences observed for Beira Rio 8, L80 and A1.

At low-altitude, the CD/NFA ratio, Beira Rio 8 showed grater value, followed by Clementino with an intermediate, and L80, A1 and Arara with the smallest values (Table 1). At high-altitude, the CD/NFA ratio, Beira Rio 8 and L80 showed greater values, while A1 and Arara showed smallest values, and Clementino did not differed of L80, A1 and Arara. Among altitudes, the values of CD/NFA in low-altitude were greater than high-altitude in the genotypes Beira Rio 8, Clementino and A1.

At low-altitude, the AntL/StaL ratio exhibited the highest values in L80, A1, and Arara, while L80 and A1 did not differ significantly from Beira Rio 8 and Clementino (Table 1). The size of the anther and stamen in Arara were similar, measuring 0.98. At high-altitude, Clementino, L80, and Arara displayed the highest Ant/StaL values, while Beira Rio 8 and A1 exhibited the smallest values. This ratio was comparable between altitudes, except for the higher values observed at high altitude in Clementino and L80.

Both at low and high-altitudes, Beira Rio 8 and L80 presented the highest values of the StgL/StyL ratio (Table 1). However, at low-altitude, the StgL/StyL ratio of Beira Rio 8 differed only from A1, while at high-altitude, Beira Rio 8 differed from Clementino, A1, and Arara. High-altitude conditions resulted in larger values of this ratio across all genotypes.

Altogether, most parameters showed that larger flowers are present at low-altitude, with exception for the StgL, StgL/StyL and AntL/StaL ratios that tended to increase with increasing altitude (Table 1). In general, a reduction in CD, TD, TL, PL, AntL, StaL, StyL, NFA and CD/NFA of all genotypes was observed with increasing altitude, with L80 and Arara not always following the general rule (interaction G x E, Table 2). Additionally, Arara genotype presented the similar values for CD, TL, PL, StaL, StyL, NFA, CD/NFA and AntL/StaL, explaining the G x E interactions.

The CVe for all the traits presented low to medium values, ranging from 2.37 to 12.74%, whereas the genetic coefficient of variation (CVg) ranged from 5.0 (NFA) to 27.74% (StyL) (Table 2). The values of the variation index (VI) showed that in the CD, PL, and StyL traits, the variations due to genetic causes were more than three times higher than those due to environmental causes. The CD, PL, and StyL traits had the highest genotypic determination coefficients (H^2), while AntL, AntL/StaL and StgL/StyL had the lowest (H^2), indicating that CD, PL and StyL were less influenced by the environment than AntL, AntL/StaL and StgL/StyL.

Table 2- Analysis of variance, means (n = 30), and estimation of genetic parameters for: corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), petal length (PL, mm), anther length (AntL, mm), stamen length (StaL, mm), stigmatic lobes length (StgL, mm), style length (StyL mm), number of floral appendages (NFA, petals or stamens), CD/NFA, AntL/StaL, StgL/StyL. E, environment; G, genotype; H^2, genotypic coefficient of determination; CVe (%), environmental coefficient of variation; CVg (%), genetic coefficient of variation; VI, variation index.

	Mean square								
Variables	G	E	G x E	Residue	Mean	H^2	CVe (%)	CVg (%)	VI (%)
CD	156**	132**	14.04**	2.49	33.20	0.91	4.75	15.23	3.20
TD	0.14**	0.96**	0.03**	0.005	1.60	0.81	4.42	9.20	2.08
TL	15.98**	14.14**	1.75*	0.44	9.98	0.85	6.64	16.12	2.43
PL	47.56**	40.46**	3.36**	0.68	16.66	0.92	4.96	16.78	3.38
AntL	4.90**	9.64**	0.76^{ns}	0.44	10.24	0.63	6.48	8.42	1.30
StaL	18.28**	28.48**	4.44**	0.69	12.09	0.81	6.90	14.17	2.05
StgL	18.9 **	8.60 **	0.31^{ns}	0.74	7.25	0.80	11.89	24.01	2.02
StyL	156**	132**	14.04**	2.49	18.24	0.91	8.66	27.74	3.20
NFA	0.45**	0.59**	0.07**	0.01	5.41	0.82	2.37	5.00	2.11
CD/NFA	3.65**	1.53**	0.58*	0.13	7.24	0.82	5.84	12.51	2.14
AntL/StaL	0.03**	0.01*	0.02**	0.003	0.86	0.62	6.51	8.24	1.27
StgL/StyL	0.16**	0.03**	0.01^{ns}	0.002	0.40	0.63	12.74	16.54	1.30

Significance of the two-way ANOVA p-values: ‘**’<0.01, ‘*’<0.05, ‘^{ns}’ not significant were indicated.

Simple and complex G × E interactions

Simple G x E interactions were observed only in CD, indicating variations due to genotype impacts in this floral trait (Figure 4a). The complex interactions were detected for TD, TL, PL, StaL, StyL, NFA, AntL/StaL, and CD/NFA, ed (Figures 4b, c, d, e, f, g, h, i). Generally, Clementino, L80, and A1 genotypes responded most divergently to each other in a great number of flower traits at different altitudes. The Arara genotype was stable for most flower traits at two altitudes, although it contributed to the occurrence of complex interactions in AntL/StaL and CD/NFA.

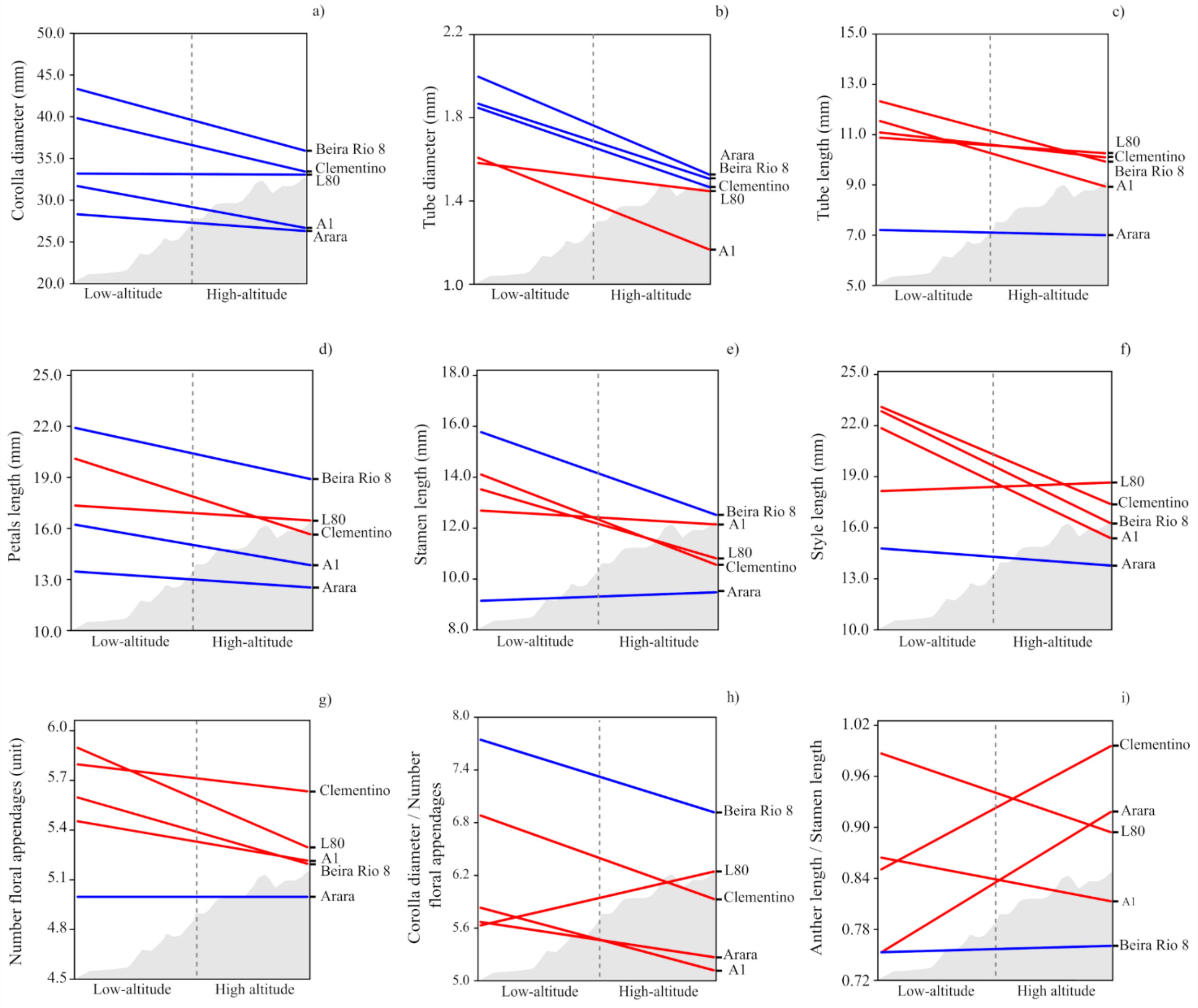

Figure 4- Simple and complex interaction effects between five coffee genotypes and two altitudes. The Arara genotype belongs to the *C. arabica*, and the Beira Rio 8, Clementino, L80 and A1, genotypes belong to the *C. canephora*. Responses of nine morphological flower traits (a-i) were compared under two altitudes, low (36 m.a.s.l.) and high (1100 m.a.s.l.). Blue line indicates simple type G x E interaction and red line indicates complex type G x E interaction.

Correlations among the floral traits at two distinct altitudes

A higher number of significant correlations of morphological traits of flowers were found at low-altitude (Figure 5a) than at high-altitude (Figure 5b), either positive or negative. At both altitudes, CD and PL were positively correlated with NFA. High and positive correlations of TL with StyL and low and negative correlations of TD with all other traits at both altitudes, were found. At low-altitude, all traits were significantly negatively correlated with AntL/StaL (with exception of TD). At high-altitude, only the correlation of StaL with AntL/StaL was significantly high and negative, indicating that when StaL increased, AntL in relation to the stamen decreased.

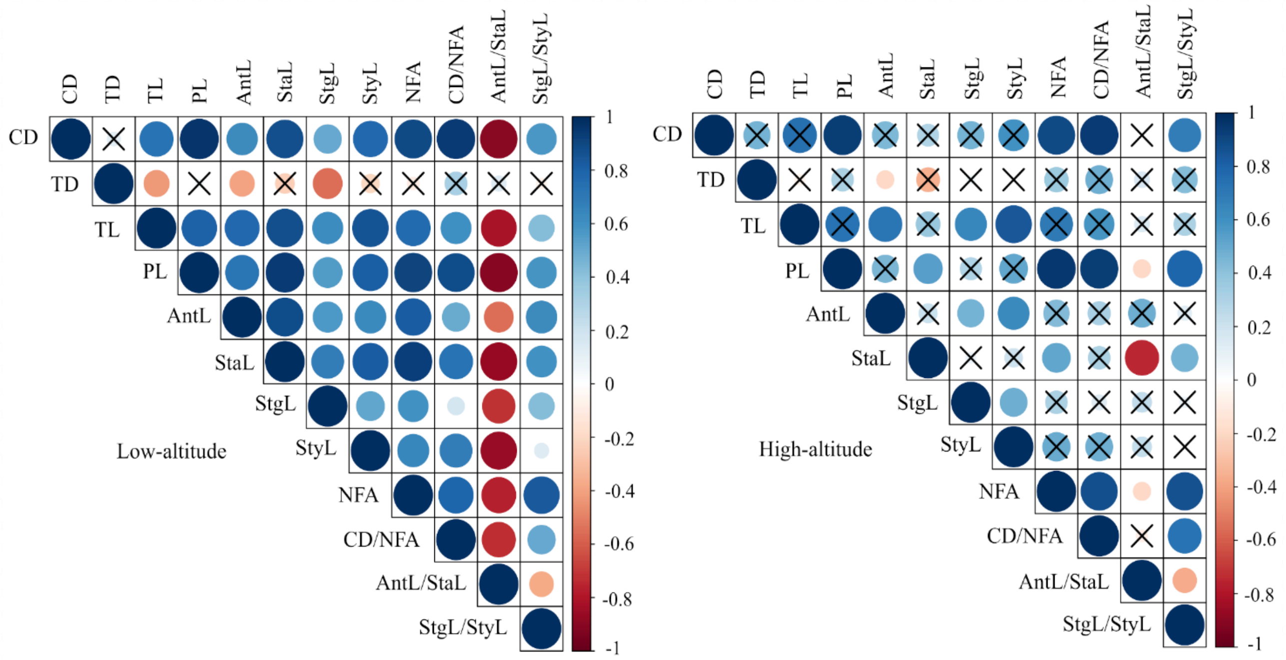

Figure 5- Graphical presentation of coefficients (values corresponding to circle size and color intensities) and *p*-values (significant without a cross, n=15, $p < 0.05$) for correlations between morphological traits of coffee flower at low-altitude and high-altitude. Corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), petal length (PL, mm), anther length (AntL, mm), stamen length (StaL, mm), stigmatic lobes length (StgL, mm), style length (StyL mm), number of floral appendages (NFA, petals or stamens), CD/NFA, AntL/StaL, StgL/StyL.

DISCUSSION

The first novelty of this study is that among evaluated genotypes, only the *C. arabica* cv. Arara presented pentamerous flowers, whereas *C. canephora* plants showed a certain degree of prevalence of six stamens and petals, but with differences between genotypes within each altitude (denoting a different genetic response), and also between altitudes for all genotypes, although significantly for Beira Rio 8, L80 e A1 (thus reflecting an environmental dependence), with greater values in low-altitude (Table 1). The number of flower petals is usually resistant to genetic and environmental variations, and rarely varies within one species (Monniaux et al., 2018). The flowers of *C. arabica* and *C. canephora* are usually composed of five petals to which the stamens are connected (Charrier and Berthaud, 1985), but both species can also have six petals per flower (Prado et al., 2019). It has not yet been reported what determines the number of petals and stamens in *Coffea* spp. but in *Rosa wichurana* Crép. (Chao et al., 2021) and in *Cardamine hirsute* L. (Pieper et al., 2015), the number of petals is determined by many genes of small effect, which is additionally impacted by temperature seasonal changes in *C. hirsuta* (McKim et al., 2017).

In *C. canephora* and *C. arabica* plants, the size of flower structures size increased at low-altitude, with exception of the the stigmatic lobes length and the ratio between stigmatic lobe length and style length and partly the AntL/StaL that show a reverse pattern of variation (Table 1). This is supported by the fact that growth and development of plants is influenced by temperature, precipitation, and light intensity, which are factors commonly associated with altitude (Körner, 2007; Hautier et al., 2009). Our findings are fully in line with the reports that show that flower traits can be significantly affected by altitude, as the width and length of the petal of *Impatiens textori* Miq., *I. noli-tangere* L. (Hattori et al., 2016), the sepal size, stigma length and number of anthers per flower of *Populus cathayana* Rehder (He et al., 2017), the sepal length and width of *Trollius ranunculoides* Hemsl. (Zhao and Wang, 2018). Therefore, altitude (likely closely associated with temperature) greatly alters the flower morphology of *C. canephora* and *C. arabica*, with likely impact on fertility and yield (Drinnan and Menzel, 1995; Camargo and Camargo, 2001).

The only *C. arabica* genotype showed a great stability for most of the studied flower morphological traits (Figure 4), what somewhat agrees with a more global high resilience (up to 37 °C) at leaf level (Rodrigues et al., 2016; Scotti-Campos et al., 2019), and because a self-pollinating species (as *C. arabica*) tend to have smaller flowers than cross-pollinating species (Krizek and Anderson, 2013). Although the Arara genotype is still under evaluation, it is indicated for high-altitude regions in the Brazilian state of Espírito Santo, as it is tolerant to low temperatures, a trait associated with these environments (Ferrão et al., 2021a). Additionally, Arara plants has shown high drought tolerance (Matiello et al., 2010), overall indicating this genotype great ability to acclimate to different environments.

On the other hand, *C. canephora* plants showed greater variability. Beira Rio 8 stood out at both altitudes, due to their highest values for all traits related to the corolla (corolla diameter, tube diameter, tube length and petal length), thus indicating bigger flowers (Table 1). Flower size is related to pollination efficiency, as it indicates the potential amount of flower nectar reward for insect pollinators (Fenster et al., 2006; Delgado et al., 2023). Therefore, the Beira Rio 8 genotype may be preferred by bees than genotypes with less floral display. Furthermore, this genotype showed long stigmatic lobes and styles at both altitudes (Table 1). The stigmatic lobes are the pollen receptive structures (Edlund et al., 2004), therefore, the larger the stigmatic lobe, the greater the contact surface to receive pollen from other plants (Montgomery et al., 2011). Long styles may also contribute to greater exposure of the stigmatic lobe, thus ensuring cross-pollination (Arroyo et al., 2002). Therefore, the dimensions of these traits can make Beira Rio 8 a good candidate for female parent.

Changes in environmental factors such as temperature, precipitation, and light intensity can impact the allocation of resources and energy to different plant structures, including flowers (Fabbro and Körner, 2004). To produce larger flowers, there is an increase in the physiological cost to the plant, and therefore, in environments with low resource availability or under environmental stress, the production of larger flowers may be compromised (Galen, 2000; Carroll et al., 2001). Flowering in low-altitude of *C. canephora* and *C. arabica* occurred during the Brazilian winter, while at high-altitude, flowering occurred at the end of winter and beginning of spring. However, the minimum temperature at high-altitude during flowering was lower than at low-altitude (Figure 1). This may have influenced the size of the flowers because although there are no estimates for ideal minimum and maximum temperatures during flowering, low minimum temperatures during flowering have been related to reduced productivity of *C. canephora* (Kath et al., 2020). Therefore, the favorable climatic conditions for the cultivation of *C. canephora* at low-altitude may have contributed to better plant development and, consequently, to the production of bigger flowers than at high-altitude.

The heightened sensitivity of *C. canephora* to environmental pressures associated with high altitude, such as increased susceptibility to ultraviolet radiation and low temperatures compared to *C. arabica* (Partelli et al., 2009; Martins et al., 2019; Bernado et al., 2021), led us to hypothesize that there would be greater variability in floral morphology among *C. canephora* genotypes at higher altitudes, driven by environmental pressures. However, the results did not support this hypothesis. Conducting studies with a larger number of genotypes across a broader range of environments (varying elevation levels) could provide further insights into the validity of this hypothesis in future research.

The specific changes observed in the morphology of *Coffea* spp. flowers may be related to the genetic traits of the evaluated plants (Silva et al., 2021), but also to environmental influence. To infer experimental accuracy and understand the contribution genetic causes to these traits variability, genetic parameters have to be estimated (Cruz et al., 2014). The CVg values, which measures the proportion of total variance due to genotypic variation (Cruz et al., 2014), were higher than the corresponding environmental coefficients of variation in each parameter. This points that the variations due to genetic basis surpassed those attributed to environmental effects, what in turn was directly reflected in the values of the genotypic determination coefficient (H^2). The high values of H^2 for the studied traits indicated a high degree of certainty in inferring genotypic values based on these phenotypic markers (Ramalho et al., 2016). Therefore, the morphological traits of coffee flowers may represent additional options for phenotypic markers in studies of genotype distinction, as also reported for other plant traits (Ferrão et al., 2021b; Schmidt et al., 2022).

The presence of G x E interactions (Figure 4) indicated inconsistent responses of genotypes at different altitudes (Baye et al., 2011). Simple type interactions related to corolla diameter indicated that the magnitude of genotype performance for this trait was reduced or increased from one altitude to another. The predominance of complex type interactions in most traits revealed that there are genotypes with performance more adapted to a specific altitude than others, which can make selection and recommendation of these genotypes at diverse altitudes/temperatures difficult (Aragão et al., 2015). It was expected that the performance of the Arara genotype for floral morphological traits would be better at high-altitude than at low-altitude, since that is the usually recommended environment for *C. arabica* cultivation (Ferrão et al., 2021a). However, the results of G x E interaction revealed that there was stability of this genotype in floral morphological traits, likely linked to its mentioned tolerance to environmental stresses as cold and drought (Matiello et al., 2010; Ferrão et al., 2021), which greatly share the acclimation mechanisms with heat stress (here represented by low-altitude) (Rodrigues et al., 2016; Dubberstein et al., 2020).

There was higher number and intensity of correlations at low-altitude than at high-altitude (Figure 5). However, it is worth noting that there were traits with consistent correlation values at both altitudes. For example, corolla diameter and petal length were positively correlated with the number of floral appendages, and corolla tube length was positively correlated with style length at both altitudes. The high correlations between the latter traits indicate that coffee flowers with long corolla tubes would also have long styles, which is consistent in *Coffea* spp. observations (Prado et al., 2019; Silva et al., 2021). At high-altitude, as stamen length increased, the anther length relative to stamen length decreased (Figure 5). The stamen consists of an anther and a filament (Scott et al., 2004). This means that the increase in the length of the stamen occurred associated with the increase in the length of the stamen filament. This structure supports the anther, transmits water and nutrients to the anther and positions it to aid pollen dispersal (Scott et al., 2004). Therefore, it is possible that as the stamen grew, the anther decreased proportionally to maintain a balanced structure. Anthers of shorter stamens tend to release fewer quantities of pollen grains at each time interval than anthers of longer stamens (Brito et al., 2021). Both at low and high-altitude, the Beira Rio 8 and Clementino genotypes presented long stamens (Table 1), which suggests that this genotype has a greater capacity to disperse pollens and be a good candidate as a male parent of *C. canephora.*

This study was crucial to understand the effect of altitude differences on the floral traits of *Coffea* spp. We discovered that the specific changes observed may be related to the genetic traits of the evaluated plants, the different environmental conditions, and/or adaptations of the plants to these conditions at each altitude. Therefore, conducting studies on morphological traits of flowers at a greater number of altitudes can provide better insights into the potential use of these traits in studies of adaptability and stability of *Coffea* spp. genotypes to different environmental conditions, and thus contribute to genetic improvement efforts to achieve resilience of coffee cultivation in the current scenarios of climate change.

CONCLUSION

It was shown that the number of petals and stamens varied among *C. canephora* genotypes, in contrast, there was no variation in the number of petals and stamens in *C. arabica*, supporting our first hypothesis. The overall size of the flowers of *C. canephora* and *C. arabica* was larger at low- than at high-altitude. However, the stigmatic lobes length and the ratio between stigmatic lobe length and style length and partly the AntL/StaL were greater at high- than at low-altitude. The studied altitude difference resulted in variations in the morphological traits of coffee flowers, thus proving our second hypothesis, but there was no greater variability in floral morphology among *C. canephora* genotypes at high- than at low-altitude rejecting our third hypothesis. The stability in the Arara genotype (*C. arabica*) revealed that the variability in morphological traits of the flowers between the two altitudes of *C. arabica* was lower than in *C. canephora*, thus proving our fourth hypothesis. The genetic and environmental flower responses, and specific environmental adaptations must be observed in the future studies, if possible, including a bigger number of altitudes, and as the relation of the variation in flower morphology to the final yield.

Acknowledgments

We thank to the Fundação de Amparo à Pesquisa e Inovação do Espírito Santo - FAPES (Proc. 2022-WTZQP for Fábio Luiz Partelli and Proc. 2022-M465D for Miroslava Rakocevic and Fábio Luiz Partelli), Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Proc. 309535/2021-2 for Fábio Luiz Partelli), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Proc. 88882.385374/2019-01 for Larícia Olária Emerick Silva). We appreciate the support done by farmer Júlio Aguilar in the logistic of experimentation.

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CHAPTER 3: TOWARD A MINIMUM NUMBER OF KEY FLOWER TRAITS IN STUDIES OF *Coffea* spp. PHENOTYPE VARIABILITY

ABSTRACT: The identification of key traits in optimization of phenotypic variability studies represents time savings in development of new cultivars. To attain this purpose, it was hypothesized that: 1) different coffee genotypes presented morphological variability in flower size and form, 2) various coffee flower size and form traits were correlated, 3) the exclusion of correlated flower morphological traits can facilitate future flower evaluations, without compromising the inference of phenotypic variability. A total of 36 *Coffea canephora* and the two *C. arabica* genotypes were studied. Nine floral traits were measured in 760 flowers. It was proven that the flower phenology can be used to differentiate *Coffea* spp. genotypes. Some *C. canephora* genotypes showed the largest floral structures (Beira Rio 8, Guarani, 8R, and Clementino), while the genotypes with the smallest floral structures were genotypes belonging to the same species (Z8, Verdim D, Ouro Negro 1, Tardio C, NV8, Ouro Negro 2, Bamburral, LB1, and Peneirão). Among the nine evaluated floral traits, the five - petal length, anther length, stamen length, style length, and stigmatic lobe length - were strongly correlated with the traits of the greatest weight for data variability. The correlated traits had little contribution to the data variability, permitting that their exclusion had not compromised the inference of morphological flower variability in *Coffea* spp. genotypes. This suggested that studies aiming at differing the *Coffea* spp. genotypes based on floral structures may consider the four studied traits, tube length, tube diameter, corolla diameter, and the number of floral appendages (petals or stamens). The reduction of nine to these four traits can optimize time and efficiency in data collection. In the future works, the association of selected flower morphological traits with traits related to *Coffea* spp. production could additionally move ahead the process of coffee breeding programs.

Keywords: Arabica coffee, Conilon coffee, Correlations, Floral characteristics, Genetic variability.

CAPÍTULO 3: RUMO A UM NÚMERO MÍNIMO DE CARACTERES PRINCIPAIS DE FLOR EM ESTUDOS DE VARIABILIDADE FENOTÍPICA DE *Coffea* spp.

RESUMO: A identificação de caracteres-chave na otimização de estudos de variabilidade fenotípica representa economia de tempo no desenvolvimento de novas cultivares. Para atingir esse objetivo, foi levantada a hipótese de que: 1) diferentes genótipos de café apresentam variabilidade morfológica no tamanho e na forma da flor, 2) vários caracteres de tamanho e forma da flor do café foram correlacionados, 3) a exclusão de caracteres morfológicos da flor correlacionados pode facilitar futuras avaliações florais, sem comprometer a inferência da variabilidade fenotípica. Um total de 36 genótipos de *Coffea canephora* e os dois de *C. arabica* foram estudados. Nove caracteres florais foram medidos em 760 flores. Foi comprovado que a fenologia da flor pode ser utilizada para diferenciar genótipos de *Coffea* spp. Alguns genótipos de *C. canephora* apresentaram as maiores estruturas florais (Beira Rio 8, Guarani, 8R e Clementino), enquanto os genótipos com as menores estruturas florais foram genótipos pertencentes à mesma espécie (Z8, Verdim D, Ouro Negro 1, Tardio C, NV8, Ouro Negro 2, Bamburral, LB1 e Peneirão). Dentre os nove caracteres florais avaliados, os cinco - comprimento da pétala, comprimento da antera, comprimento do estame, comprimento do estilete e comprimento do lóbulo estigmático - foram fortemente correlacionados com os caracteres de maior peso para a variabilidade dos dados. Os caracteres correlacionados, pouco contribuíram para a variabilidade dos dados, permitindo que sua exclusão não tenha comprometido a inferência da variabilidade morfológica das flores em genótipos de *Coffea* spp. Isso sugere que estudos visando diferenciar genótipos de *Coffea* spp. baseados em estruturas florais podem considerar os quatro caracteres estudados, comprimento do tubo, diâmetro do tubo, diâmetro da corola e o número de apêndices florais (pétalas ou estames). A redução de nove para esses quatro caracteres pode otimizar tempo e eficiência na coleta de dados. Em trabalhos futuros, a associação de caracteres morfológicos florais selecionados com caracteres relacionados a produção de *Coffea* spp. poderá, adicionalmente, avançar no processo de programas de melhoramento do café.

Palavras-chave: Café arábica, Café conilon, Correlação, Caracteres florais, Variabilidade genética.

INTRODUCTION

Originating in the African continent, the genus *Coffea* (coffee) includes more than 130 species belonging to the family Rubiaceae (Davis, 2021). Among *Coffea* species, only two have high commercial value - C. *arabica* L. and *C. canephora* Pierre ex Froehner. Coffee producing countries are dispersed in different tropical and subtropical regions of the world. The regional genetic diversification of coffee has high commercial value, being of additional importance for genetic resource conservation and research development (Macedo et al., 2021; Ferrão et al., 2021).

C. arabica is an allotetraploid (2n=4x=44) and autogamous species, with an outcrossing index of around 10% (Ferreira et al., 2020). Studies about the genetic diversity of *C. arabica*, which use morphological characteristics (Chidoko et al., 2022) and molecular markers (Antony et al., 2001; Sousa et al., 2017), show that the genetic base of this species' commercial cultivars is narrow (Scalabrin et al., 2020). On the other hand, the *C. canephora* is diploid (2n=2x=22), allogamous, and exhibits gametophytic self-incompatibility (Moraes et al., 2018), which promotes its great genetic diversity (Zaidan et al., 2023). *C. canephora* has two botanical varieties, Conilon and Robusta. Both varieties are cultivated in Brazil, with Conilon being predominant in the state of Espírito Santo and Robusta being predominant in the state of Rondônia (Partelli et al., 2020; Espindula et al., 2022). Conilon plants exhibit shrub-like growth, elongated leaves, early fruit ripening, drought tolerance, and higher susceptibility to pests and diseases (Vieira et al., 2013; Oliveira et al. 2018; Souza et al. 2021). Robusta plants have upright growth, larger leaves compared to Conilon, late fruit ripening, lower drought tolerance, and higher tolerance to pests and diseases (Oliveira et al., 2018; Souza et al., 2021). Under non-limiting conditions of minerals, water, and light, the Robusta assimilates more CO_2 and produces more plant biomass than the Conilon (Rakocevic et al., 2023).

The coffee flowers are hermaphroditic, and grouped in inflorescences that develop from serial buds, located in the leaf axils, mainly on plagiotropic branches (Hallé et al., 1978; Barthélémy and Caraglio, 2007; Rakocevic et al., 2021). The flowers have a short pedicel, an inferior ovary, and a style with the two stigmatic lobes. Each flower has five stamens attached to the corolla tube, with anthers longer than the filaments (Charrier and Berthuad, 1985). In *C. canephora* and *C. arabica*, the flowers may have five or six petals (Prado et al., 2019). Although the morphology of coffee flowers is well-defined, recent studies show that the size of floral structures can vary both among genotypes of the same species (Silva et al., 2021) and between *C. arabica* and *C. canephora* (Prado et al., 2019).

Morphological traits of flowers are used to distinguish the genotypes in various species. For example, flower length, style thickness, and number of stigmatic lobes are the traits used to distinguish pitaya genotypes (Martinez et al., 2005); flower diameter and number of petals are used to distinguish commercially valuable apple cultivars (Goncharovska et al., 2022); flower length and width are used to distinguish genotypes and flower types of pomegranates (Ikram et al., 2022), among others. To distinguish genotypes of Robusta coffee, corolla tube length, corolla tube diameter, number of stamens, lobe length, stamen length, anther length, style length, and stigmatic lobe length are used (Silva et al., 2021). Despite some examples, the morphological flower trait definitions in studies of coffee genotypes are scarce, even though flowers play a crucial role in crop yield determination and species maintenance through seed dispersal (Lazaro et al., 2015; Bailes et al., 2015).

The flowering of coffee in non-equatorial regions, such as the main coffee-producing areas in Brazil, occurs in different periods (from August to November) in two or more unsynchronized flushes (Rena and Barros, 2004). The duration of coffee flowers after opening is approximately three days, with flowers becoming less turgid and starting to fall on the third day (Souza et al., 2003; Ferrão et al., 2017). This short flower lifespan is one of the limiting factors for the exploration of flower traits in coffee research, making it impractical to evaluate many genotypes within one single flowering flush. Therefore, the question is how to increase the efficiency in the evaluation of coffee flower morphology in inclusion of these traits in phenotypic variability. A principal component analysis (PCA) can be useful in the process of trait selections because this method enables identifying the redundant traits, those that are correlated with other traits present in the analysis (Regazzi, 2002). Inter-correlated flower morphological traits have been reported in apple trees (Zhou et al., 2021), in rice (Khumto et al., 2018), and in pomegranate (Cizmovic et al., 2016). For traits with high heritability, the non-phenotypic correlation refers to likely non-genotypic correlation, indicating that the expression of non-correlated traits is the result of the action of genes located in distinct genome regions (Chen et al., 2020).

The morphological traits of coffee flowers have high heritability (Silva et al. 2021), indicating that the identification of key traits would permit the optimization of phenotypic variability study, with essential time savings in the process of new cultivars development. To attain this purpose, it was hypothesized that: **1)** different coffee genotypes would present morphological variability in flower size and form, **2)** various coffee flower size and form traits are correlated, **3)** the exclusion of correlated flower morphological traits can facilitate future flower evaluations, without compromising the inference of phenotypic variability.

MATERIAL AND METHODS

Plant material and characterization of the experimental area

In this study were evaluated 36 genotypes of *C. canephora* plus two cultivars of *C. arabica* that are part of the coffee germplasm bank, coordinated by the 'Núcleo de Excelência de Pesquisas em Café Conilon' of the Federal University of Espírito Santo (UFES), Brazil. Among the 38 genotypes (Table 1), the genotypes 1 to 10 were planted in June 2018, the genotypes 11 to 30 were planted in April 2019, and the genotypes 31 to 38 were planted in March 2021. A space of 2 m was adopted among rows and 1 m among plants, equivalent to a planting density of 5000 plants ha^{-1}. The coffee orchard was managed to maintain two stems (orthotropic axes) per plant.

Table 1 Names and internal number attributed to 38 *Coffea* spp. genotypes evaluated in the experiment.

Genotype nº	Name	Genotype nº	Name	Genotype n^{c}	Name
1	Pirata	14	AD1	27	K61
2	Verdim R	15	Graudão HP	28	JC JM
3	Bamburral	16	AP	29	Arara
4	A1	17	L80	30	785/15 Vermelho
5	Clementino	18	Peneirão	31	Tardio V
6	Beira Rio 8	19	Z21	32	Z39
7	P1	20	Ouro Negro	33	Z18
8	Verdim TA	21	Tardio C	34	Z36
9	NV2	22	P2	35	Encapa 02
10	NV8	23	LB1	36	8R
11	Bicudo	24	Verdim D	37	Guarani
12	CH1	25	Ouro Negro 1	38	22R
13	Imbigudinho	26	Ouro Negro 2		

The genotypes 29 and 30 are two *C. arabica* cultivars (‘Arara’ and ‘785/15’), while all other genotypes are of *C. canephora.* Genotypes 1-6 belong to the cultivar ‘Tributun’ (Partelli et al., 2020); genotypes 7-10 belong to cultivar ‘Andina’ (Partelli et al., 2019); genotypes 13, 14, 16, 18, 22, and 23 belong to the cultivar ‘Monte Pascoal‘ (Partelli et al., 2021a); genotypes 15, 21, 31, 34, and 35 belong to cultivar ‘Salutar’ (Partelli et al., 2021b); genotypes 11 and 17 belong to cultivar ‘Plena’ (Partelli et al., 2022a); genotype 20 belong to cultivar ‘Magnus Grano’ (MAPA, 2023), genotype 37 belong to cultivar ‘Forte Guarani’ (Partelli et al., 2022b). Genotypes 36 and 38 are grown in Rondônia, Brazil (Espindula et al., 2022). The genotypes 19, 32, and 33 were selected by one local producer by seed propagation, Nova Venécia, ES, Brazil (Partelli et al., 2022c). The genotypes 12, 25, and 26 were vegetatively propagated by one local producer from Aracruz, ES, Brazil.

All genotypes were grown in the municipality of São Mateus (latitude 18º 42’ 58” S, longitude 39º 51’ 32” W and 36 m.a.s.l.), state of Espírito Santo, Brazil. The climate is tropical, Aw savanna type by Köppen climate classification, with dry winter and rainy summer (Alvares et al., 2013). The mean annual temperature of the region is 24 ºC and precipitation is approximately 1.370 mm (INMET, 2021). The entire experimental area was benefited by drip irrigation. The irrigation hoses were located 5 cm away from the coffee trunk, in the direction of the planting rows, and the emitters were spaced every 0.5 m. The fertilizations were carried out according to the soil and productivity analysis (Paye et al., 2019). Plant needs and phenological stages were also considered, using around 300 - 500 of N, 50 - 80 of P_2O_5 and 200 - 400 K_2O_2 kg ha^{-1}, divided into six times each year, starting from flowering to early maturation phenophases.

Flower morphology measurements

The flower morphological evaluation was conducted from July to September 2021. A total of 20 flowers of each genotype (10 flowers per each of two blocks) were randomly collected from the 2nd branching order plagiotropic axes in the middle third of three plants per each genotype. The evaluations were performed in the morning, immediately after the flower opening. The evaluated traits of each flower were: corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), petal length (PL, mm), stamen length (StaL, mm), anther length (AntL, mm), stigmatic lobes length (StgL, mm), style length (StyL, mm), number of floral appendages (NFA) - petals and stamens - that always were strongly correlated, five and five, six and six (Figure 1a). These measurements were taken using a digital caliper with an accuracy of two decimals.

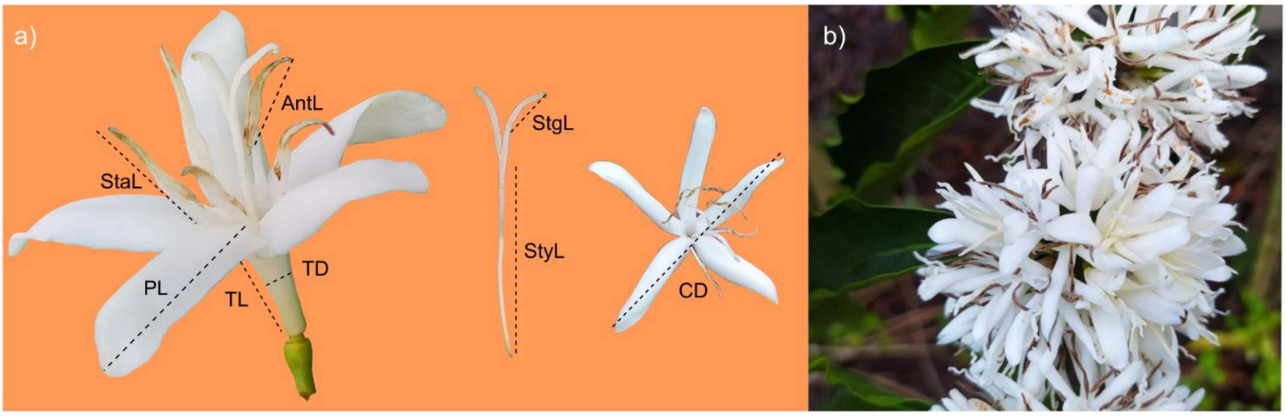

Figure 1- a) Flower morphological traits evaluated in *Coffea* spp.: Corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), petal length (PL, mm), anther length (AntL, mm), stamen length (StaL, mm), stigmatic lobes length (StgL, mm), style length (StyL mm), number of floral appendages (NFA, petals or stamens) and b) Flowers on *Coffea canephora* secondary axis.

Statistical analysis

The experimental design was a completely randomized block system with the two blocks. Flower collection was performed on three random plants in each block summing ten flowers per block. The 38 genotypes were considered as treatments. The data were firstly analyzed by the Shapiro-Wilk normality test and Bartlett's test for homogeneity of variance. Once the data met the mentioned assumptions, the one-way ANOVA by the F-test was performed ($n = 20$). After significant variability was identified among the genotypes by the ANOVA, the grouping of means was performed using the Scott-Knott method. From the means obtained for each genotype, the data were standardized and subjected to PCA (Principal Component Analysis). Based on the recommendations of Jolliffe (Jolliffe 1972, 1973), the traits whose principal component (PC) presented eigenvalue below 0.7 were discarded. Also, the process of trait discard consisted in the component corresponding to the lowest eigenvalue and discarding the flower traits associated with the highest weighting coefficient, as proposed by (Regazzi, 2002). After the discard, a new PCA was performed and the biplot with the first two PCs was constructed. To examine the possibility of traits, all trait exclusion, Pearson's correlation analysis and the correlations tested by the t-test were performed. All tests and correlations were performed at a 5% significance level with the R software (R Core Team, 2021), using the packages ‘ExpDes’ (Ferreira et al., 2021), ‘factoextra’ (Kassambara, 2020) and ‘psych’ (Revelle, 2022).

RESULTS

The *F*-test detected significant differences among the evaluated genotypes for all flower traits (Table 2). All traits showed low coefficient of variation (CV), except for the length of the stomatal lobe (StgL), which presented a moderate CV. This suggested that StgL was more impacted by environmental conditions than the other studied ones. Although with a high CV, the significant variation attributed to the genotype effect for this trait was detected. On the other hand, the number of floral appendages (NFA) showed reduced variation that was influenced by environment, with the lowest CV among all evaluated traits. The low variation related to environment plus a significant effect of the genotype variation source, indicated higher heritability for the NFA, indicating that this trait could be of interest for plant breeders.

Table 2- ANOVA, means (n = 20), for: Corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), petal length (PL, mm), anther length (AntL, mm), stamen length (StaL, mm), stigmatic lobes length (StgL, mm), style length (StyL mm), number of floral appendages (NFA, petals or stamens). CV (%), experimental coefficient of variation.

Flower traits	Mean Square			Mean	CV (%)
	Genotype	Block	Error		
CD	35.46 **	12.25	3.59	31.48	6.02
TD	0.09 **	0.02	0.01	1.53	7.10
TL	10.63 **	1.14	0.75	9.34	9.28
PL	13.95 **	4.59	1.70	16.6	7.85
AntL	2.62 **	1.12	0.87	9.86	9.46
StaL	6.01 **	0.00	1.25	11.82	9.45
StgL	4.33 **	2.82	0.90	18.99	14.99
StyL	16.74 **	3.06	1.31	6.31	6.03
NFA	0.13 **	0.07	0.01	5.23	1.31

** significant at <0.01 level according to the *F*-test.

The genotypes segregated in two groups of different means (‘a’ and ‘b’) based on anther length (AntL), three groups based on tube diameter (TD), stamen length (StaL) or and StgL, four groups based on corolla diameter (CD), tube length (TL), or petal length (PL), and five groups based on StyL or NFA (Table 3). The greatest variability among the genotypes (the greatest number of groups) was found in StyL and NFA, indicating their possible role in phenotyping.

The Clementino, Beira Rio 8, 8R, and Guarani genotypes formed the group with the highest means for CD (39.25-43.37 mm) (Table 3). The group with the highest means of TD (1.76-1.96 mm) was formed by the genotypes Clementino, Beira Rio 8, Arara and 785/15. For the TL, the group with the highest means (12.26-13.65 mm) was formed by the genotypes Beira Rio 8, NV2, Z39, 8R, Guarani and 22R. Parallelly, the three of the last-mentioned genotypes, Beira Rio 8, 8R and Guarani, also formed the group with the highest means for PL (21.95-23.56 mm). Based on the AntL means, all genotypes were divided into two groups, where the group with the highest means (10.39-11.93 mm) was formed by 36.8% of genotypes. Based on StaL, the group with the highest means (14.85-16.09 mm) was formed by the genotypes Beira Rio 8, Guarani and 8R. The group with highest means of StgL (7.34-9.37 mm) was formed by the genotypes Pirata, Beira Rio 8, Tardio V, Z39, 8R, Guarani and 22R. Interestingly, the genotype Guarani represented alone the group with the highest mean of StyL (26.08 mm). For the NFA, the group with highest means (5.75-5.90 units) was formed by the genotypes Clementino, Beira Rio 8, AD1 and L80.

Table 3- Grouping of means (n=20) for: Corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), petal length (PL, mm), anther length (AntL, mm), stamen length (StaL, mm), stigmatic lobes length (StgL, mm), style length (StyL mm), number of floral appendages (NFA, petals or stamens).

Genotype n°	Genotypes	CD	TD	TL	PL	AntL	StaL	StgL	StyL	NFA
1	Pirata	33.62 c	1.65 b	11.51 b	16.78 c	11.40 a	12.40 b	8.05 a	18.00 d	5.35 c
2	Verdim R	32.12 c	1.50 b	8.25 c	16.39 c	11.12 a	12.35 b	5.71 c	17.51 d	5.00 e
3	Bamburral	29.93 c	1.20 c	6.96 d	16.32 c	9.31 b	11.52 c	7.05 b	15.85 e	5.06 e
4	A1	31.52 c	1.62 b	11.68 b	16.50 c	11.07 a	12.71 b	5.38 c	22.01 b	5.28 d
5	Clementino	39.90 a	1.81 a	10.83 b	19.64 b	10.23 a	13.75 b	7.09 b	22.15 b	5.80 a
5	Beira Rio 8	43.37 a	1.87 a	12.41 a	21.95 a	11.89 a	15.80 a	8.98 a	22.89 b	5.60 b
7	P1	31.09 c	1.64 b	10.18 b	16.80 c	9.69 b	11.31 c	6.74 b	20.82 c	5.75 a
8	Verdim TA	34.65 b	1.26 c	8.87 c	19.44 b	10.69 a	13.61 b	6.83 b	18.95 d	5.00 e
9	NV2	30.06 c	1.35 c	12.93 a	16.08 c	9.04 b	10.63 c	6.63 b	23.24 b	5.10 e
10	NV8	27.00 d	1.46 b	9.57 c	15.03 d	9.07 b	10.86 c	5.34 c	18.22 d	5.07 e
11	Bicudo	30.64 c	1.55 b	10.21 b	16.58 c	10.81 a	12.91 b	5.62 c	20.44 c	5.20 d
12	CH1	24.49 d	1.53 b	8.86 c	13.73 d	9.04 b	9.98 c	6.38 b	15.64 e	5.19 d
13	Imbigudinho	30.81 c	1.56 b	8.45 c	15.61 c	8.75 b	11.19 c	5.13 c	18.90 d	5.35 c
14	AD1	27.23 d	1.50 b	10.93 b	14.61 d	8.98 b	9.50 c	5.20 c	20.05 c	5.75 a
15	Graudão HP	28.14 d	1.67 b	11.44 b	13.83 d	8.30 b	9.29 c	5.48 c	18.69 d	5.50 c
16	AP	33.69 c	1.42 c	7.76 c	18.82 b	10.32 a	12.88 b	6.00 c	19.53 c	5.13 d
17	L80	33.18 c	1.59 b	10.94 b	17.35 c	11.50 a	13.53 b	7.34 b	18.16 d	5.90 a
18	Peneirão	29.21 d	1.66 b	7.48 c	15.04 d	9.56 b	11.22 c	5.38 c	15.81 e	5.25 d
19	Z21	27.83 d	1.76 a	10.78 b	15.80 c	11.14 a	11.83 c	6.73 b	21.06 c	5.00 e
20	Ouro Negro	30.20 c	1.64 b	5.46 d	14.31 d	8.00 b	10.38 c	4.47 c	15.44 e	5.42 c
21	Tardio C	27.78 d	1.60 b	5.10 d	14.29 d	9.62 b	11.48 c	5.44 c	16.73 e	5.00 e
22	P2	27.81 d	1.47 b	7.34 c	12.81 d	9.26 b	10.78 c	4.92 c	18.03 d	5.18 d
23	LB1	31.13 c	1.58 b	7.47 c	15.18 d	9.72 b	11.76 c	5.33 c	16.62 e	5.06 e

24	Verdim D	33.23 c	1.31 c	8.88 c	16.68 c	9.08 b	11.84 c	5.28 c	18.09 d	5.05 e
25	Ouro Negro 1	26.64 d	1.62 b	5.75 d	13.64 d	9.72 b	10.22 c	3.73 c	17.05 d	5.08 e
26	Ouro Negro 2	31.03 c	1.59 b	6.88 d	15.81 c	9.15 b	11.33 c	5.33 c	16.40 e	5.05 e
27	K61	25.79 d	1.52 b	8.87 c	13.13 d	9.41 b	10.49 c	6.63 b	15.54 e	5.11 e
28	JC JM	31.86 c	1.61 b	11.03 b	16.24 c	11.11 a	11.68 c	7.53 b	19.72 c	5.39 c
29	Arara	26.79 d	2.07 a	6.89 d	13.30 d	8.83 b	8.98 c	3.56 c	14.40 e	5.00 e
30	785/15 Vermelho	28.69 d	1.96 a	8.37 c	13.54 d	8.02 b	8.90 c	4.63 c	15.73 e	5.00 e
31	Tardio V	32.89 c	1.34 c	6.18 d	17.54 c	11.01 a	13.19 b	9.30 a	14.51 e	5.42 c
32	Z39	34.60 b	1.60 b	13.65 a	19.77 b	9.55 b	11.34 c	7.98 a	21.05 c	5.40 c
33	Z18	28.54 d	1.09 c	8.24 c	15.77 c	7.66 b	10.14 c	5.05 c	17.86 d	5.22 d
34	Z36	32.96 c	1.33 c	8.27 c	18.23 b	9.90 b	13.05 b	6.70 b	21.52 c	5.00 e
35	Emcapa 02	36.25 b	1.17 c	8.72 c	19.64 b	9.62 b	13.96 b	6.92 b	22.52 b	5.00 e
36	8R	40.09 a	1.51 b	12.27 a	22.48 a	11.84 a	14.85 a	9.37 a	23.44 b	5.10 e
37	Guarani	39.25 a	1.49 b	13.42 a	23.56 a	11.93 a	16.09 a	8.92 a	26.08 a	5.00 e
38	22R	32.32 c	1.23 c	12.26 a	18.72 b	9.47 b	11.41 c	7.84 a	23.00 b	5.00 e

Means followed by the same letter in the columns belong to the same group.

Generally, the genotypes with the highest size of floral structures were Beira Rio 8, Guarani, 8R, and Clementino, respectively (Table 3). The greatest similarities among these genotypes were the highest values for CD and AntL. The genotypes with the lowest size of floral structures, considering the set of traits, were Bamburral, NV8, Peneirão, Arara and 785/15 Vermelho (the last two are *C. arabica*). The greatest similarities among these genotypes were the low values of AntL, StaL and StyL. The cultivars Arara and 785/15 Vermelho constituted the group with the highest means only for TD, while for TL, each of the two *C. arabica* genotypes was classified into one different group of means ('c' or 'd'), while for the other flower traits, both genotypes constituted the groups with the lowest means). These results indicated that the size of the floral structures of *C. arabica* and *C. canephora* could be similar, depending on cultivar/clone.

Table 4- Principal components (PC), eigenvalues (λ), percentage of variance explained by components (% PVC), and cumulative percentage of flower morphological traits.

PC	λ	% PVC	% PVC (accumulated)
PC1	5.02	55.74	55.74
PC2	1.38	15.39	71.13
PC3	0.95	10.57	81.70
PC4	0.71	7.92	89.62
PC5	0.48	5.30	94.92
PC6	0.30	3.29	98.21
PC7	0.09	0.98	99.19
PC8	0.04	0.46	99.64
PC9	0.03	0.36	100.00

The first four components classified by PCA explained most of the total variation in the data set (89.62%), being associated with the highest eigenvalues (λ >0.7), thus retaining the highest variance of the data (Table 4). Among the nine PCs, five presented eigenvalues lower than 0.7, which means that five of the nine evaluated flower traits can be excluded without significant losses in the representativeness of flower size and form variability. The choice of traits to be discarded followed the criterion of elimination of those ones that were the most correlated with the PCs able to explain the small part of the total variation among genotypes. Herein, the PC5 to PC9 were of low λ and low percentage of variance explained by components (% PVC).

Table 5- Weighting coefficients of flower morphological traits related to the principal components (PC) that explained the smallest percentage of the total variation. Corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), petal length (PL, mm), anther length (AntL, mm), stamen length (StaL, mm), stigmatic lobes length (StgL, mm), style length (StyL mm), number of floral appendages (NFA, petals or stamens).

	Weighting coefficients				
Traits	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
CD	0.4997	-0.2518	0.3056	-0.3801	0.5029
TD	0.1196	-0.2447	-0.2071	0.0298	-0.1230
TL	-0.2871	-0.1029	0.5407	-0.1215	-0.2059
PL	0.2801	-0.2185	0.0772	**0.8009**	-0.1710
AntL	**-0.5015**	0.4749	0.1559	0.1874	0.3706
StaL	0.1238	0.2643	-0.0153	-0.3643	**-0.6973**
StgL	-0.4962	**-0.5702**	-0.4496	-0.1414	0.0335
StyL	0.2276	0.3703	**-0.5796**	-0.0559	0.1880
NFA	0.1014	0.2465	-0.0527	0.0869	-0.0173

Traits and values marked in bold indicate the correlation value used as a criterion for discarding the trait.

In the next step, we discarded the five traits of low λ and low % PVC , PC5 to PC9 (Table 4), which also showed the highest weighting coefficients in absolute values, from the last PC towards the fifth component (Table 5). The traits with the lowest impact on the total variation were suggested for disposal. They were identified in the following order: PL, AntL, StaL, StgL, and StyL. The discard of these traits was justified additionally by their association with components that little explain the data variability, showing significant simple linear correlation with the others, providing little or no additional information (Figure 2). The PL was highly correlated with the CD, as well as in the other traits suggested for discard. The traits associated with the PC that most explained the total variation of the data were: CD, TD, TL, and NFA. These traits showed low correlations with each other. A linear positive correlation between the CD and TL was significant, with low value for correlation coefficient, while the correlation between the CD and TD was negative and not significant.

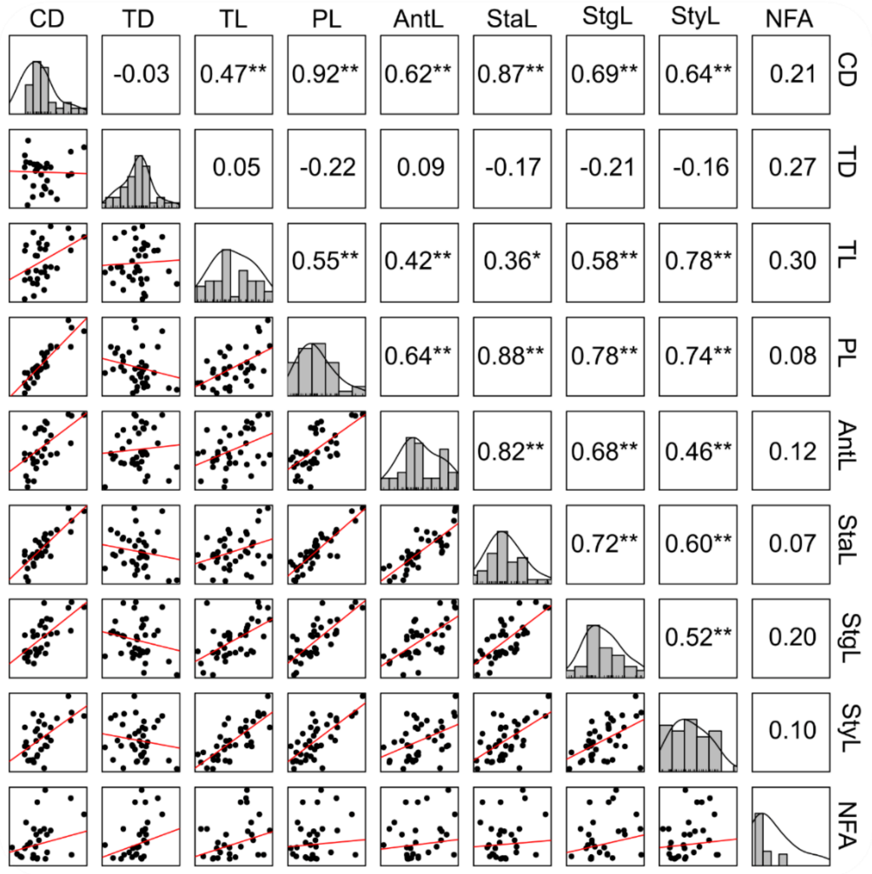

Figure 2- Pearson correlation (n=38) and p-values significance (* and **corresponded to the significance of $p < 0.05$, 0.01, respectively) for: Corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), petal length (PL, mm), anther length (AntL, mm), stamen length (StaL, mm), stigmatic lobes length (StgL, mm), style length (StyL mm), number of floral appendages (NFA, petals or stamens).

After the discard of five flower traits that showed low impacts on the total variation (based on high correlation to the traits of high impacts on the total variation), the new two PCs were formed (Fig. 3). The new PC 1 explained 42.4% of the total variation, including the most correlated floral traits with this component: TL (-0.61), CD (-0.55), and NFA (-0.52). The new PC 2 explained 28.4% of the total variation, where the TD was the trait with the highest correlation with this component (-0.78). Based on the arrangement of genotypes in the graph defined by the first two PCs, which account for 70.8% of the data variation, genotype dispersion was observed, indicating significant phenotypic variability regarding the evaluated traits. In PC1, the Beira Rio 8 and Tardio C genotypes exhibited the greatest distances. This was related to higher TL observed in Beira Rio 8 compared to the Tardio C genotype. The positioning of the Clementino, L80, and P1 genotypes in relation to PC1 was influenced by NFA, as these genotypes likely had the highest NFA. Additionally, in relation to PC1, genotypes 8R and Guarani showed the smallest distances due to their similar CD. In PC2, the cultivars

Arara and 785/15 displayed the largest distances from the Encapa 02, 22R, Verdim TA, Verdim D, and Z18 genotypes. This was probably associated with higher TD observed in the cultivars Arara and 785/15 than in Encapa 02, 22R, Verdim TA, Verdim D, and Z18 genotypes.

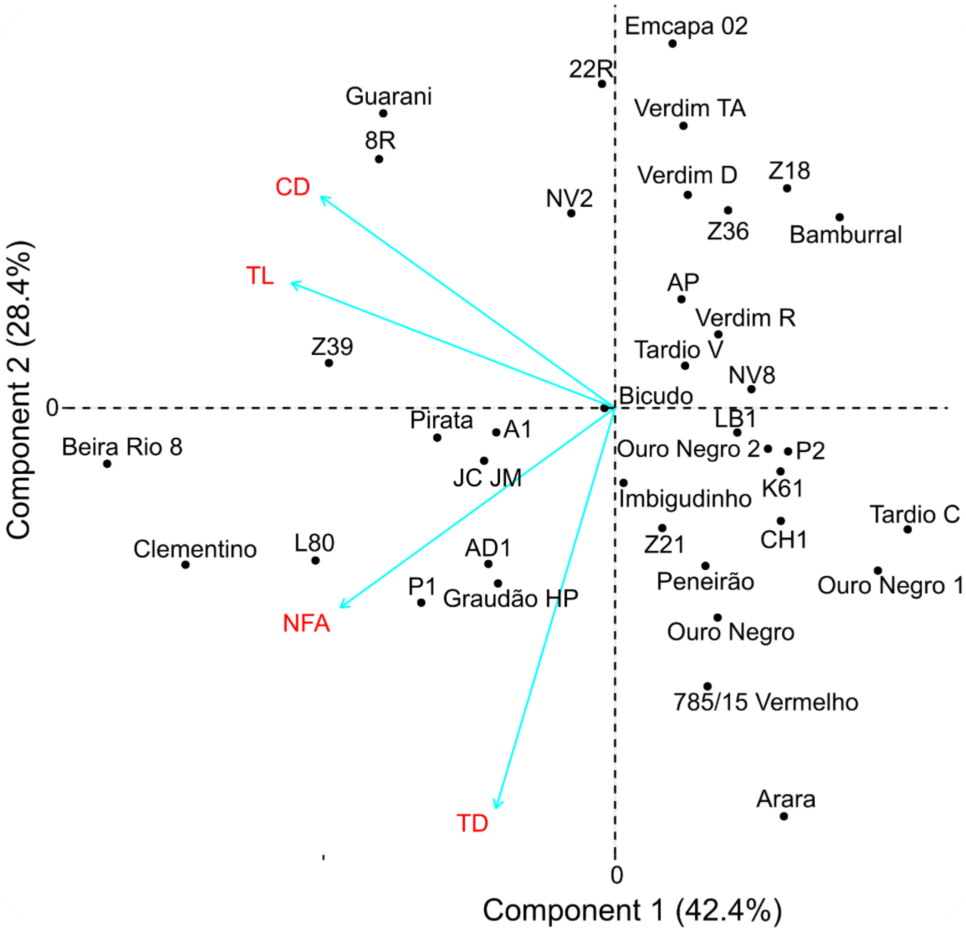

Figure 3- PCA formed after the discard of flower morphological traits for 38 coffee genotypes. Traits: Corolla diameter (CD, mm), tube diameter (TD, mm), tube length (TL, mm), number of floral appendages (NFA, petals or stamens).

DISCUSSION

Our results showed the variability among coffee genotypes regarding flower size (Table 3), confirming the first hypothesis of this study about the variations in flower traits among the genotypes. Flower size is often associated with pollination efficiency (Lázaro et al., 2015; Kaczorowski et al., 2012; Latty and Trueblood, 2020). In melon plants, hybrids with larger corolla diameter are more visited by pollinators compared to those with smaller corolla diameter (Kiill et al., 2016). The flower size of sunflower lines influences bee preference, where even a 2 mm difference in flower size can significantly increase the pollinator activity (Portlas et al., 2018). Both in *C. arabica* and *C. canephora* plantations, the presence of pollinators, especially bees, effectively contributes to fruit yield and quality (Klein et al., 2003; Saturni et al., 2016). Those statements, together with our findings, indicate that the morphology of coffee flowers can have some significant implications in coffee crop productivity, which must be studied in the future.

Larger flowers are more attractive to bees compared to smaller flowers, as they tend to offer more rewards (Vaughton and Ramsey, 1998, Kumari et al., 2020). If this would also be a case in coffee plantations, the bees may prefer to visit genotypes with larger flowers, such as Clementino, Beira Rio 8, Guarani, and 8R (Table 3). Although this study did not evaluate the pollinator visits, nor productivity, it is known that Clementino and Beira Rio 8 genotypes are a constituent of the high productivity conilon cultivar Tributun, which has an average productivity of 90.87 bags ha^{-1} $year^{-1}$ (Partelli et al., 2020). The Guarani genotype is a constituent of the cultivar Forte Guarani, which is the first conilon cultivar selected for high productivity and high caffeine bean content (Partelli et al., 2022b). The 8R genotype is not yet a part of one registered cultivar but is widely grown in coffee plantations throughout the Brazilian state of Rondônia and is already commercially cultivated by some coffee producers in the states of Espírito Santo and Bahia (Espindula et al., 2022). Thus, one possible explanation for the large flower size in genotypes that have undergone a selection process may be the potential of these genotypes to attract pollinators, which, combined with other agronomic aspects, has resulted in higher grain productivity in these materials. This statement, as the statement at the end of the previous paragraph, must be confirmed, or not, by the future studies.

C. arabica cultivars Arara and 785/15 Vermelho differed among them only in the tube length (Table 3). The general similarity between these two cultivars may be due to their origin, as their male parents are full siblings: Arara resulted from the cross of Obatã and Catuaí Amarelo, 785/15 Vermelho resulted from the cross of Icatú Vermelho and Catuaí Vermelho (Ferrão et al., 2021; Fazuoli et al., 2007). Both Catuaí Amarelo and Catuaí Vermelho (male parents) originated from the cross of Caturra x Mundo Novo. Since the genetic variability of this species is relatively narrow, it is possible that the Arara and 785/15 cultivars share common alleles for flower morphology traits, which can be studied in the future.

The low values observed for the CV (Table 2) revealed that the quantity of evaluated flowers (20 flowers per genotype) was sufficient to achieve good experimental precision (Chater et al., 2014). It is worth noting that the conditions of the experiment setup, such as the easily accessible location for monitoring of the flower opening and the maintenance of irrigation during the evaluation period, contributed to the flower sample size used in this study. In equatorial regions, coffee flowering occurs asynchronously throughout the year due to the absence of water deficit periods (Quiñones et al., 2014). However, in the main coffee production areas in Brazil, where the winter season is dry and well-defined, flower opening occurs within a shorter period (from August to November) during two or more flushes (Rena and Barros, 2004; Ronchi and Miranda, 2020). This condition poses a challenge for using morphological traits of flowers in phenotypic variability studies because the shorter the period of flower opening, the less time the researchers will have to evaluate them.

After opening, coffee flowers maintain their turgid structures for a short period (Souza et al., 2003; Ferrão et al., 2017). Within 12 hours of anthesis initiation, the flower structures already show signs of dehydration (Ramalho et al., 2014), with anthers being the first structures to undergo modifications shortly after the release of pollen (Figure 1b). To optimize the time spent on flower morphological evaluations, especially considering a great number of genotypes, we proposed performing PCA to identify correlated traits that explain little variability in the data. Traits with low variability can be excluded from the analysis and future morphological evaluations, as they contribute little to genotype discrimination and require more time and effort for evaluation (Jolliffe and Cadima, 2016). The high positive and significant correlations (Figure 2) observed among the petal length, anther length, stamen length, stigmatic lobe length, and style length with corolla diameter confirmed the hypothesis about the strongly correlated morphological traits in coffee flowers. These traits were also associated with components that explained little variability in the data (Table 5). In multivariate analyses, the identification of correlated traits is important because when two subsets of data are correlated, using only one subset of the total data is sufficient for analysis, and the second data subset becomes redundant (Rego et al., 2003). In studies of genetic diversity based on phenotypic traits, multiple traits are often used to determine which genotypes are more dissimilar (Sarif et al., 2020; Dubberstein et al., 2021; Silva et al., 2023). In these cases, the inclusion of groups of traits with high positive correlations (multicollinearity) can prevent traits with lower variation from contributing to inferences of phenotypic variability and thus bias the results.

In our study, the excluding of petal length, anther length, and stamen length could significantly contribute to reducing the time spent on data collection. The flowers evaluated in this study had 5-6 petals, anthers, and stamens, and each of these individual structures was measured (all petals, anthers, and stamens) to obtain the final average. Thus, considering that each genotype was evaluated based on 20 flowers, each required 300 to 360 measurements of petal length, anther length, and stamen length for each genotype. The short flower lifespan, particularly during simultaneous flowering, justified the methods toward the trait number reduction, to decrease the sampling effort, and enable the information of the floral morphology for a great number of coffee genotypes. In plant breeding, a range of solutions have already been proposed to increase efficiency in studies of genotype diversity based on phenotypic traits. For that are used the method of Mahalanobis distance, treating the datasets with strongly correlated traits (Arunachalam, 1967), or Singh exclusion method based on relative contribution (Singh 1981), or correlation among traits and principal components (Jolliffe, 1972). Comparing the Singh method to the PCA for the effective exclusion of redundant traits in peppers, the exclusion method based on principal components is shown to be more efficient (Rego et al., 2003).

The PCA showed that among the nine evaluated traits, the subset of five of them was able to explain only 10.31% of the variation, and the exclusion of these traits did not compromise the inference of data variability, confirming the third hypothesis of this study (Fig. 3). Although there is no fixed value previously established in genetic variability studies, there is a convention among plant breeders that the use of traits capable of expressing values above 70% of the total variation presents sufficient precision for studies of genetic divergence (Sraçli et al., 2013; Tounekti et al., 2017; Shoba et al., 2019), expressed either by the cumulative variance in PCs, or by the coefficient of cophenetic correlation, in the case of dendrogram use. The use of the first two PCs for clustering the genotypes considering only the corolla diameter, tube diameter, tube length, and number of appendages floral (petals and stamens) showed good precision (70.8%). The trait that contributed the most to the discrimination of genotypes was the tube diameter, which was highly correlated with PC2 (Fig. 3). This component was effective in discriminating among the genotypes Arara and 785/15, which were the only ones that belong to *C. arabica* among the 38 evaluated genotypes. PC1 was also effective in discriminating among the genotypes with larger corolla diameter, tube length, and number of floral appendages (petals and stamens). Therefore, in studies aiming to assess the diversity of different coffee genotypes based on these morphological traits of flowers, the researchers can concentrate their evaluations to one limited set of traits, having the similar efficiency as with one large data set of traits.

CONCLUSION

Our study showed the flower size morphological variability among different coffee genotypes, where some genotypes were segregated into group of larger flowers than others. This fact supported our initial hypothesis about the genotype variation in coffee flower traits. Among the nine of them evaluated, five - petal length, anther length, stamen length, style length, and stigmatic lobe length - were strongly correlated with traits of greater importance in explaining the data variability, confirming our second hypothesis about the existence of strong correlations among flower morphological traits in *Coffea* spp. The correlated traits that contributed little to the data variability, and their exclusion did not compromise the inference of flower morphological variability in *Coffea* spp. genotypes, confirming our third hypothesis. Studies aiming to differentiate *Coffea* spp. genotypes based on floral structures may consider only the tube length, tube diameter, corolla diameter, and the number of floral appendages (petals or stamens) as the focus of investigation. Evaluating only these traits can optimize time and efficiency in data collection. The identification of morphological traits of flowers, which tend to exhibit high genetic variability, and their relationship with genotype productivity (a low heritability trait) is essential information for enhancing genetic gains in the crop.

So, in the future, the association of selected flower morphological traits in *Coffea* spp. with traits related to production, could be a great contribution to coffee breeding programs.

Acknowledgments

We thank to the Fundação de Amparo à Pesquisa e Inovação do Espírito Santo - FAPES (Proc. 2022-WTZQP for Fábio Luiz Partelli and Proc. 2022-M465D for Miroslava Rakocevic and Fábio Luiz Partelli), Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Proc. 309535/2021-2 for Fábio Luiz Partelli), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Proc. 88882.385374/2019-01 for Larícia Olária Emerick Silva). We thank to the Núcleo de Excelência de Pesquisas em Café Conilon for its support in the logistics of the experiment.

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FINAL CONSIDERATIONS

This study was important in understanding the potential of leaf anatomical and floral morphological traits of *Coffea* spp. in genetic variability studies. We discovered that all leaf anatomical traits related to stomatal density of *C. canephora* genotypes showed potential for distinguishing and selecting genotypes for the development of new cultivars.

We also found that the specific changes observed in flower morphology may be related to the genetic traits of the evaluated plants, different environmental conditions, and/or plant adaptations to these conditions at each altitude. Therefore, conducting studies on the morphological traits of flowers at a greater number of altitudes and their relationship with productivity (a trait with low heritability) can enhance the genetic gains of the crop.

Finally, we discovered that studies aiming to differentiate *Coffea* spp. genotypes based on flower morphology but needing to save time and increase data collection efficiency may focus solely on the tube length, tube diameter, corolla diameter, and the number of floral appendages (petals or stamens). Among the nine traits studied, these four explained the most variability in the data.

Therefore, the results of this study have shown that characterizing leaf anatomy and flower morphology in *Coffea* spp. is an effective phenotypic marker option for distinguishing genotypes. Furthermore, from our results, new hypotheses have emerged that can be tested in future studies, thereby providing more information about the potential of these traits and contributing to coffee breeding programs.