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Variability of photosynthetic performance among improved genotypes of *Coffea canephora*

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ABSTRACT

This study evaluated the variability of photosynthetic performance of 27 improved genotypes of Conilon coffee, cultivated in the Southern of the Espírito Santo State. The photosynthetic performance was based on the measurement of gas exchange rates and chlorophyll in the period most favorable to the photosynthetic activity and in different stages of the reproductive cycle: flowering, fruit initiation, grain formation and fruit maturation; being expressed as the average (weighted by the number of days) along the phenological stages of the third reproductive cycle of the plants. It was possible to verify the existence of sufficient variability to differentiate the photosynthetic performance among the 27 genotypes throughout the reproductive cycle, even starting from a group of already improved genotypes. Among the physiological parameters, the rate of carbon assimilation, stomatal conductance and the transpiration rate stood out as parameters for the study of variability, mainly due to their contributions to the clustering of genotypes. The genotype 108 is highlighted due to its high photosynthetic rate, associated with higher relative content of chlorophyll, as well as reasonable water use efficiency. The genotypes 205, 206 and 305 stood out in terms of water use and carbon assimilation.

Keywords: Conilon coffee, cultivar, diversity, gas exchange, phenological cycle.

INTRODUCTION

Brazil is the world's largest coffee producer, mainly from the cultivation of *Coffea arabica* Lineu (Arabica coffee) and *C. canephora* Pierre ex A. Froehner (Conilon coffee). During the past 150 years, Conilon coffee has become an important commodity, accounting for a third of the global coffee trade.[1] In the Espírito Santo State (Brazil), coffee cultivation is the main agricultural activity, and Conilon coffee amounts to nearly 76% of the coffee produced in the state, covering an area of nearly 262,988 ha, with a 0.4% increase from the last cycle.[2]

Due to the great importance of coffee cultivation for the Brazilian economy, several research projects have been developed, including through the genetic improvement of both species.[3] The scientific knowledge constantly developed and improved for Conilon coffee has allowed the recommendation of new cultivars based on the selection and grouping of compatible genotypes.[4] Great advances have been obtained in the area of genetic improvement and variability studies[5], growth and physiology[6], fertilization and nutritional efficiency[7,8], among others. In addition, the high phenotypic and genotypic variability observed in this species has enabled the selection of genotypes for specific traits, mainly focused on higher crop yield, drought tolerance and defined ripening cycles.[5,9]

Knowledge about the photosynthetic performance of Conilon genotypes can contribute to the development of cultivars that are more efficient in physiological and productive terms, capable of evading premature aging or tolerating conditions of water deficit. For example, genotypes with poor control of their transpiration rate and stomatal conductance in response to water deficit and high temperatures may be more susceptible to rapid dehydration in drought situations.[10] By knowing the physiological performance, problems like this can be overcome by making rational choices about which genotypes are more suitable for each crop condition.

It is known that the photosynthetic rates of both Conilon and Arabica coffee are relatively low when compared with other woody species, and the greater resistances (stomatal and mesophilic) to the diffusion of CO_2 are the main mechanisms responsible for this behavior.[11] However, Conilon coffee trees can maintain greater cumulative absorption of CO_2 throughout the day, probably due to a lower stomatal sensitivity in response to the evaporative demand of the air[10], and this fact may contribute to the higher yields observed in this species when compared with Arabica coffee. It is also possible to infer that Conilon coffee can present larger accumulations of starch in the leaves, without the occurrence of metabolic retroinhibition of photosynthesis.[12]

The high crop yield of some genotypes of Conilon coffee could be associated with higher photosynthetic rates of the whole plant, depending on the maintenance of a healthy leaf area and a canopy architecture capable of optimizing the photosynthetic processes.[10]

Global predictions have pointed out the risk of severe losses and changes in areas considered suitable for growing coffee in South and Central America, mainly due to the increased occurrence of extreme weather events caused by climate changes.[13] In this context of climatic change, studies are needed to identify genotypes with higher efficiency in using available natural resources and capable of tolerating environmental stresses and sustaining higher photosynthetic rates along their phenological cycle.

The need for knowledge about the photosynthetic behavior of genotypes of Conilon coffee and the expression of variability is emphasized as a possible trait to be taken into consideration for plant breeding programs during the development of new cultivars. Therefore, the objective of this study was to evaluate the variability of photosynthetic performance of 27 improved genotypes of Conilon coffee that compose three clonal cultivars.

MATERIAL AND METHODS

Local conditions and experimental design

The experiment was conducted on a coffee plantation, located in the municipality of Castelo, southern Espírito Santo State, in the Southeast Region of Brazil. The site presents a plain terrain and is located at the latitude of 20°34'19.6"S and the longitude of 41°18'51.7"W, with an altitude of 126 m. The crop lines followed the relief and were oriented in a southeast-northwest direction. During the cycle sampled in this experiment (August 2017 to July 2018), the average air temperature was 23.9 ºC, the accumulated rainfall was 1,375 mm, and there were 143 days of rainfall (Fig. 1).

The soil was sampled and analyzed[14], being classified as an Oxysol, with a clayey-sandy texture (33% clay, 12% silt, 55% sand), soil density of 1.077 Mg m^{-3} and particle density of 2.439 Mg m^{-3}. Chemical analysis showed a pH of 5.54 (in water), 48.27 mg dm^{-3} of P, 445 mg dm^{-3} of K, 140 mg dm^{-3} of Na, 3.65 $cmol_c$ dm^{-3} of Ca, 0.8 $cmol_c$ dm^{-3} of Mg, 0 $cmol_c$ dm^{-3} of Al, 6.52 $cmol_c$ dm^{-3} of H+Al.

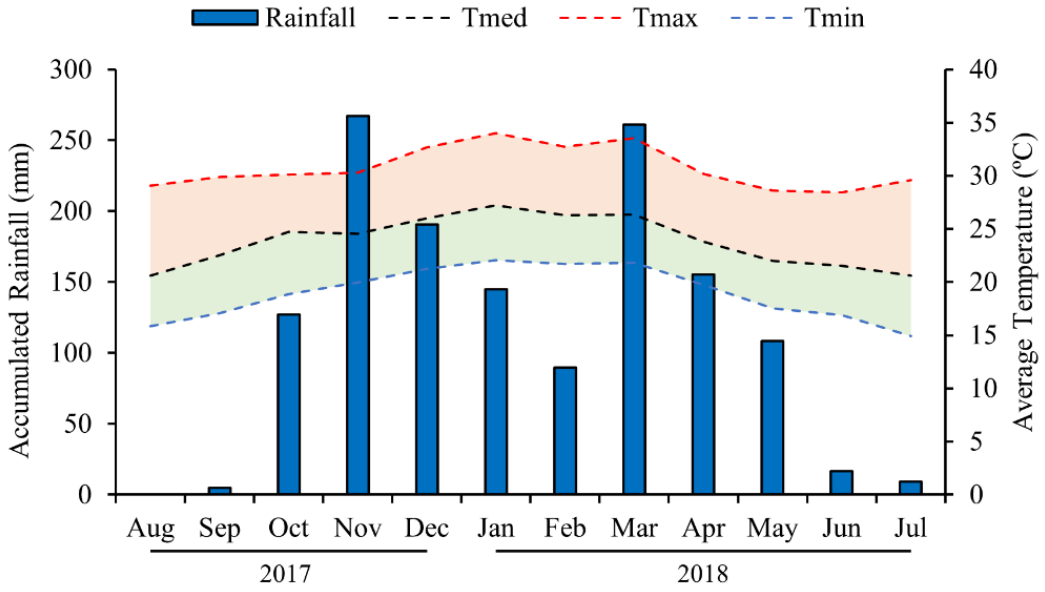

Figure 1. Monthly accumulated rainfall and average air temperature between August 2017 and July 2018 (Castelo, Espírito Santo State, Brazil).

The competition field was established in August 2014, using a plant spacing of 3×1 m (3,333 plants ha^{-1}), and the plants were managed with three orthotropic stems each (9,999 stems ha^{-1}), resulting in a population of plants and stems within the recommended level for Conilon coffee plantations.[4] The field was irrigated by mesh spraying, starting when the tension of water retention in the soil (measured by a set of tensiometers in the plantation) corresponded to the tension of 30 ± 4 kPa, in order to return the soil moisture to field capacity. All plantation management practices were carried out according to the current recommendations for Conilon coffee plantations in the Espírito Santo State.[4] The experiment followed a randomized block design, with four repetitions, where the treatments consisted of 27 genotypes of Conilon coffee, and each experimental plot was composed of six plants. In each plot, the two most representative plants were selected to evaluate the average status of growth, vigor, canopy size, sanity and yield, picked among the four central plants to avoid possible border effects over the first and sixth plant of the plot.

Selection of genotypes

The 27 genotypes of *C. canephora* used in this study compose three recent clonal cultivars certified in Brazil by SNPC (Serviço Nacional de Proteção de Cultivares) for Conilon coffee. Nine genotypes are from the cultivar "Diamante ES8112" (SNPC Certification number: 20140103) of early-ripening and will be referred to in this study as 101, 102, 103, 104, 105, 106, 107, 108 and 109. Nine genotypes are from the cultivar "Jequitibá ES8122" (SNPC Certification number: 20140104) of intermediate-ripening, referred to as 201, 202, 203, 204, 205, 206, 207, 208 and 209. The last nine genotypes are components of the cultivar "Centenária ES8132" (SNPC Certification number: 20140102) of late-ripening, referred to as 301, 302, 303, 304, 305, 306, 307, 308 and 309. These clonal cultivars were developed and registered by the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (INCAPER), and are results of compatible arrangements characterized by high crop yield and beverage quality.[4]

The difference between the ripening cycles of these genotypes is based on the period between anthesis and harvest, averaging 34 weeks for genotypes of the early-ripening cycle, 41 weeks for intermediate-ripening, and 45 weeks for late-ripening.[15]

Photosynthetic performance evaluation

In each of the two representative plants of the experimental plot, two plagiotropic branches of first production were tagged, being selected in the middle section of the canopy. The evaluations of the photosynthetic performance were performed on mature and healthy leaves from each branch (sampled from the third/fourth pair of leaves from the apex of the branch). Ten gas exchange readings were performed on each evaluated leaf, and the results were expressed as the mean reading per experimental plot.

Gas exchange parameters were monitored by evaluating the net assimilation rate of CO_2 (A, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), substomatal concentration of CO_2 (C_i, μmol mol^{-1}), instantaneous water use efficiency (A/E, μmol $mmol^{-1}$), and intrinsic water use efficiency (A/g_s, μmol mol^{-1}). These efficiency rates (A/E and A/g_s) were used in other studies with coffee.[16,17] For this purpose, instantaneous measurements were performed using an infrared gas analyzer (IRGA Licor 6400XT). The same leaves were evaluated for the relative content of chlorophyll *a* (Chl *a*, relative unit), chlorophyll *b* (Chl *b*, relative unit), ratio between chlorophyll *a*/*b* (Chl *a*/*b*) and total chlorophyll (Chl *a*+*b*, relative unit), using a portable chlorophyll meter (ClorofiLOG Falker FL1030).

The evaluations were carried out at four stages of the reproductive cycle of the third coffee harvest: (i) flowering (first stage), (ii) fruit initiation (pellet-like berry, 112 days after flowering), (iii) grain formation (194 days after flowering), and (iv) maturation (274 days for early, 313 days for intermediate, and 348 days after flowering for late genotypes). The evaluations took place during five or six consecutive days in each period of the phenological cycle due to the size of the experiment.

The photosynthetically active radiation was standardized by artificial saturating light at 1,000 μmol (photons) m^{-2} s^{-1} and the internal concentration of CO_2 in the chamber at 420 μmol mol^{-1}, in order to provide a CO_2 concentration larger than the maximum limit observed in the atmosphere in the experimental period (405.0 ppm).[18] The vapor pressure deficit (VPD), leaf and air temperatures were monitored during measurements to avoid collecting data when temperature or VPD were too high. During flowering, the average air temperature was 28.5 ± 1.8 ºC, leaf temperature was 28.1 ± 2.4 ºC and VPD was 1.73 ± 0.41 kPa (therefore, the evaluations were performed between 8:00 and 10:20 am). For fruit initiation stage, the air temperature was 31.2 ± 2.1 ºC, the leaf temperature was 31.3 ± 2.1 ºC, and VPD was 1.66 ± 0.41 kPa (between 7:30 and 10:00 am). During grain formation, the air temperature was 33.3 ± 2.3 ºC, leaf temperature was 32.8 ± 2.2 ºC, and VPD was 1.49 ± 0.73 kPa (between 7:30 and 9:50 am). For the maturation state, the average air temperature was 29.2 ± 1.6 ºC, leaf temperature was 28.4 ± 1.8 ºC, and VPD was 1.93 ± 0.39 kPa (between 7:30 a 10:10 am).

These evaluations made it possible to present the gas exchange values obtained during the period of the day favorable to the photosynthetic activity of Conilon coffee plants. Therefore, the weighted average for A, g_s, E, C_i, A/g_s, A/E, Chl *a*, Chl *b*, Chl *a*/*b*, e Chl *a*+*b* was calculated for each genotype. The ratio between the area under the curve of the parameter (based on the evaluations during the four different stages of the reproductive cycle) and the total number of days for the evaluation period (274 for genotypes of early-ripening, 313 for genotypes of intermediate-ripening, and 348 for genotypes of late-ripening) was used to estimate the performance of each genotype. Thus, the photosynthetic performance of the genotypes was based on the measurement of gas exchange at the schedule most favorable to the activity of the plants, during different stages of their reproductive cycle; being expressed as the average (weighted by the number of days) of the maximum gas exchanges sampled in the morning period and during the phenological stages from flowering to fruit maturation.

Statistical analyses

The data were subjected to an analysis of variance, by the F-test to identify the existence of significant differences between genotypes for each parameter of photosynthetic performance. In the presence of significant differences, the Scott-Knott criterion was used to analyze the means (5% probability).

The genetic parameters were estimated using the individual model: $Y_{ij} = \mu + B_j + G_i + \varepsilon_{ij}$, where: Y_{ij} represents the phenotypic value of the ijth observation; B_j represents the effect of the jth block; G_i is the fixed effect of the ith genotype; ε_{ij} is the experimental error. The methodology described by Cruz e Carneiro[19] was used to calculate the genetic parameters: mean phenotypic variance ($\hat{\sigma}^2_p$), mean environmental variance ($\hat{\sigma}^2_e$), genotypic quadratic component ($\widehat{\Phi}_g$), broad genotypic determination coefficient (H^2), coefficient of genetic variation (CV_g), and variation index (CV_g/CV).

The determined variables: A, g_s, E, C_i, Chl *a*, and Chl *b*; were selected to study the genetic divergence among the genotypes through multivariate analysis, using the generalized Mahalanobis distance (D^2) as a dissimilarity measure. The relative contribution of each previously mentioned parameter was estimated based on their standardized means.[20] The genotypes were clustered using Tocher's optimization method, by dissimilarity criteria, as described by Cruz e Carneiro[19]. Data analyses were performed using the statistical software GENES.[21]

RESULTS

Significant differences were observed among genotypes of Conilon coffee, through the F-test, for all parameters of photosynthetic performance (Table 1). The estimated values of genetic parameters showed that mean phenotypic variances ($\hat{\sigma}_p^2$) among genotypes were generated by greater contributions from the genotypic quadratic components ($\widehat{\Phi}_g$), with the only exception observed for the variable Chl *a*. As a result, the genotypic determination coefficients (H^2) were higher than 80% for the variables *A*, g_s, *E*, C_i, A/g_s, *A*/*E*, Chl *b*, and Chl *a*+*b*; with emphasis for *A*, g_s, *E* and A/g_s, since these variables presented H^2 above 90%, showing a greater proportion of the phenotypic variance being attributed to the quadratic component of genotypic variability. In addition, the estimated variation indexes (CV_g/CV) were greater than 1.00 for eight variables (g_s > A/g_s > *E* > *A* > *A*/*E* > Chl *b* > Chl *a*+*b* > C_i), indicating a favorable situation for studying the genetic diversity using these parameters and increasing the chance of success in a possible selection of genotypes aimed at improve these photosynthetic parameters (Table 1).

Sufficient variability was observed to identify phenotypic differences among genotypes for the 10 parameters of photosynthetic performance, allowing differentiation of groups of genotypes with homogeneous behavior for each variable. Five homogenous groups were observed for the net photosynthetic rate. Six groups for the stomatal conductance and intrinsic water use efficiency. Seven groups were distinguished for the transpiration rate. Four groups for the instantaneous water use efficiency. Two groups were formed for the substomatal concentration of CO_2, the relative content of chlorophyll *a*, and the chlorophyll ratio *a*/*b*. Three groups were observed for the chlorophyll *b* and the relative content of total chlorophyll (Fig. 2, 3 and 4). The variables g_s and *E* made it possible to differentiate a greater number of groups among the genotypes, being favorable parameters for studying the divergence among these genotypes.

Table 1. Estimative of phenotypic and genotypic parameters of ten traits of gas exchange and relative chlorophyll content of 27 genotypes of *Coffea canephora* (Castelo, Espírito Santo State, Brazil)

Parameter	***A*** [9]	g_s [10]	**E** [11]	C_i [12]	A/g_s [13]
$MS_{genotypes}$ [1]	3.506**	0.018**	0.814**	1511.932**	469.019**
Overall mean	6.020	0.169	2.230	314.500	47.024
CV(%) [2]	8.372	11.558	10.136	5.407	8.975
$\hat{\sigma}_p^2$ [3]	0.876	0.004	0.203	377.983	117.255
$\hat{\sigma}_e^2$ [4]	0.063	0.0001	0.012	72.305	4.453
$\widehat{\Phi}_g$ [5]	0.813	0.004	0.191	305.678	112.802
H^2 [6]	92.755	97.842	93.724	80.871	96.202
CV_g(%) [7]	14.978	38.920	19.586	5.559	22.585
CV_g/CV(8)	1.789	3.367	1.932	1.028	2.516
Parameter	***A*/*E*** [14]	**Chl *a*** [15]	**Chl *b*** [16]	**Chl *a*/*b*** [17]	**Chl *a*+*b*** [18]
$MS_{genotypes}$ [1]	0.447**	9.191*	42.518**	0.259**	85.724**
Overall mean	2.916	37.952	23.243	2.027	61.196
CV(%) [2]	8.390	5.685	10.974	14.945	6.400
$\hat{\sigma}_p^2$ [3]	0.112	2.298	10.629	0.065	21.431
$\hat{\sigma}_e^2$ [4]	0.015	1.164	1.626	0.023	3.835
$\widehat{\Phi}_g$ [5]	0.097	1.134	9.003	0.042	17.596
H^2 [6]	86.615	49.344	84.698	64.567	82.105
CV_g(%) [7]	10.672	2.805	12.909	10.087	6.854
CV_g/CV [8]	1.272	0.493	1.176	0.675	1.071

** and * significant at 1 and 5% of probability, respectively, by the F-test [1] mean square of genotypes; [2] coefficient of variation; [3] mean phenotypic variance; [4] mean environmental variance; [5] genotypic quadratic component; [6] genotypic determination coefficient; [7] coefficient of genetic variation; [8] variation index; [9] net assimilation rate of CO_2; [10] stomatal conductance;[11] transpiration rate; [12] substomatal concentration of CO_2; [13] intrinsic water use efficiency; [14] instantaneous water use efficiency; [15] relative content of chlorophyll *a*; [16] relative content of chlorophyll *b*; [17] ratio between chlorophyll *a*/*b*; [18] relative content of total chlorophyll.

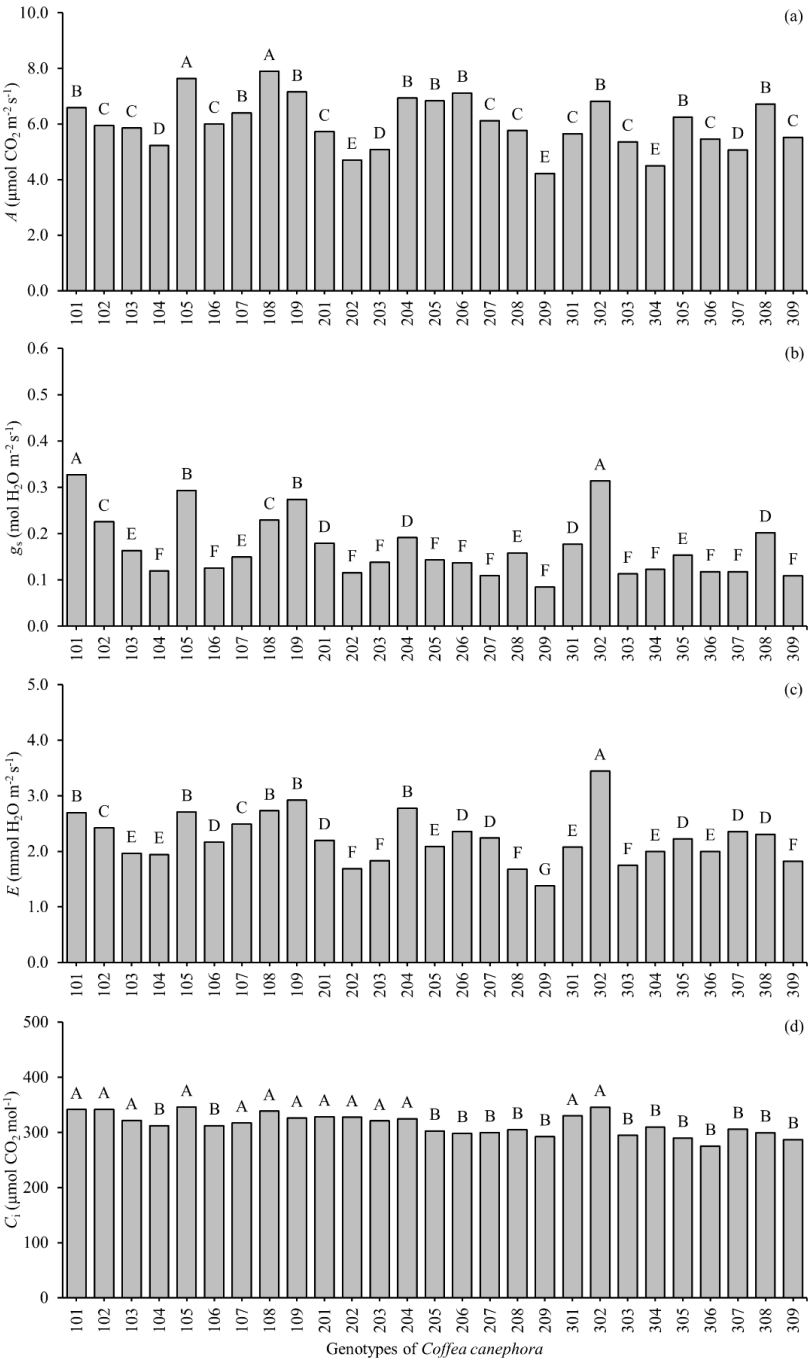

Figure 2. Weighted means for instantaneous readings of net assimilation rate of CO_2 (a), stomatal conductance (b), transpiration rate (c), and substomatic concentration of CO_2 (d) at the time most favorable to photosynthetic activity of 27 genotypes of *Coffea canephora*, throughout the reproductive cycle (Castelo, Espírito Santo State, Brazil).

For the photosynthetic rate, the genotypes 105 and 108 composed the group of higher means, while genotypes 202, 209, and 304 integrated the group with the lowest *A* (Fig. 2a). The genotypes 101 and 302 presented the highest mean stomatal conductance, while the genotypes 104, 106, 202, 203, 205, 206, 207, 209, 303, 304, 306, 307, and 309 formed the group with the lowest means (Fig. 2b). A strong or significant correlation between the crop yield and *A* was not verified; however, the phenotypic correlation was positive (ρ=0.3037). The average yield among all genotypes was 80.65 bags ha^{-1} (1 bag corresponds to 60 kg of processed coffee), ranging from 35.28 bags ha^{-1} for genotype 307 to 123.40 bags ha^{-1} for genotype 108, showing considerable variation among genotypes.

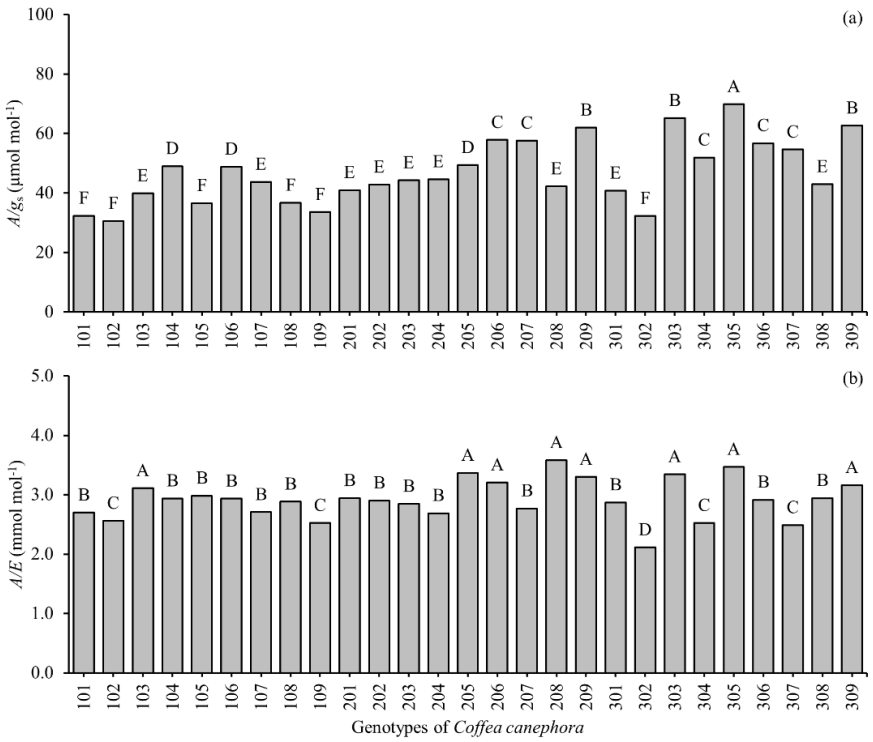

Figure 3. Weighted means for instantaneous readings of intrinsic water use efficiency (a) and instantaneous water use efficiency (b) at the time most favorable to photosynthetic activity of 27 genotypes of *Coffea canephora*, throughout the reproductive cycle (Castelo, Espírito Santo State, Brazil, 2017-2018).

Regarding the transpiration rate, genotype 302 alone composed the group with the highest mean, and genotype 209 was isolated in the group with the lowest mean (Fig. 2c). Genotypes 101, 102, 103, 105, 107, 108, 109, 201, 202, 203, 204, 301, and 302 formed the group of higher means for substomatal concentration of CO_2, while the others were grouped and presented lower means (Fig. 2d).

For intrinsic water use efficiency, genotype 305 formed the group with highest mean, while the group with the lowest means was formed by genotypes 101, 102, 105, 108, 109, and 302 (Fig. 3a). For instantaneous water use efficiency, the genotypes 103, 205, 206, 208, 209, 303, 305, and 309 formed the group with the highest means. The genotype 302 stood out for the lowest mean for *A*/*E* (Fig. 3b).

Regarding the relative content of chlorophyll *a*, the group with higher means was formed by genotypes 101, 102, 104, 106, 108, 109, 201, 203, 205, 206, 207, 208, 301, 302, 303, 304, 305, and 306 (Fig. 4a).

For chlorophyll *b*, the genotypes 101, 102, 104, 106, 108, 109, 203, 205, 206, 208, 301, 302, 305, and 306 formed the group with the highest contents; while genotypes 105, 202 and 308 integrated the group with lower means of Chl *b* (Fig. 4b). For the ratio between the relative contents of chlorophyll *a* and *b*, genotypes 101, 103, 105, 202, 204, 209, 303, 304, 305, 306, 307, 308, and 309 were included in the group of higher means (Fig. 4c). Genotypes 101, 102, 104, 106, 108, 109, 201, 203, 205, 206, 207, 208, 301, 302, 305, and 306 composed the group with the highest means for relative content of total chlorophyll, while genotypes 105, 202, and 308 formed the group with the lowest means (Fig. 4d).

Based on the determined characteristics (*A*, g_s, *E*, C_i, Chl *a* and Chl *b*), it was possible to estimate the dissimilarity measures between pairs of genotypes, which ranged from 1.51 to 202.32. The maximum distance was observed between genotypes 209 and 302 (from different groups for the ripening cycle), and the shortest distance was observed between genotypes 303 and 309 (from the same group for the ripening cycle) (Table 2). However, overall, there was a complexity of behaviors between genotypes, regardless of the ripening cycles to which they belong.

The relative contribution of each trait to the genetic divergence among genotypes was estimated based on the Mahalanobis dissimilarity matrix. Thus, it was verified that g_s was the variable that contributed the most to the overall divergence. The order of contribution of the variables was

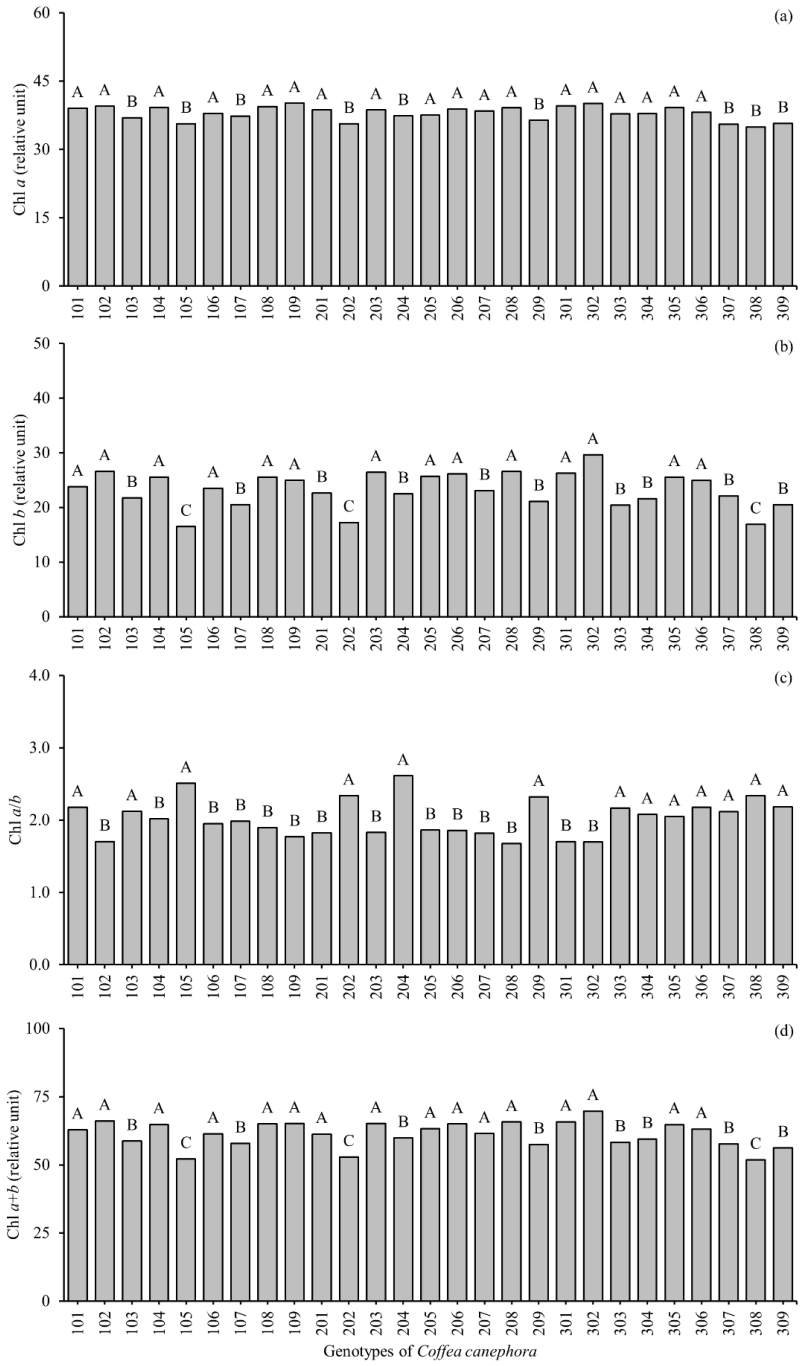

Figure 4. Weighted means for instantaneous readings of relative content of chlorophyll *a* (a), chlorophyll *b* (b), the ratio between chlorophyll *a*/*b* (c), and relative content of total chlorophyll (d) at the time most favorable to photosynthetic activity of 27 genotypes of *Coffea canephora*, throughout the reproductive cycle (Castelo, Espírito Santo State, Brazil).

as follows: g_s (62.23%) > *E* (12.56%) > *A* (11.22%) > Chl *b* (9.62%) > C_i (4.01%) > Chl *a* (0.36%). The higher number of different groups observed for the variables g_s and *E* during the previous comparison of means (univariate statistics) corroborates these results, as the multivariate analysis confirms that these variables significantly contributed to the genetic divergence. The clustering analysis allowed for the observation of the formation of ten groups of genotypes based on the dissimilarity between their pairs.

DISCUSSION

The differences observed among genotypes of Conilon coffee for the photosynthetic performance can be explained by the wide genetic variability found within the species,[9]

Table 2. Dissimilarity measures between pairs of genotypes, obtained by Mahalanobis distance and estimated from the study of characteristics of gas exchanges (A, g_s, E, and C_i) and relative chlorophyll contents (Chl *a* and Chl *b*), from 27 genotypes of *Coffea canephora*, throughout the reproductive cycle (Castelo, Espírito Santo State, Brazil)

Genotypes	102	103	104	105	106	107	108	109	201	202	203	204	205	206	207	208	209	301	302	303	304	305	306	307	308	309
101	29	78	117	20	115	100	46	15	63	141	96	62	104	116	140	85	169	62	20	137	120	89	123	124	63	138
102		19	33	30	34	31	17	13	10	56	22	16	34	41	50	23	72	8	38	52	40	25	43	43	29	54
103			8	48	7	9	28	45	3	15	6	14	10	18	16	6	23	5	105	10	14	9	14	18	12	11
104				90	3	11	47	69	10	14	3	23	13	16	7	11	13	10	130	7	7	9	6	10	33	8
105					77	59	25	16	42	97	78	35	68	76	95	64	131	49	51	93	94	63	93	94	26	93
106						4	37	62	10	17	8	15	7	8	2	13	20	13	127	6	11	6	6	10	23	6
107							30	49	9	21	17	7	13	11	6	22	32	16	109	12	15	8	12	11	15	12
108								13	24	79	42	12	24	25	47	31	98	22	55	61	67	25	52	63	26	61
109									32	100	59	22	54	56	77	51	122	33	20	86	78	41	70	75	33	87
201										20	7	9	16	22	19	11	32	2	81	17	13	10	17	17	14	19
202											17	43	36	45	22	30	8	28	174	8	8	33	23	17	36	11
203												24	15	22	17	6	17	4	113	13	10	12	11	16	32	15
204													16	15	21	26	59	14	65	32	30	11	25	23	13	31
205														3	11	8	36	15	121	16	31	5	12	29	22	15
206															8	17	45	22	123	20	35	5	13	29	27	19
207																22	22	24	148	7	15	9	6	12	30	7
208																	28	7	113	16	25	9	14	33	24	18
209																		36	202	7	10	32	16	20	50	8
301																			78	23	19	10	19	24	23	25
302																				166	135	100	136	126	93	164
303																					9	14	7	15	26	2
304																						20	12	5	34	11
305																							5	18	17	13
306																								11	27	5
307																									33	12
308																										24

D^2 maximum: 202.32 (209 and 302); D^2 minimum: 1.51 (303 and 309).

and this fact is reinforced by the estimated genetic parameters found in this study, which allowed the clustering of the genotypes for photosynthetic traits. This fact shows that using weighted means throughout the cycle may be a valuable descriptor to differentiate genotypes, as environmental factors normally significantly contribute to determine gas exchanges parameters meansured in instantaneous readings.

By analyzing the photosynthetic performance, it was also possible to observe the higher importance of some physiological variables in the study of genetic variability among genotypes of Conilon coffee, mainly due the results for g_s, E, A, and A/g_s, since they presented high genotypic determination coefficients (H^2) and variation indexes (CV_g/CV). Additionally, these photosynthetic parameters presented higher contributions to the genetic divergence, based on the dissimilarity between pairs of genotypes, which shows their potential value for variability studies and breeding programs focused on improving photosynthetic performance.

The methodology proposed in the present study made it possible to understand the photosynthetic performance and observe the existing variability between Conilon genotypes in a general and simplified way, since the objective was to analyze the existing variability and not the behavior of photosynthesis over time. It has already been established in the literature that photosynthesis of coffee trees is influenced by the seasonal variation of climatic aspects and phenological stages, with higher photosynthetic rates in rainy periods (fruit development) and lower in drought periods (vegetative rest).[12,22] However, it is expected to find different behaviors regarding the amplitude of this variation due to the large number of genotypes available for cultivation and the high genetic variability of *C. canephora*, as observed in the results of this experiment. This reinforces the prerogative that it is possible to analyze the genotypic differences for photosynthetic performance, in order to characterize the improved genotypes which are already available and to generate data for breeding programs to explore this important trait.

The highest photosynthetic rates were obtained from early-ripening genotypes (105 and 108), which can be explained by the strong demand for photoassimilates due to the faster development of the fruits (stronger metabolic sinks). Early-ripening genotypes have less time to complete the entire fruit development process than genotypes of later ripening cycles.[12] However, such increases in A were supported by higher g_s, E, and C_i, resulting in low intrinsic water use efficiencies and average values for instantaneous water use efficiency.

Genotypes 205, 206, and 305 presented considerable values of A, but lower g_s, E, and C_i, which allowed them to sustain carbon assimilation with greater instantaneous use efficiency of the transpired water, as well as greater relative content of total chlorophyll. This could be an important result for the selection of genotypes focusing on photosynthetic efficiency. The same could be observed for genotypes 103, 207, 208, 303, 306, and 309, however, with lower values of A.

Genotypes of Conilon coffee that present high water use efficiency and photosynthetic rate may be interesting alternatives to be explored in breeding programs for developing new cultivars capable of sustaining higher yield or tolerating drought conditions. It is noteworthy that genotypes such as 109 and 302, even with considerable values of A, presented increased values of g_s and E, resulting in decreased water use efficiencies (A/g_s and A/E). Genotypes like these may not be as sensitive to variations in the evaporative demand as others and may be subject to rapid dehydration in drought situations.[23] However, genotypes with higher stomatal conductance may present better evaporative cooling and, therefore, could show better response to warmer conditions.

Among the genotypes with the highest C_i, those that integrated the first two homogeneous groups for A stood out, such as 101, 105, 107, 108, 109, 204, and 302. For these genotypes, this fact may have sustained, at least partially, the increases in A.[24,25] It is reported in the scientific literature that, in general, coffee trees present low values of photosynthetic rates, mainly due to a mechanism of greater resistance to the diffusion of CO_2 in the stomata and leaf mesophyll.[11,26,27] In this context, the higher values of C_i associated with considerable values of A (*e.g.*, genotypes 101, 105, 107, 108, 109, 204, and 302) may be indicative of genotypes that present lower resistance to diffusion of CO_2 within the species.

Based on the relative chlorophyll content of leaves, it is inferred that the differences in Chl $a+b$ were more strongly influenced by the effect of the Chl b. However, it was not possible to observe a direct relationship between higher levels of relative chlorophyll content and higher rates of carbon assimilation. Chlorophyll plays a fundamental role in the processes of receiving and transferring light energy for photosynthesis.[28,29] In this context, the genotypes 101,

305, and 306 stood out in terms of relative chlorophyll content (Chl *a*, Chl *b*, Chl *a*/*b*, and Chl *a*+*b*), but reached modest results for *A*.

Another relevant point concerns the genotypes 202, 209, and 304 as they presented the lowest values of *A* and relatively low levels of Chl *a*+*b*, the latter being caused by a decreased Chl *b* since Chl *a*/*b* ratios were high. The decreased content of photosynthetic pigments in the leaf tissues may have caused lower absorption and use of light energy, which can reduce the rate of electron transport through the photosystems[30], leading to decreases in the assimilation of CO_2. This finding has already been reported in coffee under water deficit.[31]

The multivariate analysis, based on the study of determined characteristics of photosynthetic performance, made it possible to better understand the overall behavior of the genotypes. Sampling pairs of genotypes with the greatest distances in the dissimilarity matrix (*e.g.*, $D^2 \geq 100$) revealed that only four genotypes (101, 202, 209, and 302), were involved in the greatest distances. The smallest distances (*e.g.*, $D^2 \leq 20$), however, occurred as a function of nine genotypes: 103, 104, 106, 107, 201, 203, 303, 305, and 306. Both the most similar and dissimilar genotypes originated from all three groups regarding ripening cycles (early, intermediate, and late), leading to the conclusion that physiological performance may not be entirely linked to the ripening cycle. The variability among genotypes within each group is high enough to observe several patterns of similarity within and between groups regarding ripening cycles. This behavior has been previously reported for crop yield, bienniality, and several other agronomic traits.[5,32]

The duration of the ripening cycle is directly related to the length of time being exposed to environmental factors, as well as its capacity to influence the expression of variability among the genotypes. However, the sample of genotypes selected in this study appears to be clustered according to the similarity of photosynthetic performance, regardless of their maturation cycle. The absence of a trend of superiority among groups of coffee genotypes of different ripening cycles for several agronomic traits is a result of the high variability found among genotypes within each group.[5] Possibly for these reasons, genotypes from widely different maturation cycles (*e.g.*, similar results for *A*, g_s, and C_i of genotypes 101 and 302) showed similar behavior in photosynthetic performance, since the photosynthetic response may be more directly related to the strength of the metabolic sinks[12] than with the fruit maturation cycle.

Based on the Tocher optimization method, ten groups were identified among genotypes, clustering genotypes of different maturation cycles (early, intermediate, and late) regarding their photosynthetic characteristics. Group I was composed of the genotypes 105, 108, 207, 302, and 304, which presented extreme (highest and lowest values) results for *A*, g_s, and *E*. Group II was composed of the genotypes 106, 107, 203, and 205, which were grouped due to overall average results of *A* and low g_s. Group III clustered the genotypes 109 and 204, which also presented average values of *A* but associated with higher *E*. Group IV was formed by the genotypes 201, 202, and 305, which presented overall average or lower values of *A* and lower values of g_s and *E*.

Group V was composed of the genotypes 206, 301, and 308, characterized by average *A*; average to lower values of g_s; and low C_i. Group VI was formed by the genotypes 208, 209, and 309, which shared lower results for C_i. Group VII was composed of the late genotypes 303 and 306, which present late ripening cycle and average *A* and lower values of g_s, *E*, and C_i; as well as higher Chl *a*. Group VIII was composed of the early genotypes 101 and 102, which presented higher levels of Chl *a* and Chl *b*, associated with higher C_i. Group IX was composed of genotypes 103 and 104, which also present an early ripening cycle, but show average levels of Chl *a* and Chl *b*. Group X was formed by the genotype 307 alone, which presented lower *A*, g_s, and C_i, as well as lower Chl *a*.

CONCLUSIONS

It was possible to verify an expressive variability among the 27 genotypes for photosynthetic performance, even starting from a group that already comprises improved cultivars selected for several agronomic traits.

Among the physiological parameters, net CO_2 assimilation rate, stomatal conductance, and transpiration rate stood out due to their contribution to the overall variability and for allowing higher differentiation among genotypes.

Genotype 108 is highlighted due to the high photosynthetic rate, associated with higher relative content of chlorophyll, as well as a reasonable water use efficiency. Genotypes 205, 206, and 305 stood out in terms of water use efficiency and carbon assimilation.

The use of photosynthetic performance characteristics is an effective tool to study the variability among genotypes of Conilon coffee, which is an important result for breeding programs aiming to improve this trait.

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