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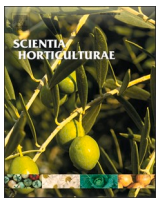

Genotypic diversity of *Coffea canephora* cv. Conilon identified through leaf morpho- and eco-physiological traits

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ABSTRACT

The knowledge about the genetic variability of a population is essential to increase the selection efficiency of promising genotypes to breeding programs. This study evaluated the genetic diversity among nine *Coffea canephora* genotypes based on leaf morphophysiological traits, *e.g.*, dry mass (DM), leaf mass per unit area (LMA) and the relative water content (RWC%), as well as the performance of the photosynthetic apparatus, through PSII functioning and electron transport, performed in summer and winter in three periods of the day. Three distinct groups were formed based on chlorophyll *a* fluorescence (Chl*a*F) parameters, demonstrating the heterogeneity of the genetic constitution of the evaluated population, what is quite relevant for the analysis of genetic divergence and breeding purposes, having the potential to identify superior genotypes. In summer and winter, the initial fluorescence (F_0) tended to increase in the morning and noon, while the quantum yield of the primary photochemistry (ϕP_0) increased in the afternoon. Leaf traits increased in the summer period in all groups. Group 1 showed more significant dissimilarity when compared to the others, with a higher mean of the most variable fluorescence parameters and a lower mean of the design index. The multivariate analyses showed that the leaf traits are correlated with the OJIP-test parameters concerning the variability in the periods and times studied. Our findings showed that the leaf traits can be adequately used to study genetic diversity in coffee. Additionally, ChlaF revealed to some extent some physiological differences among coffee genotypes associated with the two annual periods studied.

1. Introduction

The selection of new coffee varieties, through genetic improvement programs, aims to develop and select superior genotypes with characteristics such as high yields, stability of grain production, adaptability to growing conditions in different environments, tolerance to biotic and abiotic events, among other desirable agronomic characteristics (Partelli et al., 2019; Ferreira et al., 2020; Pontes et al., 2020). Despite selecting genotypes with desirable characteristics, there might be a variation associated with local and regional environment pressure. The phenotypic variation observed in a given population is due to the joint action of the environment and the genotype (Peloso et al., 2017). The phenotypic plasticity expressed by this interaction is vast and ranges from the thickness of the leaves and fruit skins to the size of the plant and

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the reproductive cycle (Partelli et al., 2014).

In Brazil, specifically in spring-summer, temperatures are usually higher than 30 °C along a substantial part of the day (Silva et al., 2004), negatively impacting the photosynthetic performance of plants. Under these conditions, there are interferences in the physiology of the coffee tree and its vegetative and reproductive development (DaMatta, 2018; Lemordant and Gentine, 2019). During this period, high evapotranspiration rates and greater water vapour deficit between air and leaves can induce stomatal closure (Melo et al., 2014; Medauar et al., 2021). This will reduce the use of light energy through photochemistry, what together with high incident irradiance on the leaves, likely increase the production of reactive oxygen species (ROS), with the potential to cause damage to the photosynthetic apparatus (Awasthi et al., 2015). However, despite these adverse events, coffee trees can recover after this period of stress due to physiological mechanisms of adaptability (Batista et al., 2010).

In the autumn-winter period, characterized by shorter days, milder temperatures, reduced light availability, and low relative humidity that will reduce vegetative growth of plants and affecting leaf morphological, anatomical, and physiological traits, associated with reduced photosynthetic activity and stomatal conductance (Partelli et al., 2010; Stirbet et al., 2018; Medauar et al., 2021). These events can ultimately affect coffee production (Camargo, 2010; Dubberstein et al., 2021).

Physiological indicators associated with edaphoclimatic variations in the environment-plant relationship have been used in improvement programs (Yusuf et al., 2010; Nascimento et al., 2019) and in understanding the mechanisms of action of various factors that can cause stress in plants (Bayat et al., 2018; Rastogi et al., 2019). In this context, the measurement of Chl *a* fluorescence, a technique that allows for an assessment of disturbances caused to the photosynthetic apparatus of plants, provides detailed information on photosystem II-PSII (Chen et al., 2015; Rosa et al., 2018), and can be helpful to choose promising genotypes. Furthermore, morphophysiological parameters such as leaf mass per area (LMA), density (DEN), succulence (SUC), leaf thickness (THI), and relative water content (RWC) (Chaturvedi et al., 2014) can vary according to the environment and its adaptations (Mendes et al., 2022). Therefore, environmental variations can be reflected in more sensitive organs, such as the leaves (Tripathi et al., 2020). Given the above, this study aimed to evaluate the genotypic diversity among nine genotypes of *C. canephora* based on morphophysiological traits and Chl *a* fluorescence parameters in the summer and winter periods at different times of day in the full-field sun. We undertook an investigation to answer the following set of questions (i) Is there genetic diversity among the *Coffea canephora* genotypes for leaf traits? (ii) Can ChlaF transient parameters be used for analysing diversity genetic in *C. canephora* under field conditions? We hypothesize that both leaf traits and Chl*a*F transient parameters can become useful tools to identify genetic diversity in *C. canephora* under field conditions.

2. Material and methods

2.1. Area, plant material, and experimental design

The research was conducted in the experimental area of the Federal University of Espírito Santo, in the city of São Mateus, ES, Brazil. The average altitude is 36 m, latitude 18° 40′ 25″ S, and longitude 40° 51′ 23″ W. The region has a tropical climate characterized by hot and humid summers, dry winters, and rainy spring-summer classified as Aw according to Köppen (Alvares et al., 2013). The soil is classified as an Argisol with a sandy loam texture, with undulating relief (Santos et al., 2018).

The planting of *C. canephora* plants (transferred from the nursery at the stage of 5 pair of leaves) took place in June 2018, using a spacing of 1.0 m in the row and of 2.0 m between rows under full Sun conditions. Fertilization management was performed according to Paye et al. (2019), together with cultural practices, such as weed control using herbicides and mowing, preventive control of pest and disease measures, liming, and drip irrigation. The climate indicators (maximum and minimum temperature and radiation) were measured at least 3–4 times per season through weather stations located 200 m away (https://portal.inmet.gov.br/) and the photosynthetic data were analyzed based on them. The data of maximum and minimum temperature, relative air humidity, and radiation were obtained from weather stations located 200 m away (https://portal.inmet.gov.br/) on November, 04 (summer 2019) and June 08 (winter 2020) period.

Table 1
Identification of the nine studied genotypes of *Coffea canephora* cv. Conilon (18 months old) in São Mateus, ES, Brazil.

Genotype	Clone	Cultivar	Maturation
G1	Pirata	Tributum	Early
G2	Verdin R	Tributum	Early/Medium
G3	Bamburral	Tributum	Medium/Late
G4*	A1	Andina/tributum	Medium
G5	Clementino	Tributum	Medium
G6	Beira Rio 8	Tributum	Early/Medium
G7	P1	Andina	Late
G8	Verdin TA	Andina	Medium
G9	NV2	Andina	Early

Note: Genotype G1 a G6 belong to cv. 'Tributun' (Partelli et al., 2020), whereas genotypes G4, G7, G8 and G9 integrate cv. 'Andina' (Partelli et al., 2019).

The experiment was performed using a randomized block design with three blocks, each with three plants per genotype. A total of nine plants per each of the nine genotypes (Table 1) were used. The nine studied clones belong to the two newly released cultivars Andina (Partelli et al., 2019) and Tributun (Partelli et al., 2020), with Pirata, Verdin R, Bamburral, A1, Clementino and Beira Rio 8 clones integrating cv. Tributun, whereas A1, P1, VerdinTA and NV2 integrates the cv. Andina. The nine coffee clones are the genotypes that make up the first two UFES cultivars, with high productivity. The first cultivar for the Espírito Santo region (below 500 m in altitude) and the Andean region for altitudes above 700 m. Both, cultivars available as treatments in the same environment and age.

2.2. Leaf traits

2.2.1. ChlaF analysis – transients Ojip and JIP-test

Chl*a*F measurements were performed with a portable fluorometer (Handy-PEA Model, Hansatech Instruments, King's Lynn, Norfolk, UK) to perform a JIP-Test analysis, according to a simple model of energy flow through PSII (Strasser et al., 2004; Chen et al., 2015), accurately used for coffee leaves (Rodrigues et al., 2016). Measurements were carried out in three plants per block at three times throughout the day, in the morning (7 h), noon (12 h), and in the afternoon (17 h), at least once a month (summer 2019 and winter 2020), using the third leaf of the fully expanded plagiotropic branch of the upper middle third of the coffee tree. To obtain the transients OJIP, leaves were previously dark-adapted for 30 min using leaf clips (Hansatech, UK) to turn the reaction centers into an "open" state (Q_A is oxidized).

The transients OJIP were induced by 1 s pulses of red light (650 nm, 3000 μmol m^{-2} s^{-1}), and the Chl*a*F kinetics (F_0 to F_M) was recorded from 10 s to 1 s. The Chl*a*F signal recorded at 20 μs (O-step, F_0) indicates the minimal fluorescence value immediately reached at the onset of illumination. The maximum fluorescence (P-step, F_M) was registered around 300 ms. J (2 ms) and I (30 ms) are inflection points between the O and the P levels. These Chl*a*F signals were used to calculate the parameters of JIP-test (Strasser and Strasser, 1995). A detailed description of parameters and their meaning can be found elsewhere (Rodrigues et al., 2016), and briefly addressed in Table 1.

2.2.2. Morphophysiological analyses

Three leaves of each genotype were collected from the 2nd pair of

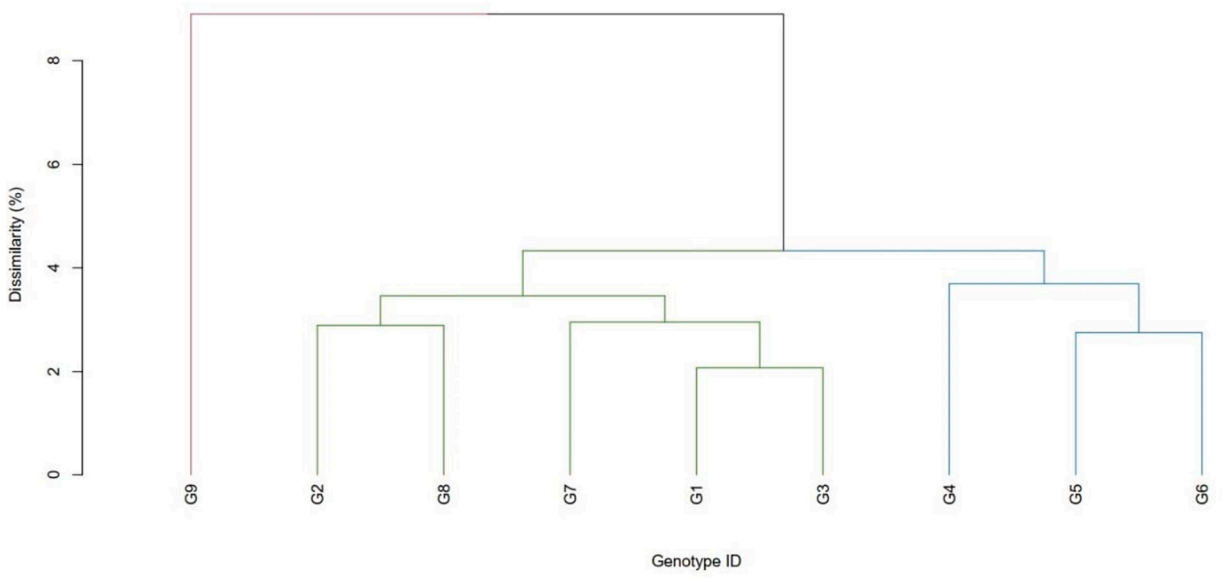

Fig. 1. Dendrogram representing the genetic dissimilarity among the nine studied genotypes of *Coffea canephora*, obtained by the UPGMA clustering method, using the Euclidean distance, considering the chlorophyll *a* fluorescence parameters.

completely expanded leaves from plagiotropic branches in the upper middle third part of the plant (well illuminated) in the morning (7 h) at least once a month. The samples were stored in clearly labeled paper bags, placed in ice boxes in order to reduce the water loss, and taken to the laboratory, where three discs were removed (total area = 0.78 cm^2) from each leaf blade which was weighed together, obtaining the fresh mass of the tissue (FM). After, the discs were placed in Petri dishes and hydrated with water distilled for 24 h to obtain the turgid mass (TM). The leaf thickness (mm - THI) was measured on turgid discs using a digital caliper (Digimess® 100.174BL 150 mm/6). Finally, dry mass (DM) of the leaf discs was obtained after drying the leaf discs for 72 h under 60° until constant weight. From the values of FM, TM, and DM obtained, the following leaf traits were calculated: i) leaf succulence (g m^{-2}) (SUC), obtained by the difference between TM and DM divided by the leaf disk area (Kluge and Ting, 1978), ii) and leaf mass per unit area (g m^{-2} - LMA), measured through ratio between DM and leaf area, iii) Leaf density (mg m^{-3} - DEN) obtained as DEN = LMA / THI (Witkowski and Lamont 1991), iv) Sclerophilia index values (g mm^2 - SCL) were calculated using the following formula: SCL = (DM/2) x leaf area (Rizzini, 1997), and v) the relative water content (RWC%), which was obtained using the formula: RWC = [(FM-DM) / (TM-DM)] x 100 % (Barrs and Weatherley, 1962).

2.3. Statistical analysis

For statistical analysis, the generalized Euclidean distance matrix was calculated as a measure of disparity to assess the genotype clustering using the hierarchical method of the Unweighted Pair Group Method using arithmetic means (UPGMA) being combined with photosynthetic indicators. Furthermore, principal component analyses (PCA) were performed to identify correlations between ecophysiological parameters. Spearman correlation analysis was performed between the standardized effect size of the raw data and the sample size. All statistical analyses were performed using the R software (R Core Team, 2020).

3. Results

3.1. Genetic dissimilarity

The UPGMA hierarchical method produced a dendrogram that illustrates the genetic distance between the genotypes studied for all parameters of transient Chl*a*F evaluated (F_0, V_J, V_I, F_M, φP_0, φE_0, φD_0, ABS/RC, TR_0/RC, ET_0/RC, DI_0/RC, RC/CS_0, PI_{ABS}), in two periods of the year and at three times of the day (Fig. 1). The maximum limit chosen, 45 % from the maximum fusion point of dissimilarity between the genotypes, was the value used for segregation, promoting the constitution of three groups. The first group was formed only by genotype NV2. In contrast, the second group integrated five genotypes (Verdin R, Verdin TA, P1, Pirata, Bamburral), and the third group another three (A1, Clementino, and Beira Rio 8).

After obtaining the groups by the UPGMA method, the mean of the parameters F_0, F_M, V_J, V_I, ϕP_0, ϕE_0, ϕD_0, ABS/RC, TR_0/RC, ET_0/RC, DI_0/

Table 2
Formulas of terms used in the JIP-test obtained from the chlorophyll *a* fluorescence transient evaluation (O-K-J-I-P).

Fluorescence Parameters Derived Parameters and OJIP Parameters	Description
$F_0 = F_{20\mu s}$	Minimal fluorescence when all FSII reaction centers are open
F_M	Maximum fluorescence when all FSII reaction centers are closed
Fv/Fm	Maximum quantum efficiency of photosystem II
$V_J = (F_{2ms}-F_0)/(F_M-F_0)$	Variable efficiency relative to 2 ms
$V_I = (F_{3ms}-F_0)/(F_M-F_0)$	Variable efficiency relative to 3 ms
Quantum Yield and Efficiency	
$\phi P_0 = TR_0/ABS = (1- F_0/ F_M) = F_V-F_M$	Primary photochemical maximum quantum yield at $t = 0$
$\phi E_0 = ET_0/ABS = (1-F_0/F_M)x(1-V_J)$	Q_A^- electron transport quantum yield for the electron acceptor intersystem
$\phi D_0 = F_0/ F_M = 1- \phi P_0$	Quantum yield at $t = 0$ for energy dissipation
$RC/CS_0 = \phi P_0(V_J/M_0)x(ABS/CS_0)$	Cross-section active Q_A reducing reaction centers in the FSII
Specific Energy Fluxes by FSII Reaction Center	
$ABS/RC = M_0 \times (1/V_J) \times (1/\varphi P_0)$	Absorption flux per reaction center
$TR_0/RC = M_0/V_J$	Energy flux captured per reaction center
$ET_0/RC = (M_0/V_J) \times \psi E_0 = (M_0/V_J) \times (1 - V_J)$	Electron transport flux per reaction center
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	Dissipated energy flux per reaction center
Performance Index	
$PI_{ABS} = (RC/ABS) \times (\phi P_0/(1 - \phi P_0)) \times (\psi E_0/(1 - \psi E_0))$	Vitality index for energy conservation for intersystem reduction

Source: STRASSER et al. (2000; 2004); CHEN et al. (2015). RODRIGUES et al., 2016.

Table 3
Mean JIP-test parameters per group of *Coffea canephora* genotypes as obtained by the UPGMA method. For parameter definition please refer to Table 1.

Parameters	Group 1	Group 2	Group 3
F_0	651.6250	629.8998	653.7641
F_M	2776	2837	2967
V_J	0.5517625	0.501046	0.489858
V_I	0.7987	0.780685	0.76954
ϕP_0	0.71655	0.590185	0.738498
ϕE_0	0.3238875	0.37009	0.366537
ϕD_0	0.283525	0.262593	0.264887
RC/CS_0	308.4874	317.938	317.3534
ABS/RC	2.530000	2.344428	2.403034
TR_0/RC	1.775487	1.705839	1.747738
ET_0/RC	0.7816	0.846831	0.866127
DI_0/RC	0.754725	0.636626	0.655325
PI_{ABS}	9.85050	13.87412	13.15191

RC, RC/CS_0, and PI_{ABS} were measured and calculated (Table 3), thus obtaining a better visualization for the discussion of the possible differences that led to the formation of the groups. The NV2 genotype (Group 1) presented the highest values of the parameters V_J and V_I and the lowest performance index (PI_{ABS}) values. The second group had higher F_0, ϕP_0, ABS/RC, and DI_0/RC values. The third group showed highest mean values of F_0, F_M,ϕP_0, and ET_0/RC and the lowest V_J values (Table 3). Some parameters were similar in the three groups, such as ϕD_0, while ϕE_0, ET_0/RC, RC/CS_0, and PI_{ABS} were closer in Groups 2 and 3.

3.2. Climate

In the summer period, temperatures were higher than those observed in winter (Fig. 2A). In summer, in the early morning, at 5:00, the temperature varied between 21.1 to 19.9 °C with radiation on average of 1.3 KJm^2 (Fig. 2B). At 7 h and 12 h, the temperature increased, reaching 24 °C and 32 °C, respectively. At 17 h, there was a sharp drop in temperature, which reached values close to 28 °C.

In winter, the temperature and solar radiation were milder compared to the summer period. Early in the morning, at 5:00, the temperature was around 18.6 to 19.2 °C, with no record of radiation at this time. Signs of radiation were registered only around 7 h when temperatures were around 19.8 °C. At 12 h, the temperature increased to 28.5 °C, followed by a minor decay at 17 h, when 23.7 °C was recorded (Fig. 2A).

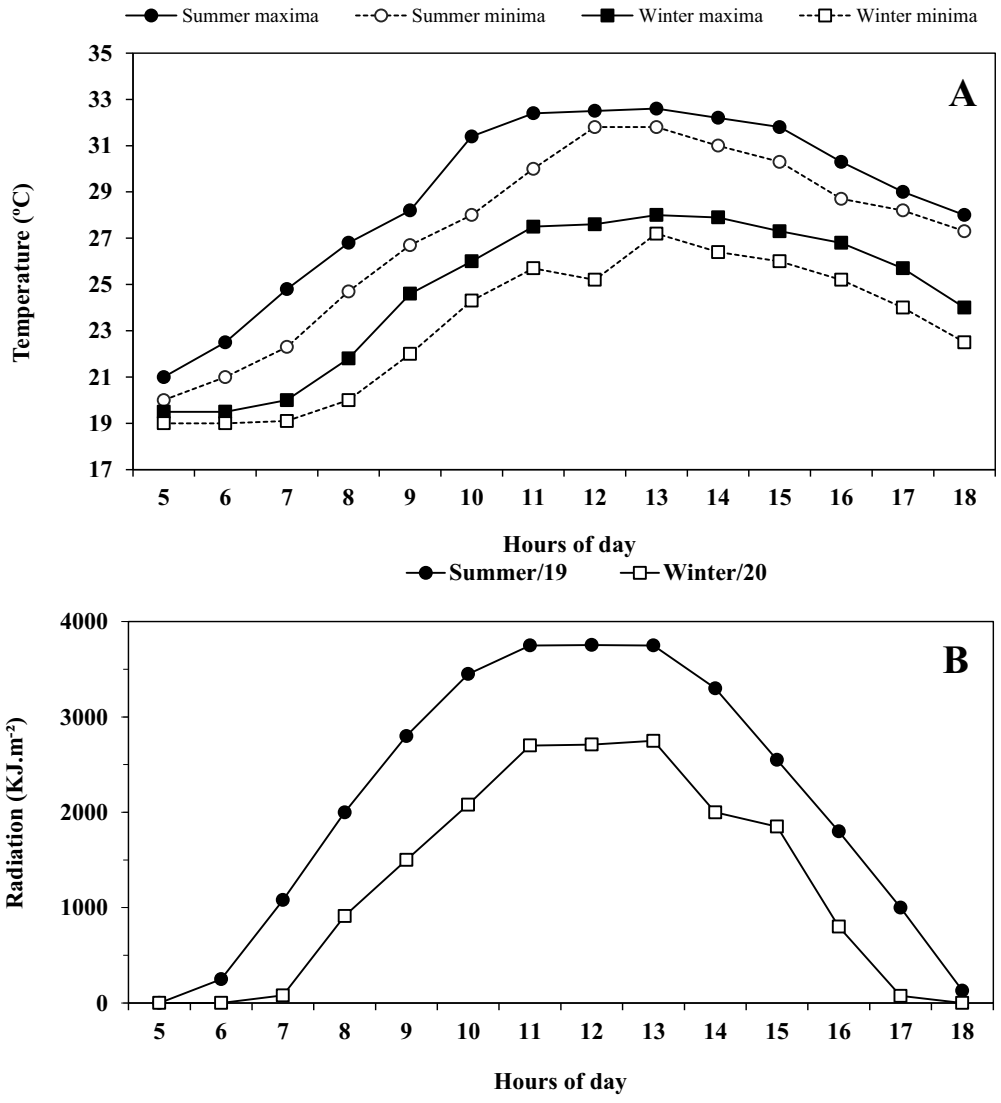

Fig. 2. Daily curve of Maximum and minimum temperature (A) and radiation at the times of the experiment (summer 2019 and winter 2020) (B) in São Mateus, ES, Brazil.

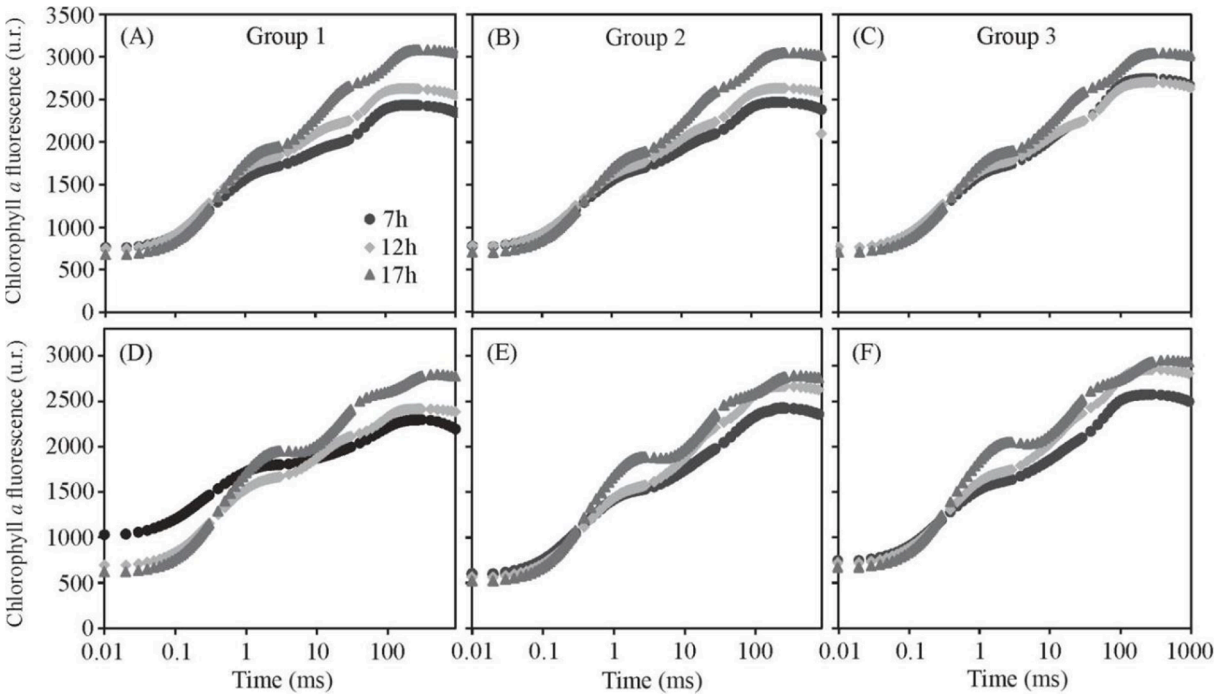

Fig. 3. Chlorophyll *a* fluorescence transient curve (OJIP) according to groups formed by UPGMA clustering of *Coffea canephora* genotypes in two periods of the year: summer 2019 (A-C) and winter 2020 (D – F) at three times of day: in the morning: (7 h), noon (12 h), and afternoon (17 h). Time is represented in the logarithmic scale (ms). Dark adaptation before the onset of measurements was 30 min.

3.3. OJIP curves

Regardless of the time of year, the OJIP curves obtained showed a characteristic polyphasic increase, starting from initial fluorescence level (F_0) to the maximal level (F_M), with well-defined intermediate J and I-steps (Fig. 3A–F). For Group 1, in summer, increased F_0 and reduced F_M values occurred in all periods, except at 17 h IJOP crves obtained at 12 h and 17 h showed sharp increases from step-J to P-step (Fig. 3A). In contrast, in winter, F_0 values reduced at 12 h and 17 h, with increases of J-step at 17h However, a partial suppression of OJIP transients was observed at 7 h and 12 h (Fig. 3D). For Group 2, step-J increased at 17 h during both summer and winter, with reductions in P-step at 7 h and 12 h in summer and 7 h in winter (Fig. 3B). During summer, there was an increase I-step and P-step at 17 h for Group 3 plants (Fig. 3C). In winter, J-step increased 17 h but I-step and P-step were similar to those reported at 12h P-step was suppressed at 7 h (Fig. 3F).

Group 1 showed a positive deviation in O-J and J-I phases in summer, where ΔVOP showed a slight drop (Fig. 4B). In phases O-I and I-P, negative deviations are observed at 17h For winter, positive O-J and negative O-I phase were observed at 17 h (Fig. 5B). For Group 2, negative phases were observed at 12 h and 17 h in summer (Fig. 4D) with negative deviations in O-I and J-I phases at 17 h in winter (Fig. 5D). In Group 3, there was positive deviations of O–I phase at 12 h and negative at 17 h in summer (Fig. 4F). For winter, positive and negative O-I phases were found at 17 h and 12 h, respectively (Fig. 5F).

3.4. JIP-Test parameters

For the three groups, the F_0 parameter tended to have higher morning and noon values. On the other hand, the F_M values were higher only in the afternoon in both periods (Fig. 6). Increases in V_J were observed in winter at 12 h in all groups evaluated, while higher values of V_I occurred only at 17 h (Fig. 6). Regardless of the group or period of the year, ϕP0 was higher at low light times (17 h). In contrast, ϕD_0 declined at 17 h in both summer and winter (Fig. 7). ϕE0 showed a tendency to decrease at 17 h only in winter (Fig. 7).

ABS/RC and TR_0/RC values showed trends towards higher values in the two-year periods, at 7 h and 12 h whilwe ET_0/RC tended to increase in winter at 7 h in all groups. For DI_0/RC, similar pattern for all groups was observed, with tendency to decrease and increase only for Group 1 at 7 h in winter (Fig. 8). The upward trend observed sometimes for DI_0/RC, associated with lower ET_0/RC values, led to a decrease of PI_{ABS}. In all groups, times and periods tended to have higher RC/CS_0 values.

3.5. Morpho-agronomic analyses

In general, leaf traits (leaf density, sclerophilia index, leaf thickness, leaf mass per unit area, and leaf succulence) showed a trend of lower values in winter and higher values in summer for all groups, with the exception of RWC that followed an opposite trend (Fig. 9).

3.6. Correlations

As shown in Fig. 10, THI values were tightly correlated with LMA (0.95), SCL (0.95), and CRA (−0.95) and moderately correlated with F_0 (0.59), ABS/RC (0.50), and DI_0/RC (0.58). SUC did not correlate with the other parameters. LMA was tightly correlate with SCL (1.00). DEN showed a positive correlations with SCL (0.96) and with RWC (−0.94), ϕP0 (−0.94), and ϕD0 (0.94). SCL showed a tightly negative correlation with RWC (−0.98).

For the JIP-test parameters, F_0 values were positively correlated with DI_0/RC (0.92) and F_M with ϕP_0. V_J was negatively correlated with ET_0/RC (−0.86) and ϕE_0 (−0.88) and PI_{ABS} (−0.90). On the other hand, V_I was negatively correlated with ϕE_0 (−0.85). The quantum yield parameters showed only a negative correlation (−1.00) between ϕP_0 and ϕD_0. Also, positive correlation among DI_0/RC x ϕD_0 (0.95) and negative among ABS/RC x RC/CS_0 (−0.94) were reported. PI_{ABS} was tightly correlated with V_J (−0.90) and with ϕE_0 (0.90).

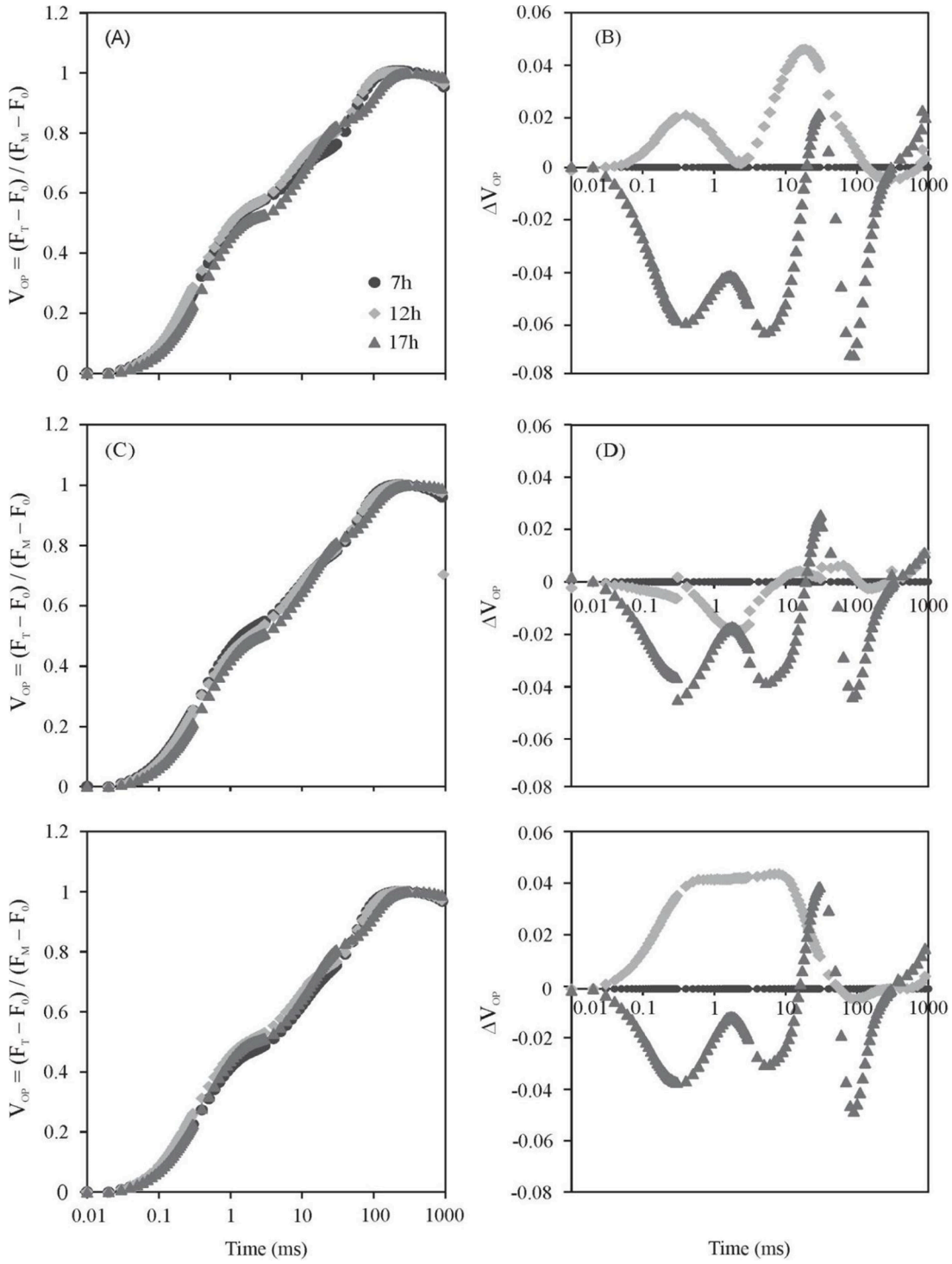

Fig. 4. Relative variable fluorescence $V_{OP} = (F_t - F_0)/(F_M - F_o)$ and kinetic differences (ΔV_{OP}) established between 0.01 and 1000 ms according to groups formed by UPGMA clustering of *Coffea canephora* genotypes in the summer period, at three times of day: in the morning (7 h), noon (12 h), and afternoon (17 h). (A,B) Group 1, (C,D) Group 2, (E,F) Group 3. Time is represented in the logarithmic scale (ms).

3.7. Principal component analysis

The first two PCA explained 77.2 % of the total variation of the data as a function of morphological and physiological characteristics and the two evaluation periods, divided by component 1 (53.3 %) and 2 (23.9 %) (Fig. 11). From the distribution of genotypes on axes 1 and 2, two distinct groups were formed: on the left side, genotypes influenced by summer, and on the right side, genotypes influenced by winter (Fig. 11A).

For the morphological and physiological characteristics (Fig. 11B), the first axis identified with parameters mainly of Chl*a*F. The variables with the highest correlation with this axis are ϕE_0, ET_0/RC, V_J, F_M, and V_I. The second axis was related to leaf traits such as RWC, THI, SCL, SUC, LMA, DEN, and photochemical traits ϕP_0, F_V/F_0, ABS/RC, ϕD_0, F_0/F_M, DI_0/RC, and F_0. The TR_0/RC parameter had a smaller contribution.

PCA was also performed using the physiological variables of the nine genotypes of *C. canephora*, showing a relationship between the evaluations carried out throughout the day (three hours) and in two-year

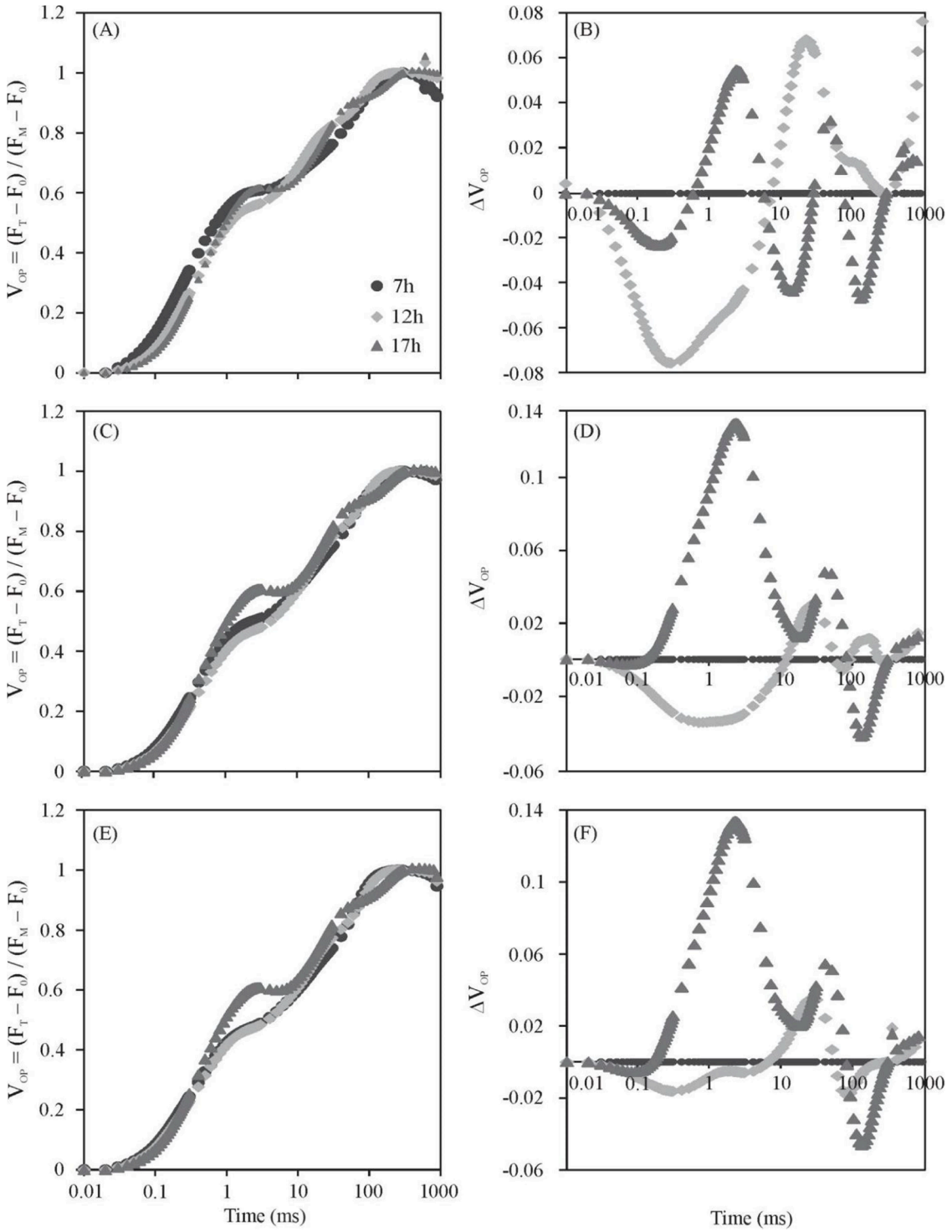

Fig. 5. Relative variable fluorescence $V_{OP} = (F_t - F_0)/(F_M - F_o)$ and kinetic differences (ΔV_{OP}) established between 0.01 and 1000 ms according to groups formed by UPGMA clustering of *Coffea canephora* genotypes in the winter period, at three times of day: in the morning (7 h), noon (12 h), and afternoon (17 h). (A,B) Group 1, (C,D) Group 2, (E,F) Group 3. Time is represented in the logarithmic scale (ms).

collection periods (summer and winter) (Fig. 12). In this case, only Chl*a*F data was used since the morphological data (RWC, THI, SCL, SUC, LMA, DEN) were analyzed just once a day.

The PCA explained 90.2 % of the total set variation, with axis 1 explaining 68 % of the data variance, and axis 2 accounted for another 22.2 % (Fig. 12). According to the genotype distribution on the axes, an intense variation in the formation of groups was observed. On the lower left side, the formation of a large group consisting of the afternoon time (H3) in the winter period stood out. Above, the formation of a group composed of the afternoon schedule, but referring to summer. On the right side, there was groups' formation, especially in the morning (H1) and noon (H2) times, in the summer and winter periods (Fig. 12).

4. Discussion

Gathering insights associated with the extent of genetic variability of

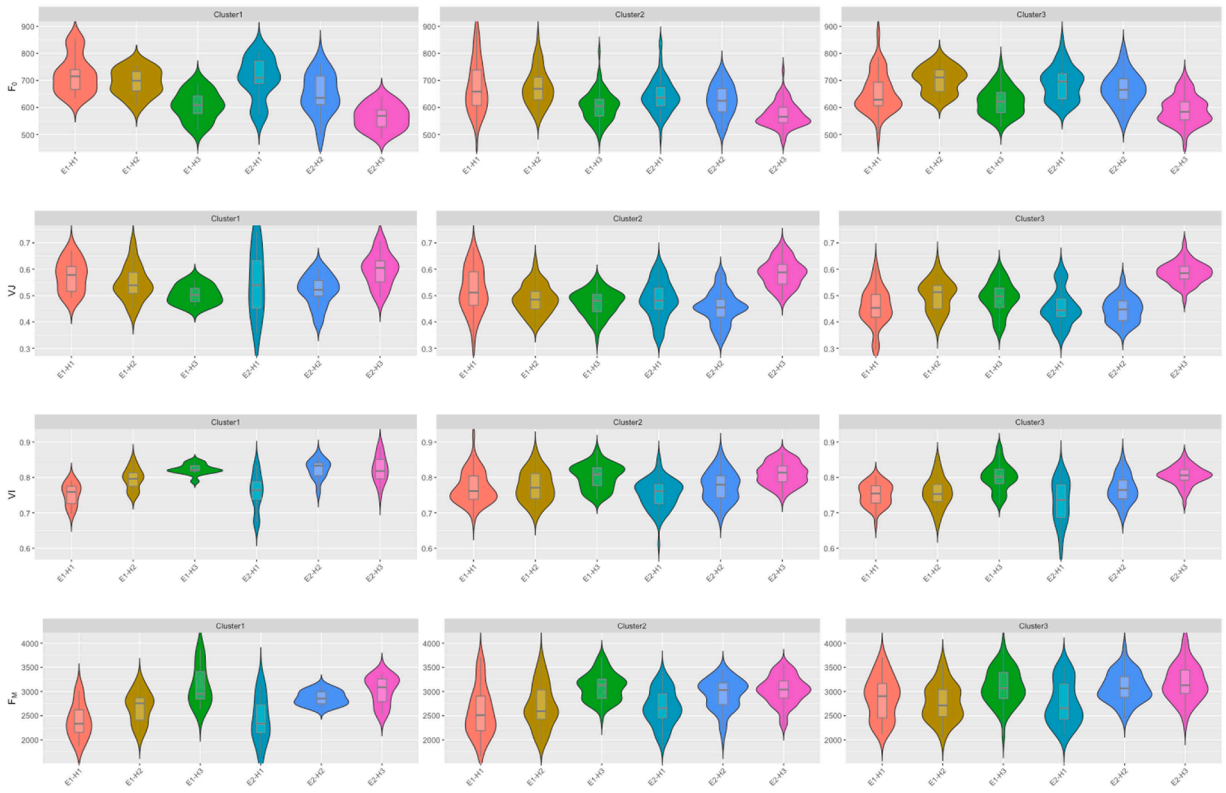

Fig. 6. Distribution of F_0, VJ, VI, and F_M in the visualization of groups defined as Group 1 (G9: NV2), Group 2 (G1:Pirata; G2: Verdin R; G3: Bamburral; G7: P1; G8: Verdin TA), and Group 3 (G4: A1; G5: Clementino; G6: Beira Rio 8) in two periods of the year (summer and winter) and at three times during the day when data were collected. Each colour represents a condition (period x time of day). The horizontal lines represent the 0.25 and 0.75 percentile from bottom to top, the inner line represents the median, and the vertical line represents the overall distribution. Periods of the year: E1 – Summer; E2 – Winter. Times: H1 – morning (7 h); H2 – noon (12 h); H3: afternoon (17 h).

a species or population provides essential information for use in selection and breeding programs, as such variability is vital to select/obtain varieties with high-stress resilience and yield performance (Oluoch et al., 2018). Genetic diversity studies of *C. canephora* genotypes have been done in recent years (Dalcomo et al., 2015; Covre et al., 2016; Giles et al., 2018, 2019; Martins et al., 2019; Dubberstein et al., 2020; 2021), but the actual study used morph physiological characteristics and Chl*a*F parameters to estimate the genetic diversity among *C. canephora* in both winter and summer periods.

The clustering by the UPGMA hierarchical method formed three genotype groups. Other authors have also reported this method (Covre et al., 2016; Giles et al., 2018, 2019; Dubberstein et al., 2020; 2021), while establishing the maximum limit of dissimilarity between genotypes. This method highlighted similar characteristics or genetic diversity in the groups formed (Chong et al., 2013; Goes et al., 2020), and the study of genetic diversity through multivariate techniques can be crucial to the planning of breeding programs (Guedes et al., 2013; Machado et al., 2017). Additionally, the JIP-test parameters were successfully used in the assessments in different periods and groups' formation or clusters in other crops, such as maize (Chiangoa et al., 2021).

The OJIP transients, and calculated parameters based on the curves, provide essential information about the structure and functioning of the photosynthetic apparatus of plants (Yusuf et al., 2010), including in *C. arabica* and *C. canephora* genotypes under high elevation and variable water deficit conditions along the year (Rodrigues et al., 2016). OJIP transients are very sensitive to light, and, in this study, it is clear that the periods of greater luminosity (7 h and 12 h) showed a decline in steps J, I, and P, in agreement with previous reports (Gonçalves et al., 2010; Zivcak et al., 2015; Serôdio et al., 2021). This suppression can be mainly attributed to the inhibition of electron transport on the donor side of the PSII or reduction in the size of the plastoquinone A (Q_A) pool, resulting in a partial blockage of energy flux (Mehta et al., 2010).

Environmental conditions, such as light, temperature, and air humidity, can affect photosynthesis's photochemical and biochemical processes (Kalaji et al., 2012; 2016; Rodrigues et al., 2016; Taiz et al., 2017). Also, plants can develop changes in the functional state of chloroplast thylakoid membranes, and structural changes in leaves, which can be quantified by Chl*a*F signals and leaf traits (Baker and Rosenqvist, 2004; Stirbet and Govindjee, 2011). Studies indicate that PSII is the most affected by high- and low-temperature stresses (Falqueto et al., 2010; Perboni et al., 2015).

The normalizations of Chl*a*F allow a better evaluation of the polyphasic behaviour of the OJIP curves (Strasser et al., 2010). The appearance of the positive band is considered a good indicator for identifying physiological disorders, even before they present visual manifestations. A positive band implies the smallest energy grouping among the PSII units (Strasser et al., 2004; Xiang et al., 2013), and the more positive, the lower the connectivity. Regardless of the period, despite the temperature variation, *C. canephora* genotypes showed tolerance similar to those observed in studies dealing with cold, thus related to temperature seasonality (Fortunato et al., 2010; Batista--Santos et al., 2011; Partelli et al., 2011; Ramalho et al., 2014). Seasonal variation in coffee plantations induces temperature fluctuations between 13 °C and 17 °C. Together with water restrictions, it can cause damage and affect the photosynthetic components of these plants, reducing stomatal conductance, photosynthetic rate, and photochemical efficiency of PSII (Partelli et al., 2013). In the present study, the lowest temperature recorded was 18.6 °C.

In the hotter months of the region (Alvares et al., 2013), the negative deviations resulted in an increased ability to reoxidize the Q_A pool to Q_B (Martins et al., 2015). A negative shift was found under colder conditions, with an increase in Q_A in the oxidized state, suggesting an increase

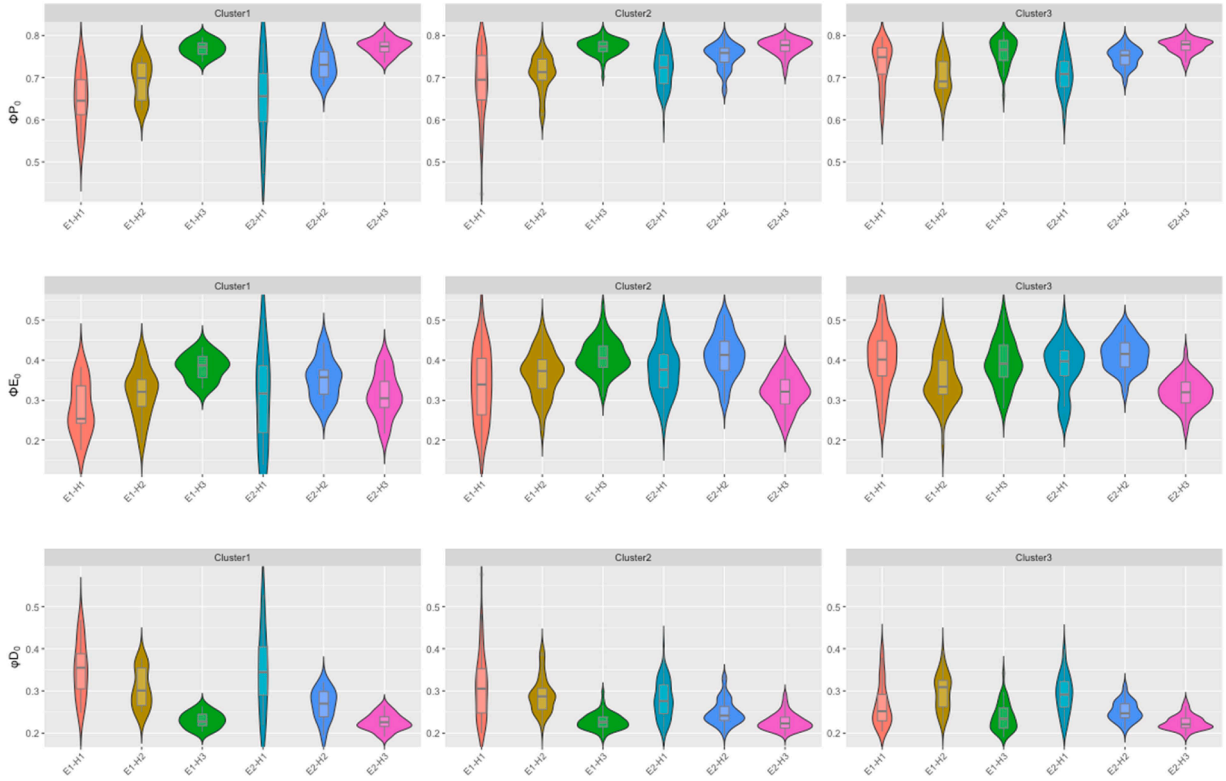

Fig. 7. Distribution of ϕP_0, ϕE_0, and ϕD_0 in the visualization of groups defined as Group 1 (G9: NV2), Group 2 (G1:Pirata; G2: Verdin R; G3: Bamburral; G7: P1; G8: Verdin TA), and Group 3 (G4: A1; G5: Clementino; G6: Beira Rio 8) in two periods of the year (summer and winter) and at three times during the day when data were collected. Each colour represents a condition (period x time of day). The horizontal lines represent the 0.25 and 0.75 percentile from bottom to top, the inner line represents the median, and the vertical line represents the overall distribution. Periods of the year: E1 – Summer; E2 – Winter. Times: H1 – morning (7 h); H2 – noon (12 h); H3: afternoon (17 h).

in the ability to reoxidize the quinone pool. The positive bands in hotter times indicate a disturbance in the thylakoid membranes, reducing the connectivity between the PSII reaction centers. F_0 and F_M values are also good indicators of effects caused by environmental stresses on photosynthetic machinery (Kalaji et al., 2017), with the increase of F_0 and the concomitant decline of φP_0, being associated with PSII impairments, as reported in *Coffea* genotypes under high irradiance (Ramalho et al., 2000), and heat (Rodrigues et al., 2016), with F_0 being also associated with over fluidity of chloroplast membranes (Tovuu et al., 2013) under stress exposure. Also, increased F_0 values may indicate the destruction of the PSII reaction center (RC) or a certain inability to transfer energy from the antenna to the RC (Baker and Rosenqvist, 2004). Therefore, the flow of electrons between quinone A (Q_A) and quinone B (Q_B) decreases, resulting in a reduction in PSII energy capture (Paunov et al., 2018). Signs of photoinhibition resulting from environmental conditions can cause some kind of stress (Perboni et al., 2015), even in plants grown in full sun as in this experiment, in which the maximum temperature was 31 °C for the hottest months (November to February) in the region, with a higher incidence of luminosity due to the period being characteristic of summer (longer days), favouring an increase in F_0 (Brestica et al., 2012; Chen et al., 2015).

Additionally, the maximum quantum yield of PSII (ϕP_0), initial fluorescence (F_0), and maximum fluorescence (F_M), the energy flux dissipated by RC (DI_0/RC), and the performance index (PI_{ABS}) are considered parameters sensitive to possible variations, such as temperature and radiation, being the most used in the identification of photoinhibition (Han et al., 2009; Ohada et al., 2011; Schansker et al., 2014), Increased F_M in the afternoon revealed that plants that receive lower light intensity do not affect the plastoquinone pool in the electron transport chain (Janusauskaite and Feiziene, 2012). However, reductions in periods of greater luminosity, such as noon, may indicate that the plants suffered some type of stress, resulting in a reduction in energy efficiency and the plastoquinone pool (Paunov et al., 2018).

The region where the genotypes were cropped is characterized by presenting marked summer and winter periods, with long days in summer and short days in winter, and temperature, RH% and radiation observed in Fig. 1. When combined with other environmental factors, high radiation decreases photosynthetic capacity, leading to inhibition (Ramalho et al., 2020; Dalmolin et al., 2015), which can influence plant development in different ways (Caron et al., 2014).

The ϕP_0 values represent the photochemical efficiency of the PSII (Chekanov et al., 2018), and its reduction indicates a deficiency in electron transfer (Jiang et al., 2008) and lowered photochemical use of energy. Reductions found in ϕE_0 may indicate photoinhibition, from a reversible photoprotective regulation to irreversible inactivation of PSII (Jiang et al., 2008). Furthermore, according to Mathur and Jajoo (2014), this reduction may be the result of an inadequate transmission of electrons and may be reflected in the increase in energy loss by dissipation (ϕD_0) (Hermans et al., 2003; Oukarroum et al., 2009). The increase in the total energy flux dissipated as heat by the reaction center (DI_0/RC) and ϕD_0 in the morning (7 h), in winter may indicate a reduction of active reaction centers that failed to direct the electrons to the plastoquinone (Kalaji et al., 2017). The dissipation of excitation energy in the form of heat (ϕD_0) prevents photoinhibition, working as a photoprotective mechanism (Chen et al., 2015; Kalaji et al., 2017), which was observed in coffee genotypes due both to increases in non-photochemical quenching processes and of the presence of zeaxanthin and lutein, which are known thermal dissipative and photoprotective pigments under cold, heat, and drought (Ramalho et al., 2000; Rodrigues et al., 2016; Ramalho et al., 2018).

The reduction observed in ET_0/RC and ϕE_0 indicate that the electron transport chain has been compromised (Redillas et al., 2011). Reduced

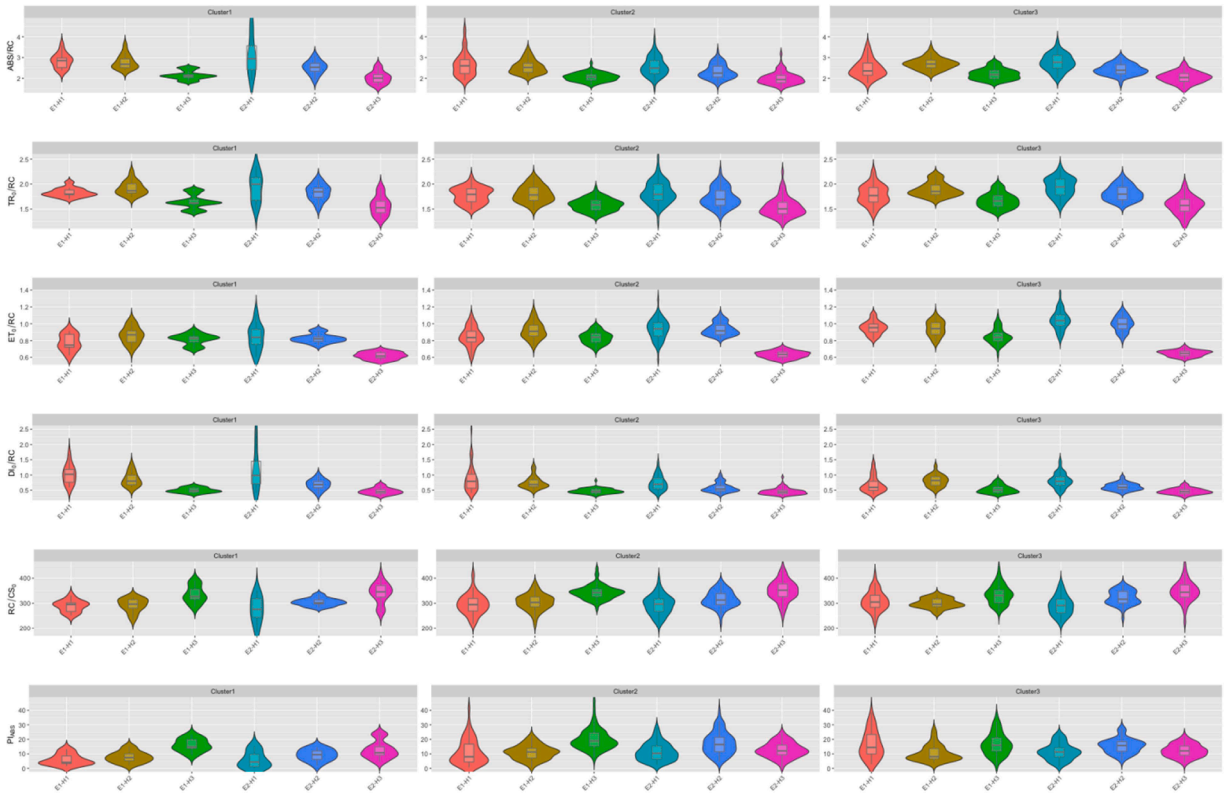

Fig. 8. Distribution of ABS/RC, TR_0/RC, ET_0/RC, DI_0/RC, RC/CS_0, and PI_{ABS} in the visualization of groups defined as Group 1 (G9: NV2), Group 2 (G1:Pirata; G2: Verdin R; G3: Bamburral; G7: P1; G8: Verdin TA), and Group 3 (G4: A1; G5: Clementino; G6: Beira Rio 8) in two periods of the year (summer and winter) and at three times during the day when data were collected. Each colour represents a condition (period x time of day). The horizontal lines represent the 0.25 and 0.75 percentile from bottom to top, the inner line represents the median, and the vertical line represents the overall distribution. Periods of the year: E1 – Summer; E2 – Winter. Times: H1 – morning (7 h); H2 – noon (12 h); H3: afternoon (17 h).

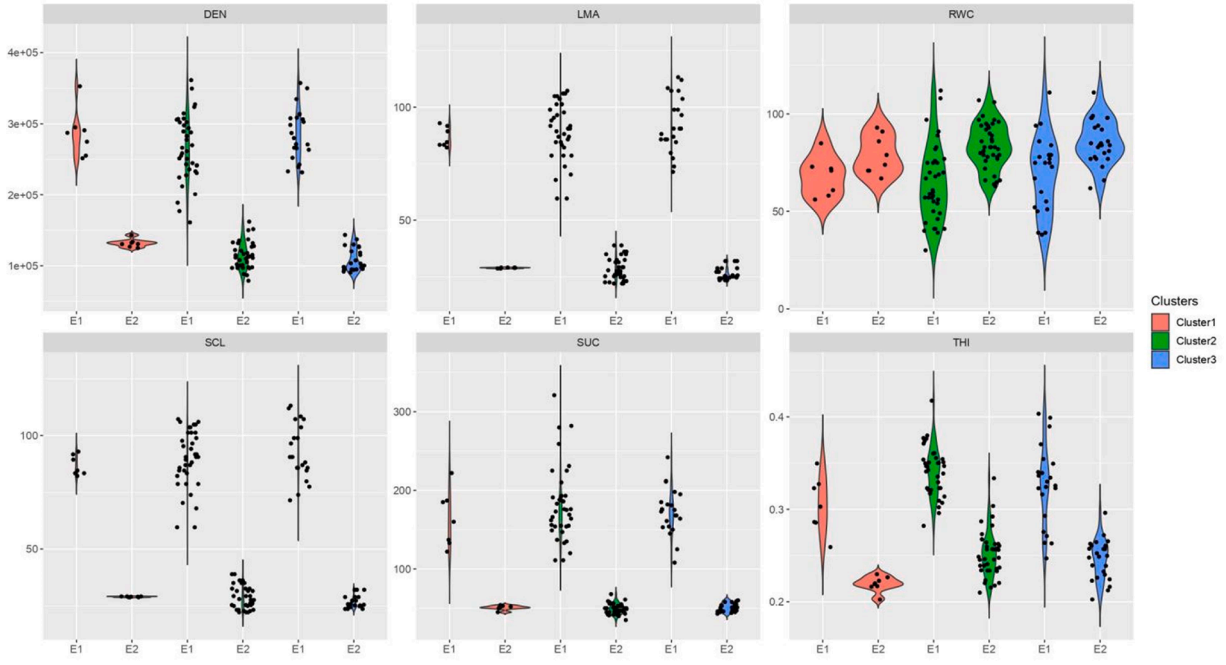

Fig. 9. Distribution of leaf density (DEN), leaf mass per unit area (LMA), relative water content (RWC), sclerophilia index values (SCL), leaf succulence (SUC), .and leaf thickness (THI) in the groups defined as Group 1 (G9: NV2), Group 2 (G1:Pirata; G2: Verdin R; G3: Bamburral; G7: P1; G8: Verdin TA), and Group 3 (G4: A1; G5: Clementino; G6: Beira Rio 8) in two periods of the year (summer and winter). The horizontal lines represent the 0.25 and 0.75 percentile from bottom to top, the inner line represents the median, and the vertical line represents the overall distribution. Periods of the year: E1 – Summer; E2 – Winter. Times: H1 – morning (7 h); H2 – noon (12 h); H3: afternoon (17 h).

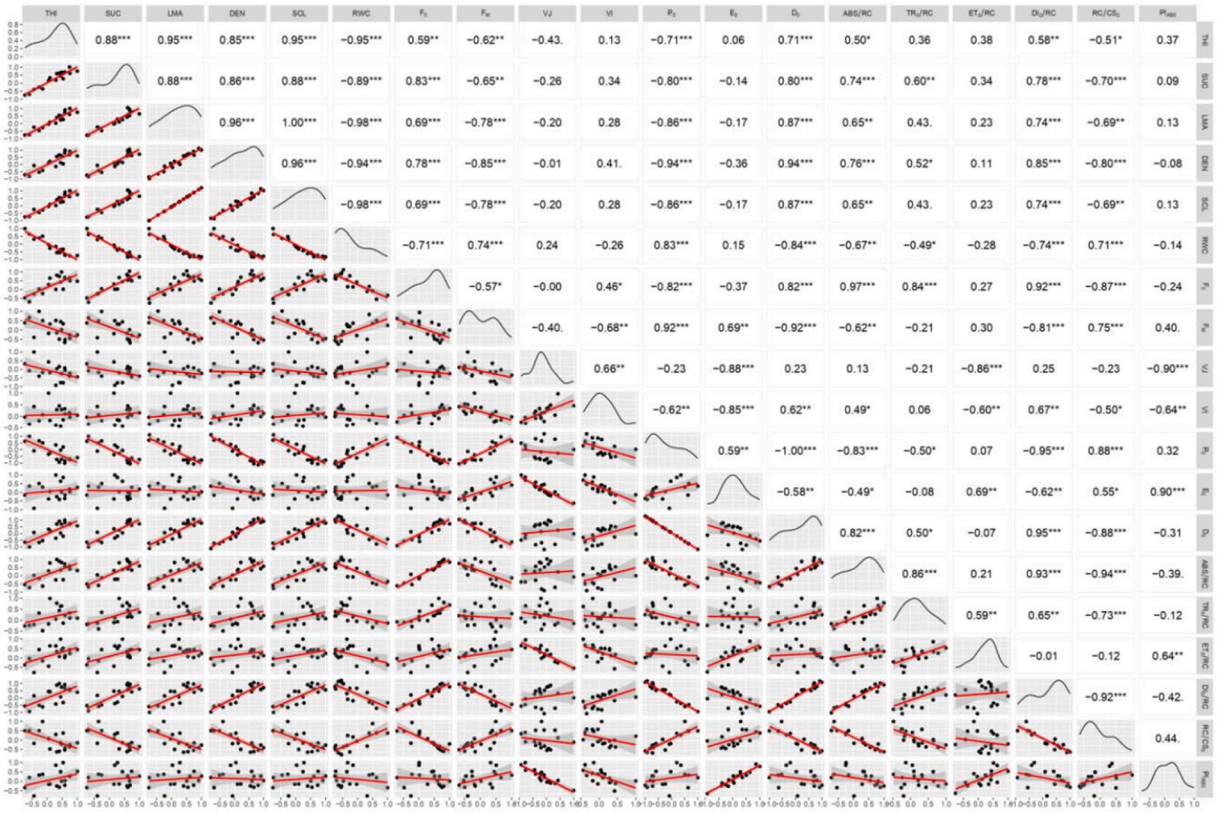

Fig. 10. Correlation between physiological and morphological characteristics studied among nine coffee genotypes.

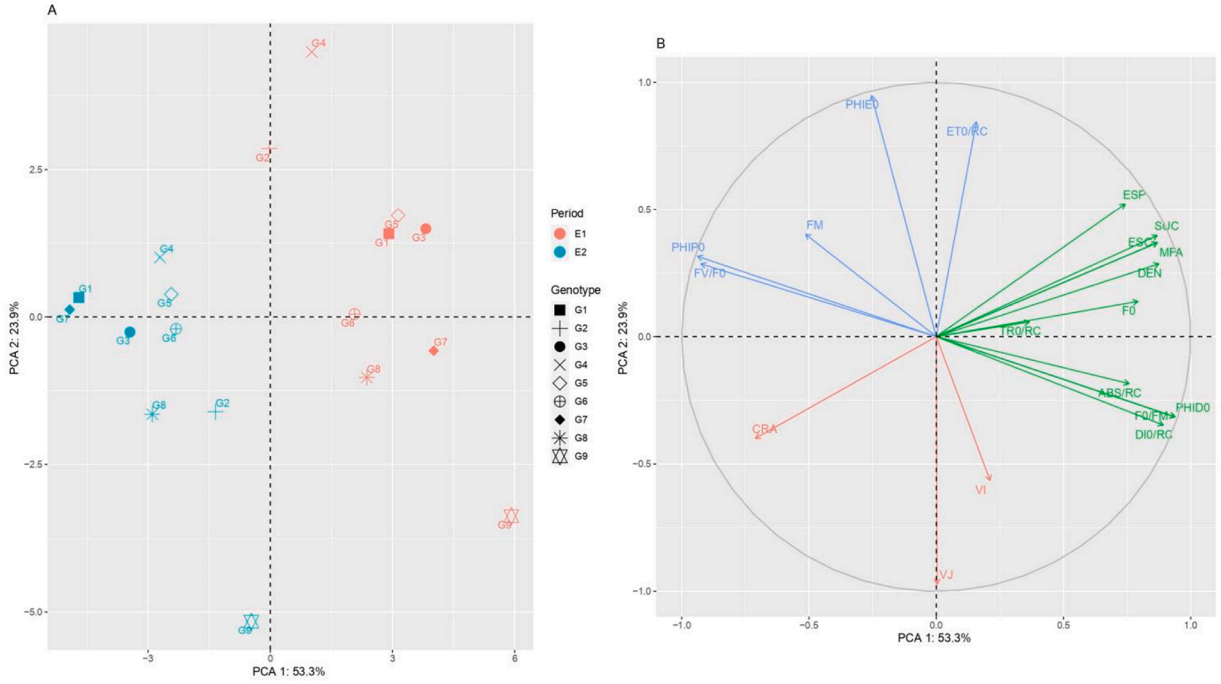

Fig. 11. Principal components analysis of morphological and physiological characteristics of nine coffee genotypes in two periods of the year. Nineteen variables allow separating period 1 (summer) from period 2 (winter) (A). The arrows represent each variable at various lengths based on the impact of each characteristic on genotype clusters (B). E1: summer; E2: winter.

ABS/RC parameter in the afternoon and noon indicate a defense against possible light stress. This stress causes changes in the size of the PSII antenna system, causing the inactivation of reaction centers (Kalaji et al., 2018), and is directly related to RC/CS_0. An increase in TR_0/RC proportional to the increase in ABS/RC may indicate a compromise in the oxygen evolution complex (Takahash and Murata, 2008). Also, while the increase in TR_0/RC induced that the entire Q_A was reduced, it could not oxidize back due to some stress factor. Thus, reduced quinone

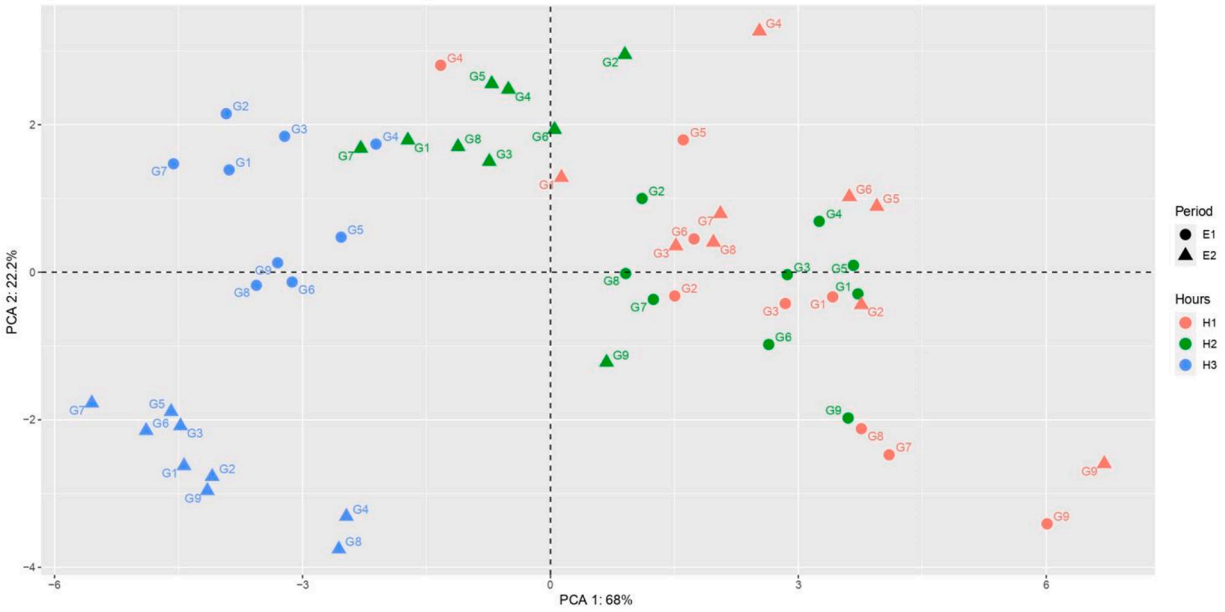

Fig. 12. Principal components analysis of physiological variables of nine coffee genotypes throughout the day in two periods. E1: summer; E2: winter. H1: morning (7 h); H2: noon (12 h); H3: afternoon (17 h).

A reoxidation ($Q_{\bar{A}}$) is inhibited, with no accumulation, and unable to transfer electrons to QB efficiently (Rathod et al., 2011).

PI_{ABS} is the JIP-test parameter regulated by energy absorption (ABS), excitation energy capture (TR), and excitation energy conversion (ET) (Chen et al., 2014). This parameter represents the performance of all photochemical processes related to PSII and is considered an indicator of plant vitality, especially concerning the effects of various environmental conditions (Strasser et al., 2004; Redillas et al., 2011; Stirbet et al., 2014). The reduction of PI_{ABS} in the morning suggests a decrease in photosynthetic performance associated with reduced electron transport capacity (Kalaji et al., 2018). Furthermore, the reduction can indicate a negative strain on the system, resulting in damaged PSII and PSI activity (Yusuf et al., 2010).

In general, leaf traits through their characteristics may reflect an environmental variation of the plant and be directly related to processes such as productivity and nutrients (Reich et al., 1992; Díaz et al., 2004). Leaf thickness is directly related to succulence, and is associated with the capacity of leaf tissues to store water (Melo Jr. and Boeger, 2016). In our case, this trait increased in the leaves collected and evaluated in the summer, a period marked by greater luminosity and high precipitation. Rosado and De Mattos (2007) analyzed some leaf characteristics, such as THI, SUC, LMA, and others, in a plant community in a Restinga area and concluded that there were higher values for these traits in the driest months of the year, similar to that found in the present study, except for RWC.

The data demonstrate the variability in each genotype group as a response to environmental stimuli throughout the day and the periods (summer and winter). Furthermore, they show how OJIP curves can be modified under different situations (Mehta et al., 2010; Yusuf et al., 2010; Kalaji et al., 2018; Paunov et al., 2018). Essential characteristics are presented through leaf traits (leaf characteristics), allowing inferences about the availability of resources and outstanding characteristics in genotypes in the study area (Díaz et al., 2004; Chaturvedi et al., 2014).

Plant phenotyping is the comprehensive assessment of complex plant traits, so that such tool should be fast, reproducible, and non-destructive approach. In fact, Chl*a*F parameters have been extensively used as a tool for plant phenotyping, especially under environmental stresses conditions (Chen et al., 2015; Rosa et al., 2018). However, *C. canephora* grown under non-soil water stress conditions, leaf traits such as DEN and LMA proved to be more sensitive, therefore, recommended for assessing genetic diversity under field conditions. On the other hand, these traits are time consuming and thus may limit the number of genotypes that can be evaluated on the same experiment. Finally, it should be taken into account that the experiment was carried out in a single location and with a low number of genotypes. Therefore, further field experiments should be performed with these tools to confirm our findings.

5. Conclusions

Genetic divergence was evidenced among the nine *Coffea canephora* genotypes evaluated, thus with this population denoting potential to be used for breeding purposes. Our results partially supported our initial hypothesis, since the leaf traits efficiently proved be a good traits to study genetic diversity. Nevertheless, Chl*a*F transient parameters revealed physiological differences among genotypes related to the year's two periods to some extent. Genotype 9 (NV2), belonging to Group 1 showed more significant dissimilarity, presenting itself as a cluster (group) isolated from the other genotypes. The parameters of the JIP-test showed increasing and decreasing trends for the diurnal periods evaluated, with more significant variation for the afternoon (17 h), regardless of the period of the year. Overall, leaf traits increased in the summer period in all groups. The correlation and principal components analysis showed a more significant correlation in this study for leaf traits and parameters of the JIP-test.

Credit author statement

We declare that all the authors have contributed, revised and agree with the text of the manuscript, both in the previous version as well in this resubmission.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data is on the manuscript.

Acknowledgments

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