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Hydraulic conductivity and photosynthetic capacity of seedlings of *Coffea canephora* genotypes

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Abstract

The aim was to investigate the morphological, photosynthetic, and hydraulic physiological characteristics of different genotypes of *Coffea canephora* under controlled cultivation conditions. Growth, conductance, and hydraulic conductivity of the root system of 16 *C. canephora* genotypes were evaluated in Experiment 1 (November 2013). In Experiment 2 (December 2014), in addition to the previous characteristics, gas exchange, photochemical efficiency, leaf water potential, and leaf hydraulic conductivity were investigated in five genotypes. No significant differences were observed in specific leaf hydraulic conductance, stomatal density, or gas exchange. The correlation between root hydraulic conductance and leaf area and dry mass indicates a physiological balance, reflecting the root system's ability to supply water to the aerial parts and maintain leaf water potential and photosynthetic activity during periods of high atmospheric evapotranspiration. These characteristics are important for genotypes cultivated under low water supply and high evaporative demand, even under irrigation.

Keywords: coffee robusta; conductance; photosynthesis; root system; water transport.

Introduction

Coffea arabica and *C. canephora* species (varieties Conilon and Robusta) dominate the global coffee market, accounting for 99% of global production, with Brazil as the leading producer (Pham *et al*. 2019). *Coffea canephora*, known for its stronger and more bitter taste compared to *C. arabica*, has stood out in recent years amidst climate change due to its robust characteristics and high heat tolerance (Rodrigues *et al*. 2016, Vilas-Boas *et al*. 2023).

Highlights

- *Coffea canephora* genotypes vary in hydraulic and photosynthetic efficiency, especially in root development
- More dry mass in the roots benefits the photosynthetic system
- Root conductance and conductivity are influenced by leaf area and the produced dry mass

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Abbreviations: AR – number of adventitious roots; CDM – cutting dry mass; CDS – cross-sectional diameter of the stem; DM – shoot dry mass; *E* – transpiration rate; F_v/F_m – PSII quantum yield; g_s – stomatal conductance; J – sap flow; K_L – leaf specific hydraulic conductance; K_r – root hydraulic conductance; $K_{r.LA}$ – hydraulic conductivity normalized by leaf area; $K_{r.RDM}$ – hydraulic conductivity normalized by root dry mass; $K_{r.RV}$ – hydraulic conductivity normalized by root volume; LA – leaf area; LDM – leaf dry mass; NL – number of leaves; PH – plant height; PI – photosynthetic performance index; P_N – net photosynthetic rate; RDM – root dry mass; RDM/DM – root/shoot dry mass ratio; RV – root volume; SD – stomatal density; SDM – stem dry mass; SLM – specific leaf mass; SPAD – Soil–Plant Analysis Development; Ψw – leaf water potential.

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In Brazil, the main producing regions of *C. canephora* have faced long periods of drought and high temperatures, along with lower mean annual precipitation (Venancio *et al*. 2019, Max *et al*. 2023). Consequently, in recent years, genetic improvement programs have focused on selecting genotypes adapted to environmental variations (Silva *et al*. 2010). Characterizing the physiology of this species is crucial for identifying genotypes with promising physiological traits capable of adapting to future environmental changes while maintaining high productivity (Semedo *et al*. 2021, Max *et al*. 2023).

Under water-restricting conditions, *C. canephora* genotypes show variations in vigor, leaf senescence, leaf water potential (Ψ_W), and productivity. Studies on *C. canephora* highlight the importance of maintaining Ψ_W as an indicator of water stress tolerance, attributing this capability to efficient stomatal control or effective water absorption by the root system (Pinheiro *et al*. 2004, Ronchi and DaMatta 2007). However, knowledge regarding the physiology of the coffee root system is limited due to difficulties in accessing this organ under natural conditions (Ronchi and DaMatta 2007).

Studies on roots are essential for water and nutrient extraction from the soil, as well as for contributing to plant stability and anchorage (Atkinson 2000, Schmidt *et al*. 2022). Genotypes vary considerably in their root and vegetative growth characteristics (Schmidt *et al*. 2022), with few studies simultaneously addressing multiple genotypes concerning growth, gas exchange, chlorophyll fluorescence, and conductance and conductivity, thereby limiting comprehensive understanding of these aspects in plants. Machado Filho *et al.* (2021) analyzed the hydraulic characteristics of roots and stems in three genotypes with different drought tolerance levels. Tolerant and moderately tolerant genotypes showed higher conductance and conductivity in roots and stems, correlating with greater root growth.

Understanding the root system and above-ground part enhances the selection of productive and resistant genotypes adapted to water and nutrient scarcity, thereby increasing the efficacy of fungicides and insecticides applied to the soil (Franco and Inforzato 1946, Rena and Guimarães 2000, Carvalho *et al*. 2008, Schmidt *et al*. 2022). The objective is to investigate the morphological, photosynthetic, and hydraulic physiological characteristics of distinct genotypes of *Coffea canephora* under controlled cultivation conditions.

Materials and methods

Plant material (seedling production): Genotypes of the *C. canephora* variety of the cultivar 'Vitória Incaper 8142' were evaluated, consisting of a group of 13 genotypes (1V–13V), in addition to the genotypes classified as sensitive (109a) and tolerant (14/86 and 120) to drought (Lima *et al*. 2002, DaMatta *et al*. 2003, Pinheiro *et al*. 2004, 2005; Praxedes *et al*. 2006). The seedlings came from the experimental farm of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), located in the municipality of Marilândia, State of Espírito Santo, Brazil (latitude 19°24'19"S, longitude 40°32'20"W).

The plants were grown in black polypropylene tubes measuring 190 mm in length and 63 mm in diameter, with a capacity of 0.28 L, filled with a substrate composed of a commercial mixture of pine bark, peat, charcoal, and vermiculite (*Basaplant*®). The substrate was enriched with 5% worm humus and 3 g of controlled-release NPK 19-6-10 fertilizer (*Osmocote*®) per tube, with a release period of 5 to 6 months.

Cultivation took place in an environment with 50% shading and intermittent sprinkler irrigation. After reaching commercial size, with three to four pairs of leaves, the seedlings were transported to the Universidade Estadual do Norte Fluminense – UENF, located in the municipality of Campos dos Goytacazes, State of Rio de Janeiro, Brazil (latitude 21°45'40"S, longitude 41°17'21"W). The plants were maintained at field capacity by irrigating twice a day.

Experiment 1: Sixteen *C. canephora* genotypes were evaluated, with four plants from each genotype assessed, totaling 256 seedlings. The evaluations took place in November 2013, 170 to 175 d after the cuttings were rooted. During the four days of measurements, the conditions in the greenhouse were as follows: relative humidity (RH) of 20–30%, air temperature (T_{air}) of 30–38°C, air vapor pressure deficit (VPD) of 3.5–4.5 kPa, and the PAR was 1,237; 1,285; 1,674; and 1,705 µmol(photon) m^{-2} s^{-1}.

Root hydraulic conductance and hydraulic conductance normalized by leaf area, dry mass, and root volume: The hydraulic conductance parameters were obtained according to the methodology described by Liu *et al.* (2001) and Li and Liu (2010), with the capture of exudate obtained by sectioning the aerial part. Briefly, after cutting, the exudate was captured using absorbent paper under different pressures, which was weighed on a precision balance at each applied pressure. The plants had their shoots removed under water to avoid embolism, leaving *ca.* 5 cm of the basal stem (Lopes *et al*. 2019, Machado Filho *et al.* 2021). Before root and stem conductance measurements, an initial pressure of 0.03 MPa was applied for 60 s to remove the remaining gas bubbles from the xylem and stabilize water flow (Lopes *et al*. 2019, Machado Filho *et al*. 2021). Thereafter, the pressure was gradually increased, *i.e*., 0.1, 0.2, 0.3, and 0.4 MPa (60 s at each pressure) inside a 'Scholander-type' pressure chamber (model *PWSC-3005*, *Soilmoisture Equipment Corp.*, USA). Sap obtained at each pressure step was collected and weighed on a precision balance (± 0.001 g precision).

The sap flow (J [kg s^{-1}]) was determined based on the mass obtained as a function of sap collection time (60 s). Sap flow was expressed in kilograms of water and plotted against the pressures applied [MPa] so that the slope of the curve was the K value [kg(H_2O) s^{-1} MPa^{-1}]. The root hydraulic conductance (K_r [kg s^{-1} MPa^{-1}]) was obtained by the relationship between the sap flow (J [kg s^{-1}]) and the pressure variation (P [MPa]).

Root hydraulic conductivity was calculated by normalizing the value of root hydraulic conductance by

dry mass ($K_{r.RDM}$ [kg s^{-1} kg^{-1} MPa^{-1}]), root volume ($K_{r.RV}$ [kg s^{-1} L^{-1} MPa^{-1}]), and leaf area ($K_{r.LA}$ [kg s^{-1} m^{-2} MPa^{-1}]) (Becker *et al*. 1999). With the J data obtained for each pressure applied under the root system of each evaluated seedling, the angular coefficients of the linear equations of root hydraulic conductance, root dry mass normalized hydraulic conductivity, root volume normalized hydraulic conductivity, and leaf area normalized hydraulic conductivity were determined (Becker *et al*. 1999, Knipfer *et al.* 2007, Gambetta *et al*. 2012).

Growth analysis: The root volume (RV) was obtained by immersing the roots in water in a graduated cylinder and determined by measuring the variation of the liquid column. The number of adventitious roots (AR) was determined by counting the roots that emerged from the cutting. Additionally, the number of leaves (NL) was also counted, and the leaf area (LA) was obtained using a *LI-3100* benchtop leaf area meter (*LICOR*, Lincoln, NE, USA) and expressed in cm^2.

After the destructive evaluations, the plants were placed in a forced ventilation oven at 70°C for 72 h until they reached a constant mass and weighed on an analytical balance. The following measurements were obtained: leaf dry mass (LDM), root dry mass (RDM), stem dry mass (SDM), cutting dry mass (CDM), specific leaf mass (SLM), shoot dry mass (DM), and the root/shoot dry mass ratio (RDM/DM). The specific leaf mass was calculated by dividing the leaf dry mass by the leaf area (SLM). The sum of the dry masses of stems, leaves, and cuttings was used to obtain DM. The root/shoot dry mass ratio was determined by dividing RDM by SDM.

Experiment 2: In the second experiment, conducted in December 2014, five genotypes selected based on the first trial were studied: 5V, 12V, 13V, 14/86, and 120. Each genotype was evaluated with 8 seedlings, totaling 40 seedlings. Growth characteristics, root and leaf hydraulic conductance, gas exchange, photochemical efficiency, leaf water potential, specific leaf hydraulic conductance, and stomatal density were analyzed.

The conditions in the greenhouse where the plants were acclimated during the four reading days were as follows: RH with an average of 48% at 8:00 h and 30% at 12:00 h; T_{air} ranged from 29°C at 8:00 h to 37°C at 12:00 h; DPV was between 2.1 and 4.4 kPa at 8:00 h and 12:00 h, respectively; PAR was a maximum of 642 and 1,312 µmol(photon) s^{-1} m^{-2} at 8:00 h and 12:00 h, respectively; and on this occasion, the greenhouse was covered with a screen with *Sombrite*® 50% light interception in addition to the plastic cover.

Hydraulic conductance: In this step, the method described by Liu *et al.* (2001) and Li and Liu (2010) for the determination of hydraulic conductance was modified as recommended by Gambetta *et al*. (2012). In this way, the aerial part was sectioned on the emitted branch of the rooting, cutting 5 cm from it, with the stem immersed in water to avoid air entry by reflux at the time of cutting. An initial pressure of 0.03 MPa was applied so that the flow started for a minimum of 3 min. Subsequently, the analysis pressures were applied to respect the minimum period of 3 min for flow stabilization, and the exudate was collected during 4 min of application of each pressure. During the root hydraulic conductance measurements, the seedlings were placed in a growth chamber with light conditions [200 µmol(photon) m^{-2} s^{-1}, relative humidity around 45%, and air temperature of 25°C] for 2 h before starting the process, as recommended by Emery and Salon (2002).

Leaf gas exchange: Gas exchanges were evaluated in two periods: between 08:00 and 09:00 h and between 12:00 and 13:00 h. Subsequently, the variations between these two moments for the obtained parameters were determined. The analyses were carried out with the aid of a portable infrared gas analyzer (IRGA *LI-6400 XT*, *Li-Cor*, USA), obtaining rates of net photosynthetic rate (P_N), transpiration (E), and stomatal conductance (g_s) in completely expanded leaves (third pair of leaves counted from the apex). A 6 cm^2 LED chamber was used, adjusted with a light intensity (PAR) of 1,000 µmol(photon) m^{-2} s^{-1}, an airflow of 500 mol s^{-1}, a reference CO_2 concentration of 400 ppm through the use of the CO_2 mixer, and a block temperature varying according to the environment.

Analysis of chlorophyll *a* fluorescence and SPAD: Chlorophyll (Chl) fluorescence parameters were obtained from readings, each one taken at 9:00 h and 13:00 h with the aid of a *Pocket PEA* fluorimeter (*Plant Efficiency Analyzer*, *Hansatech*, UK). After determining the area of the leaves, they were pre-adapted to the dark for 30 min with the aid of tweezers (*Hansatech*) so that all the reaction centers reached an 'oxidized' state. The reading was carried out on the same leaves where the gas exchanges were analyzed. The parameters of F_v/F_m (relationship between variable fluorescence and maximum fluorescence) and the photosynthetic performance index (PI) were obtained. In the same leaves in which the fluorescence of chlorophyll *a* was evaluated, the intensity of green (SPAD) related to the total Chl contents was determined using the *Portable Chlorophyll Meter*, model *SPAD-502* (*Konica Minolta*, Japan). Ten readings were performed, and the average per plant was determined.

Leaf water potential: Leaf water potential (Ψ_w) was measured at 5:00 and 13:00 h, according to the method described by Scholander *et al.* (1965), using a pressure chamber (Model *1000*, *PMS Instrument Co.*, Albany, OR, USA). For measurements taken at 5:00 h, leaves from the third pair of leaves were used, while for measurements taken at 13:00 h, the same leaves as those used for gas-exchange measurements were used. With the data obtained, the difference between the two evaluation times was determined. The leaf hydraulic conductivity (K_L) was obtained according to Ribeiro *et al*. (2009) by measuring leaf transpiration (E) by IRGA, measured at 13:00 h, divided by the difference between Ψ_W (5 h) and Ψ_W (13 h), and expressed in mmol m^{-2} s^{-1} MPa^{-1}.

According to the previous methodology, data on sap flow, root hydraulic conductance, leaf area (LA), number

of leaves (NL), root volume (RV), number of adventitious roots (AR), leaf dry mass (LDM), root dry mass (RDM), stem dry mass (SDM), cutting dry mass (CDM), specific leaf mass (SLM), shoot dry mass (DM), and root/shoot dry mass ratio (RDM/DM) were obtained, along with plant height (PH) and stem cross-sectional diameter (CDS). The diameter of the stem cross-section was determined on the branch issued from the rooting cutting by cutting five centimeters from it with the aid of a Starrett caliper.

Statistical analysis and experimental design: The design adopted in the first experimental trial was randomized blocks divided over time according to the evaluation days, employing four replications. In both experiments, the data were subjected to analysis of variance, and the means were compared using the *Scott-Knott* test at a 5% probability level. In the second trial, the design was randomized blocks with eight replications for each of the five evaluated genotypes. Root hydraulic conductance, and root hydraulic conductivity normalized by root dry mass, root volume, and leaf area were subjected to simple linear regression analysis using matrices in the System for Statistical Analysis (*Saeg*) software (*Funarbe*, BR). Additionally, a study of simple linear correlation between variables was conducted to construct a correlation matrix. *Pearson*'s coefficients were evaluated according to Callegari-Jacques (2003), which suggests that values between 0.00 and 0.30 indicate a weak linear correlation, between 0.30 and 0.60 indicate a moderate linear correlation, between 0.60 and 0.90 indicate a strong linear correlation, and between 0.90 and 1.00 indicate a very strong linear correlation. Statistical analyses were performed using *Assistat* software *version 7.7*.

Results

In the first experimental trial, significant differences were observed in the analyzed variables among the 16 genotypes (Table 1). Regarding leaf area, genotypes 1V, 2V, 3V, 4V, 5V, 11V, and 12V showed the highest means, while the remaining genotypes had the lowest means and did not differ significantly from each other (Table 1). For leaf dry mass, the genotypes were divided into three groups: the group with the highest values included 1V, 4V, 5V, 8V, 11V, and 13V; the intermediate group consisted of 2V, 3V, 7V, 10V, 12V, 14/86, and 109a; and the group with the lowest values encompassed genotypes 6V, 9V, and 120. For specific leaf mass, genotypes 1V, 2V, 3V, 7V, and 120 had the lowest means, while the other genotypes had the highest means, which did not differ significantly. In terms of the number of leaves, two groups were also formed, with genotypes 1V, 2V, 3V, 4V, 5V, 11V, and 12V showing the highest means.

The mean shoot dry mass values were distributed into four distinct groups. The genotypes 1V, 2V, 3V, 4V, 5V, 8V, 11V, 12V, and 13V showed the highest means. The genotypes 7V, 10V, 14/86, and 109a had intermediate means, followed by the genotypes 6V and 9V. The genotype 120 had the lowest mean. Root dry mass was divided into three distinct groups. The genotypes 2V, 3V, 4V, 5V, 8V, 11V, 12V, and 14/86 showed the highest means, followed by the genotypes 1V, 7V, 9V, 10V, 13V, and 109a. The genotypes 6V and 120 had the lowest mean. For the root volume variable, the highest means were observed in the genotypes 2V, 3V, 4V, 5V, 8V, 11V, 12V, and 14/86, followed by the other genotypes, which did not differ.

Table 1. Morphological and biomass characteristics of seedlings of 16 *Coffea canephora* genotypes. LA – leaf area; LDM – leaf dry mass; SLM – specific leaf mass; NL – number of leaves; DM – shoot dry mass; RDM – root dry mass; RV – root volume; AR – number of adventitious roots; RDM/DM – root/shoot dry mass ratio. Means (n = 6) followed by *the same letter* in the column do not differ according to the *Scott-Knott* test, at the 5% probability level. CV – coefficient of variation.

Genotype	LA [cm^2]	LDM [g]	SLM [g m^{-2}]	NL	DM [g]	RDM [g]	RV [cm^3]	AR	RDM/DM
1V	282.2^A	1.47^A	50.2^B	9.8^A	1.69^A	0.57^B	4.37^B	6.5^B	0.34^B
2V	255.6^A	1.30^B	50.8^B	9.5^A	1.63^A	0.70^A	5.37^A	4.8^C	0.42^B
3V	273.3^A	1.32^B	48.3^B	10.7^A	1.81^A	0.80^A	6.15^A	6.7^B	0.45^B
4V	263.8^A	1.40^A	52.5^A	10.7^A	1.68^A	0.72^A	6.07^A	10.5^A	0.43^B
5V	324.7^A	1.70^A	52.3^A	9.5^A	2.13^A	0.75^A	6.21^A	8.2^B	0.36^B
6V	169.3^B	0.95^C	56.7^A	8.3^B	1.12^C	0.43^C	3.77^B	3.2^D	0.38^B
7V	242.2^B	1.17^B	48.4^B	7.8^B	1.32^B	0.53^B	3.95^B	4.5^C	0.40^B
8V	280.4^B	1.56^A	55.1^A	8.7^B	1.83^A	0.72^A	6.30^A	7.6^B	0.39^B
9V	199.3^B	0.96^C	51.8^A	8.9^B	1.11^C	0.53^B	4.06^B	7.1^B	0.48^A
10V	225.4^B	1.22^B	53.6^A	9.3^B	1.42^B	0.53^B	4.10^B	5.0^C	0.37^B
11V	277.1^A	1.52^A	55.2^A	11.3^A	1.80^A	0.78^A	6.62^A	7.7^B	0.42^B
12V	251.5^A	1.32^B	52.6^A	9.6^A	1.62^A	0.88^A	7.16^A	9.8^A	0.54^A
13V	257.3^B	1.43^A	55.3^A	8.3^B	1.66^A	0.62^B	4.58^B	4.8^C	0.37^B
14/86	233.7^B	1.23^B	52.0^A	8.9^B	1.41^B	0.81^A	6.55^A	8.7^A	0.57^A
109a	209.9^B	1.19^B	56.4^A	7.9^B	1.34^B	0.55^B	4.37^B	6.0^C	0.42^B
120	168.0^B	0.74^C	43.0^B	8.0^B	0.82^D	0.35^C	2.89^B	7.0^B	0.43^B
CV [%]	13.94	15.90	18.30	13.94	17.07	21.76	20.56	20.85	12.65

For the number of adventitious roots, the means were distributed into four distinct groups: genotypes 4V, 12V, and 14/86 had the highest means; the second group included genotypes 1V, 3V, 5V, 8V, 9V, 11V, and 120; the third group was composed of genotypes 2V, 7V, 10V, 13V, and 109a; and genotype 6V had the lowest mean. Concerning the root/shoot dry mass ratio, the highest means were found in genotypes 9V, 12V, and 14/86, while the other genotypes showed means without significant differences among them.

Table 2 shows the analyses of conductance and hydraulic conductivity. The mean K_r values indicate that genotypes 3V, 5V, and 8V showed the highest means, while genotypes 9V and 120 exhibited the lowest values. For $K_{r.RDM}$, genotypes 1V, 2V, 3V, 5V, 6V, 8V, and 109a obtained the highest mean values, followed by genotypes 7V, 11V, 12V, 13V, and 120, whereas genotypes 4V, 9V, and 14 showed the lowest values. Regarding $K_{r.RV}$, genotypes 1V, 2V, 3V, 5V, 6V, 7V, 8V, 10V, 13V, and 109a achieved the highest mean values, followed by other genotypes that do not differ significantly. For $K_{r.LA}$, the highest means were observed in genotypes 2V, 3V, 5V, 6V, 8V, 10V, 11V, 12V, and 109a, followed by other genotypes that do not differ significantly.

The second experimental test was conducted with 5V, 12V, 13V, 14/86, and 120 due to their contrasting root hydraulic capacity. Therefore, in Table 3, only genotype 12V showed the highest values across all variables. For the number of adventitious roots, no statistical differences were found among genotypes. However, genotypes 5V, 12V, and 14/86 stood out in terms of root volume, while genotypes 12V, 13V, and 14/86 exhibited the highest means for the cross-sectional diameter of the stem.

In Table 3, regarding root dry mass, the highest means were observed in genotypes 12V and 14/86, followed by genotypes 5V and 120, while the lowest values were found

Table 2. Hydraulic conductance analysis in *Coffea canephora* seedlings. K_r – root hydraulic conductance; $K_{r.RDM}$ – root dry mass normalized hydraulic conductivity; $K_{r.RV}$ – root volume normalized hydraulic conductivity; $K_{r.LA}$ – leaf area normalized hydraulic conductivity of seedlings of 16 genotypes of *Coffea canephora*. Means (n = 6) followed by *the same letter* in the column do not differ according to the *Scott-Knott* test, at the 5% probability level. The square of the correlation coefficient (R^2) obtained in each linear regression was evaluated, verifying a mean value of 0.98 with a confidence interval of ± 0.2%. CV – coefficient of variation.

Genotype	K_r [kg s^{-1} MPa^{-1}]	$K_{r.RDM}$ [kg s^{-1} kg^{-1} MPa^{-1}]	$K_{r.RV}$ [kg s^{-1} L^{-1} MPa^{-1}]	$K_{r.LA}$ [kg s^{-1} m^{-2} MPa^{-1}]
1V	$1.28 \times 10^{-06\ B}$	$2.33 \times 10^{-03\ A}$	$3.03 \times 10^{-04\ A}$	$4.66 \times 10^{-05\ B}$
2V	$1.37 \times 10^{-06\ B}$	$2.13 \times 10^{-03\ A}$	$2.73 \times 10^{-04\ A}$	$5.43 \times 10^{-05\ A}$
3V	$1.61 \times 10^{-06\ A}$	$2.06 \times 10^{-03\ A}$	$2.66 \times 10^{-04\ A}$	$6.06 \times 10^{-05\ A}$
4V	$9.97 \times 10^{-07\ C}$	$1.57 \times 10^{-03\ C}$	$1.82 \times 10^{-04\ B}$	$3.87 \times 10^{-05\ B}$
5V	$1.72 \times 10^{-06\ A}$	$2.36 \times 10^{-03\ A}$	$2.87 \times 10^{-04\ A}$	$5.50 \times 10^{-05\ A}$
6V	$9.07 \times 10^{-07\ C}$	$2.17 \times 10^{-03\ A}$	$2.53 \times 10^{-04\ A}$	$5.44 \times 10^{-05\ A}$
7V	$9.81 \times 10^{-07\ C}$	$1.89 \times 10^{-03\ B}$	$2.49 \times 10^{-04\ A}$	$4.11 \times 10^{-05\ B}$
8V	$1.86 \times 10^{-06\ A}$	$2.72 \times 10^{-03\ A}$	$3.07 \times 10^{-04\ A}$	$6.71 \times 10^{-05\ A}$
9V	$6.94 \times 10^{-07\ D}$	$1.39 \times 10^{-03\ C}$	$1.78 \times 10^{-04\ B}$	$3.93 \times 10^{-05\ B}$
10V	$1.11 \times 10^{-06\ C}$	$2.33 \times 10^{-03\ A}$	$2.91 \times 10^{-04\ A}$	$5.25 \times 10^{-05\ A}$
11V	$1.32 \times 10^{-06\ B}$	$1.86 \times 10^{-03\ B}$	$2.16 \times 10^{-04\ B}$	$4.88 \times 10^{-05\ A}$
12V	$1.47 \times 10^{-06\ B}$	$1.77 \times 10^{-03\ B}$	$2.23 \times 10^{-04\ B}$	$5.95 \times 10^{-05\ A}$
13V	$1.04 \times 10^{-06\ C}$	$1.92 \times 10^{-03\ B}$	$2.47 \times 10^{-04\ A}$	$4.22 \times 10^{-05\ B}$
14/86	$9.21 \times 10^{-07\ C}$	$1.24 \times 10^{-03\ C}$	$1.53 \times 10^{-04\ B}$	$3.99 \times 10^{-05\ B}$
109a	$1.09 \times 10^{-06\ C}$	$2.09 \times 10^{-03\ A}$	$2.58 \times 10^{-04\ A}$	$5.40 \times 10^{-05\ A}$
120	$6.10 \times 10^{-07\ D}$	$1.84 \times 10^{-03\ B}$	$2.23 \times 10^{-04\ B}$	$3.74 \times 10^{-05\ B}$
CV [%]	14.95	21.72	20.35	15.16

Table 3. Evaluation of morphological traits in *Coffea canephora* seedlings. LA – leaf area; NL – number of leaves; RV – root volume; AR – number of adventitious roots; PH – plant height; RDM – root dry mass; LDM – leaf dry mass; DM – shoot dry mass; CDM – cutting dry mass; RDM/DM – root dry mass/shoot dry mass ratio from seedlings of five genotypes of *Coffea canephora*. Means (n = 8) followed by *the same letter* in the column do not differ according to the *Scott-Knott* test, at the 5% probability level. CV – coefficient of variation.

Genotype	LA [cm^2]	NL	RV [cm^3]	AR	PH [cm]	RDM [g]	LDM [g]	DM [g]	CDM [g]	RDM/DM
5V	333.14^B	9.00^B	9.50^A	9.25	17.08^B	1.09^B	2.38^B	3.90^B	0.80	0.28^B
12V	406.41^A	13.20^A	10.63^A	9.38	22.48^A	1.49^A	3.09^A	5.47^A	1.15	0.27^B
13V	317.43^B	6.75^B	6.50^B	6.63	19.09^B	0.95^C	2.14^B	3.77^B	0.92	0.25^C
14/86	329.82^B	9.75^B	11.25^A	9.13	18.24^B	1.37^A	2.29^B	3.96^B	0.89	0.35^A
120	301.96^B	9.25^B	8.13^B	8.63	19.56^B	1.02^B	1.94^C	3.40^C	0.79	0.29^B
CV [%]	10.83	17.84	16.02	20.21	10.92	14.23	10.38	16.71	18.19	17.77

in genotype 13V. Genotype 12V had the highest average for the shoot dry mass and leaf dry mass, while the lowest averages were observed for genotype 120. There was no significant difference in cutting dry mass among the studied genotypes. However, for the root/shoot dry mass ratio, genotype 14/86 showed the highest mean, followed by genotypes 5V, 12V, and 120, while genotype 13V exhibited the lowest mean.

The leaf area and leaf dry mass showed strong to very strong correlations with most variables, except for specific leaf mass, stem cross-sectional diameter, and K_r (Table 4). The stem cross-sectional diameter strongly correlated only with stem dry mass. Both K_r and specific leaf mass showed weak to moderate negative correlations with all observed variables. Root volume and root dry mass exhibited significantly strong correlations with most variables, except for specific leaf mass, cuttings dry mass, stem cross-sectional diameter, and K_r. Shoot dry mass showed very strong correlations with leaf area and stem and leaf dry mass.

There were no significant differences in gas-exchange parameters (P_N, g_s, and E) among the genotypes studied at 08:00 and 12:00 h (Table 5).

Concerning Chl *a* fluorescence data, the genotypes did not show statistical differences between each other for F_v/F_m and PI at both time points (Table 6). However,

Table 4. *Pearson* correlation analysis of morphological and hydraulic traits in five genotypes of *Coffea canephora* seedlings. LA – leaf area; LDM – leaf dry mass; SLM – specific leaf mass; CDM – cutting dry mass; CDS – cross-sectional diameter of the stem; SDM – stem dry mass; DM – shoot dry mass; RV– root volume; RDM – root dry mass; K_r – root hydraulic conductance. ** significant at the 1% probability level, * significant at the 5% probability level (*t*-test).

	LA	LDM	SLM	CDM	CDS	SDM	DM	RV	RDM	K_r
LA		0.92	0.01	0.64	0.39	0.81	0.90	0.64	0.68	0.37
LDM	**		0.39	0.62	0.55	0.89	0.97	0.67	0.75	0.31
SLM	ns	*		0.11	0.47	0.40	0.36	0.21	0.34	–0.04
CDM	**	**	ns		0.44	0.63	0.78	0.38	0.57	0.21
CDS	*	**	**	**		0.62	0.59	0.33	0.56	0.06
SBDM	**	**	**	**	**		0.94	0.62	0.77	0.24
SDM	**	**	*	**	**	**		0.64	0.78	0.31
RV	**	**	ns	*	*	**	**		0.80	0.08
RDM	**	**	*	**	**	**	**	**		0.20
K_r	*	*	ns	ns	ns	ns	**	ns	ns	

Table 5. Photosynthetic characterization of five genotypes of *Coffea canephora* seedlings. P_N – net photosynthetic rate; g_s – stomatal conductance; E – transpiration rate. Values are means, $n = 8$. There was no statistical difference between genotypes and times of evaluation by the *Scott-Knott* test, at the 5% probability level. CV – coefficient of variation.

Genotype	P_N [μmol(CO_2) m^{-2} s^{-1}]		g_s [mmol(H_2O) m^{-2} s^{-1}]		E [mmol(H_2O) m^{-2} s^{-1}]	
	8:00 h	12:00 h	8:00 h	12:00 h	8:00 h	12:00 h
5V	5.83	5.71	0.071	0.094	2.05	3.75
12V	7.44	6.52	0.091	0.100	2.50	3.97
13V	7.00	6.37	0.110	0.091	2.80	3.68
14/86	5.97	5.88	0.081	0.076	2.30	3.17
120	6.23	6.45	0.059	0.106	1.90	4.31
CV [%]	21.8	27.9	45.8	47.1	41.2	45.58

Table 6. Analysis of photosynthetic parameters in five *Coffea canephora* genotypes. F_v/F_m – PSII quantum yield; PI – photosynthetic performance index; SPAD – Soil–Plant Analysis Development. Values are means, $n = 8$. Values followed by *the same uppercase* (column) or *lowercase* (row) *letter* do not differ according to the *Scott-Knott* test, at the 5% probability level. CV – coefficient of variation.

Genotype	F_v/F_m		PI		SPAD
	9:00 h	13:00 h	9:00 h	13:00 h	
5V	0.73	0.70	1.19	0.92	36.4^{A}
12V	0.76	0.72	1.45	0.83	34.4^{A}
13V	0.74^{a}	0.68^{b}	1.28^{a}	0.71^{b}	37.5^{A}
14/86	0.74	0.70	1.05	0.62	32.3^{B}
120	0.73	0.69	1.00	0.59	28.6^{B}
CV [%]	7.2		15.8		11.8

genotype 13V exhibited a significant reduction in F_v/F_m and PI at 13:00 h compared to values obtained at 9:00 h. Considering SPAD values, genotypes 5V, 12V, and 13V had the highest means.

Genotype 12V had the highest K_r value, followed by genotypes 5V and 120, and genotype 14/86, with genotype 13V having the lowest value (Table 7). Concerning $K_{r.RDM}$, genotype 5V obtained the highest value, followed by 120, 13V, 12V, and 14/86, which differed among themselves. For $K_{r.RV}$, genotype 13V showed the highest conductivity, followed by genotypes 5V, 12V, and 120, while genotype 14/86 had the lowest value. For $K_{r.LA}$, genotypes were divided into only two groups, with the group with the highest average formed by genotypes 5V, 12V, and 120.

No significant differences among genotypes were found for specific leaf hydraulic conductance and stomatal density (Fig. 1*A*,*C*). For Ψ_w obtained in the morning period (5:00 h), there were no significant differences among genotypes (Fig. 1*B*). However, at 13:00 h, genotype 120 exhibited the highest Ψ_w value, followed by genotypes 5V, 12V, and 14/86, while genotype 13V showed the lowest value. These differences resulted in a lower $\Delta\Psi_w$ decline in genotype 120.

Discussion

The genotypes studied in this article were selected for their high productivity, disease resistance, and drought tolerance, displaying genetic characteristics closely related to the 'Robusta' and 'Conilon' groups.

Table 7. Hydraulic conductance and conductivity parameters of five *Coffea canephora* genotypes. K_r – root hydraulic conductance; $K_{r.RDM}$ – root dry mass normalized hydraulic conductivity; $K_{r.RV}$ – root volume normalized hydraulic conductivity; $K_{r.LA}$ – leaf area normalized hydraulic conductivity. Data were analyzed using analysis of variance, *F* test, at the 1% probability level, then simple linear regression analysis was performed using matrices, where values followed by *the same letter* in the column have the same regression equation. For this reason, the value of R^2 assumes little relevance.

Genotype	K_r [kg s^{-1} MPa^{-1}]	$K_{r.RDM}$ [kg s^{-1} kg^{-1} MPa^{-1}]	$K_{r.RV}$ [kg s^{-1} L^{-1} MPa^{-1}]	$K_{r.LA}$ [kg s^{-1} m^{-2} MPa^{-1}]
5V	$5.51 \times 10^{-7\,B}$	$6.09 \times 10^{-4\,A}$	$7.19 \times 10^{-5\,B}$	$1.79 \times 10^{-5\,A}$
12V	$7.17 \times 10^{-7\,A}$	$4.93 \times 10^{-4\,D}$	$7.19 \times 10^{-5\,B}$	$1.79 \times 10^{-5\,A}$
13V	$4.58 \times 10^{-7\,D}$	$5.16 \times 10^{-4\,C}$	$8.32 \times 10^{-5\,A}$	$1.45 \times 10^{-5\,B}$
14/86	$4.90 \times 10^{-7\,C}$	$3.74 \times 10^{-4\,E}$	$4.48 \times 10^{-5\,C}$	$1.49 \times 10^{-5\,B}$
120	$5.51 \times 10^{-7\,B}$	$5.37 \times 10^{-4\,B}$	$7.19 \times 10^{-5\,B}$	$1.79 \times 10^{-5\,A}$
R^2	0.87	0.64	0.57	0.79

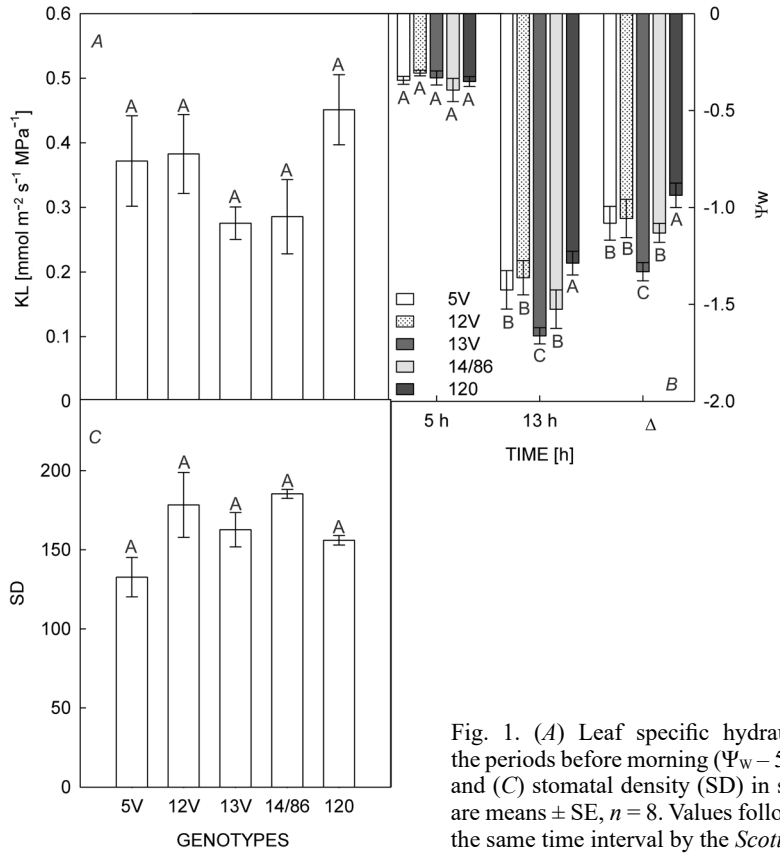

Fig. 1. (*A*) Leaf specific hydraulic conductance (K_L), (*B*) leaf water potential in the periods before morning (Ψ_W – 5:00 h), at noon (Ψ_W – 13:00 h) and its variation ($\Delta\Psi_W$), and (*C*) stomatal density (SD) in seedlings of five *Coffea canephora* genotypes. Values are means ± SE, $n = 8$. Values followed by *the same letter* do not differ from each other in the same time interval by the *Scott-Knott* test at the 5% probability level.

The materials in the first experimental test showed significant variations in root hydraulic conductance. This parameter reflects the root system's ability to supply water to the plant's aerial part, which is crucial for seedling development, although its complete influence is not fully clarified yet (Becker *et al*. 1999). Therefore, root hydraulic conductance was normalized (by root volume, leaf area, and root dry mass). Thus, the traits related to drought tolerance and sensitivity and data on root hydraulic conductance normalized by root dry mass were crucial in selecting clones for the second study. Hence, genotypes 5V, 12V, 13V, 120, and 14/86 were chosen.

Although *C. canephora* is known for its drought and heat tolerance, the main producing regions are subject to irregular rainfall and lower precipitation, significantly influencing plant phenology and production (Venancio *et al.* 2019, Max *et al.* 2023). Therefore, the water maintenance capacity of these genotypes is crucial for future studies on adaptation and productivity under water stress conditions (Silva *et al*. 2010).

Regarding the parameters of conductance and conductivity, the results of the second experiment confirm the genotype order observed in the first. However, values were reduced by approximately 60–70% in the second trial. This decrease is attributed to the interference of non-active barriers, as reported by Knipfer *et al.* (2007), who recommend a stabilization period for flow measurements (Gambetta *et al*. 2012). Nevertheless, when evaluating numerous genotypes, this stabilization period may not be feasible due to the influence of sap flow (J) by external conditions over time (Emery and Salon 2002).

The genotypes 120 and 14/86 are known for their drought resistance, while genotype 109a is considered sensitive (Lima *et al*. 2002, DaMatta *et al*. 2003, Pinheiro *et al*. 2004, 2005; Praxedes *et al*. 2006). However, no significant difference was observed in the two experimental tests presented in this study when comparing the three genotypes for root hydraulic conductance and conductivity. This indicates that this parameter alone is not sufficient to explain such characteristics. Thus, other plant components should be considered regarding hydraulic conductivity investigation (Pallardy *et al*. 1995, Sperry 1995) and water-use efficiency.

Another issue concerns the possibility of normalizing root hydraulic conductance, as several authors assert that this parameter is influenced by aquaporin activity, which cannot be measured through gravimetry or volumetry (Tyerman *et al*. 2002, Maurel *et al*. 2008, Vandeleur *et al.* 2009, Gambetta *et al*. 2012). However, root hydraulic conductance significantly correlated with morphological measures such as root dry mass.

The significant correlations between root hydraulic conductance and leaf area supported that leaf area normalized hydraulic conductivity could be an indicator of water sufficiency from roots to shoots (Whitehead *et al*. 1984, Tyree *et al*. 1998, Roubelakis-Angelakis 2009). However, it is important to note that leaf area-normalized hydraulic conductivity showed higher values for genotypes known to be drought-sensitive (109a) compared to those considered more tolerant (14/86 and 120). Therefore, it is not recommended as a definitive indicator of this characteristics.

The results indicate that the genotypes varied significantly in their leaf water potential at 13:00 h, with genotype 120 showing the best performance in this aspect, possibly due to its high drought tolerance, classified as drought-tolerant by Menezes-Silva *et al*. (2015). Conversely, genotype 13V was the most affected, showing reductions in photosynthetic efficiency based on decreases in F_v/F_m and PI parameters, indicating greater sensitivity to increased evaporative demand (Pinheiro *et al*. 2004, Ronchi and DaMatta 2007). Additionally, the low water potential combined with low root hydraulic conductivity suggested increased physical tension in the xylem due to transpirational water loss in genotype 13V (Tyree *et al.* 1994). Plant hydraulic conductivity under water deficit conditions typically decreased as well (Tyree *et al*. 1994).

According to Bolhàr-Nordenkampf *et al*. (1989), F_v/F_m values between 0.75 and 0.85 are considered optimal, corresponding to excellent PSII efficiency. However, in this study, many F_v/F_m values found at 9 h were below 0.75. It should be noted that values in the range of 0.70 to 0.75 have been reported for *C. canephora* under controlled conditions (Partelli *et al*. 2009, Rodrigues *et al*. 2016). Additionally, as light intensity increases, this variable is expected to decrease (Valentini *et al*. 1994). Despite all the mitigating factors mentioned, we could identify a decrease in F_v/F_m and PI between the two scheduled times for genotype 13V.

In the present study, SPAD levels below 40 were considered optimal due to the higher F_v/F_m values (Torres Netto *et al*. 2005). The SPAD index revealed significant differences between the materials, with genotypes 120 and 14/86, known for their drought tolerance, showing lower values. This variation can be attributed to the genetic diversity among the genotypes studied, especially those associated with the 'Conilon' group, characterized by smaller plants, lighter and thicker leaves (resulting in a lower SPAD index), and more elongated leaves (Fonseca *et al*. 2015).

The value of Ψ_W, which was reached, was not sufficient to detect significant differences in P_N, g_s, and E among the genotypes and between the two evaluation times. This can be attributed to the fact that the values obtained were not within the critical range of –1.7 to –2.2 MPa, where turgor loss occurs (DaMatta and Ramalho 2006), suggesting that the plants were not under significant water stress, except for genotype 13V, which was closer (–1.66 MPa).

Additionally, no significant differences were detected between the genotypes in specific leaf hydraulic conductance or stomatal density, which exhibited typical values for coffee (Batista *et al.* 2010). Variations in stomatal density are influenced by shading and water conditions during leaf development (Castro *et al.* 2009) but were not observed among the materials studied at the seedling stage under similar cultivation conditions.

Although the dimensions of the root system do not fully explain its efficiency in water uptake and conduction, its morphological parameters, especially in correlation

with dry mass, are important. The relationships between root volume and root dry mass, as well as leaf area and shoot dry mass, showed important, strong, and significant correlations. A high root/shoot ratio indicates the movement of a significant proportion of photoassimilates to the root system (Knipfer and Fricke 2011).

The root/shoot dry mass ratio plays a significant role when observing the greater investment of genotype 14/86 in root dry mass, even in the seedling phase, with 57% root dry mass relative to shoot dry mass in the first trial and 35% in the second, consistently higher than the other genotypes in the experiments. Genotype 13V also exhibited a significantly lower root/shoot dry mass ratio. This result highlights the importance of a well-developed root system in maintaining leaf water potential (Ψ_W). This characteristics is crucial in water-restricted situations for genotypes that still exhibit high yields under such conditions through the establishment of a deep root system and more efficient stomatal control (Franco and Inforzato 1946, Rena and Guimarães 2000, Ronchi and DaMatta 2007, Carvalho *et al*. 2008, Lopes and Reynolds 2010).

Inferences from experiments with coffee plants conducted with seedlings in the adult phase or under field conditions have been widely discussed. Several authors emphasize that the results are quite promising and that this practice represents significant savings in time and resources (Ronchi and DaMatta 2007, Cavatte *et al.* 2008).

Conclusion: Among the genotypes studied, no significant differences were observed in leaf hydraulic conductivity, stomatal density, and gas-exchange parameters, indicating that these variables may not explain the differences in leaf water status maintenance capacity among the genotypes under controlled cultivation conditions. Under conditions without water stress and with high vapor pressure deficit, our results suggest that the lower root/shoot dry mass ratio observed in genotype 13V is associated with inferior photosynthetic performance and lower root conductivity compared to other genotypes, especially drought-tolerant ones such as 14/86 and 120.

The significant correlation between K_r (root conductivity), leaf area, and dry ass suggests that the root system plays a crucial role in the plant's ability to supply water to the shoot. This relationship appears to be important for maintaining leaf water potential and photosynthetic activity under high evaporative demand.

These characteristics, particularly higher root hydraulic conductivity, are important for ensuring adequate water supply to the leaves, potentially contributing to future studies for a better understanding of the adaptations of *C. canephora* in adverse environments with high atmospheric evaporative demand.

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