

Physiological and Biochemical Responses of Robusta Coffee to Water Deficit under Field Conditions

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Editor: Samuel Pereira de Carvalho

Received in August 6, 2025 and approved in December 15, 2025

ABSTRACT

The sustainability of *Coffea canephora* cultivation in regions prone to water stress depends on the selection of cultivars adapted to water management conditions and environmental variability. This study aimed to evaluate the physiological and biochemical parameters of robusta coffee cultivars under different soil water tensions. The experiment was conducted in a split-plot design, combining five soil water tensions (20 kPa, 40 kPa, 60 kPa, 100 kPa, and no irrigation) and six robusta coffee cultivars (BRS 1216, BRS 2299, BRS 2314, BRS 3210, BRS 3213, and BRS 3220), allocated as main plots and subplots, respectively. The experimental design was randomized blocks with three replicates. Over two years, plants in the growth (2021) and production (2022) phases were evaluated for gas exchange, chlorophyll-a fluorescence, membrane damage, relative water content, and levels of photosynthetic pigments and metabolites. Non-irrigated young robusta plants exhibited lower photosynthetic rate (A) and transpiration (E), along with reduced stomatal conductance (gS). The photochemical efficiency of PSII and energy dissipation mechanisms, except for NPQ, were also lower without irrigation. Soluble carbohydrate levels were negatively correlated with photosynthesis. During the growth phase, cultivars BRS 1216 and BRS 3210 showed higher photosynthetic productivity, while BRS 2314 had the lowest. It was concluded that stomatal regulation and light absorption adjustments in PSII, through reductions in Fv/Fm and photosynthetic pigment levels, are key mechanisms in robusta coffee plants to mitigate water stress damage. Additionally, the cultivars maintained photosynthetic performance under high soil water tension, suggesting their suitability for cultivation under low irrigation levels.

Key words: *Coffea canephora*; Robustas Amazônicos; Water deficit; Irrigation.

1 INTRODUCTION

Coffee is one of the most widely cultivated agricultural crops worldwide. Among the 124 species classified in the genus *Coffea* L., *Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner are the most commercially important (Davis et al., 2011). In Brazil, the world's largest producer and exporter of coffee (ICO, 2025), 30% of production corresponds to *C. canephora* (Companhia Nacional de Abastecimento - CONAB, 2025).

C. canephora is cultivated in lower altitude regions (below 800 m) with higher average annual temperatures ranging between 22-26°C. It exhibits greater tolerance to short drought periods and is well-adapted to regions with annual water deficits between 150-200 mm (Ronchi & Damatta, 2017; Espindula et al., 2022). In the Amazon region, Brazil's second-largest producer of robusta coffee, most plantations consist of hybrid plants popularly known as Robustas Amazônicos. These hybrids result from natural or induced crosses between Conilon and Robusta (*C. canephora*) varieties, with predominance of the latter (Teixeira et al., 2020). However, coffee cultivation in this region is affected by irregular rainfall distribution throughout the year, making coffee plants susceptible to water deficit stress (Silva et al., 2015; Espindula et al., 2022).

Water deficit stands out as one of the most significant environmental stressors affecting crop growth and productivity (Yamane et al., 2022). Visible symptoms in water-limited plants

include leaf wilting, reduced height growth, decreased leaf number and area, and delayed bud and flower formation (Santos et al., 2022). These symptoms result from various physiological and metabolic changes occurring at the cellular level (Chekol et al., 2024). Generally, water deficit affects photosynthetic and transpiration rates in coffee plants. These alterations may stem from stomatal regulation, CO₂ absorption, and photoassimilate mobilization (Torres et al., 2024; Borgo et al., 2025). Increased resistance to CO₂ uptake is associated with reduced leaf water potential due to decreased soil water content or in response to high atmospheric demand (Torres et al., 2024). Prolonged stress leads to more significant metabolic changes involving photochemical and biochemical processes (non-stomatal effects) (Yamane et al., 2022).

Plants have developed mechanisms to mitigate water stress impacts. Resistance or tolerance strategies involve physiological, morphological, and biochemical changes, including: maintenance of cellular water content through compatible solute accumulation (Borgo et al., 2025), increased antioxidant enzyme activity (Yamane et al., 2022), ABA accumulation (Melo et al., 2020), and plant morphological adaptations (Santos et al., 2022; Machado Filho et al., 2021). These mechanisms regulate water use efficiency and consequently control reactive oxygen species (ROS) accumulation, ensuring plant survival (Melo et al., 2020). In coffee plants, stomatal closure at the first signs of water restriction is a primary strategy to minimize dehydration damage (Torres et al., 2024).

This rapid stomatal response results from low hydraulic conductivity that quickly reacts to declining leaf water potential (Ψ_w) (Bonomo et al., 2016; Santos et al., 2022; Borgo et al., 2025). The critical leaf Ψ_w threshold for coffee plants ranges from -2.0 to -2.5 MPa. Values below this range are considered detrimental (Deuner et al., 2011). Under water deficit conditions, yield reductions can reach 10% in tolerant genotypes and up to 90% in sensitive ones, depending on stress intensity and duration (Bonomo et al., 2016).

Irrigation has become a key strategy for mitigating drought stress and improving agricultural productivity. According to Borgo et al. (2025), annual rainfall distribution and water deficit occurrence/severity are among the climatic variables most affecting different phenological stages of coffee cultivation. Even in regions considered suitable for robusta coffee production, irrigation is justified as it eliminates risks from occasional droughts while enhancing vegetative vigor and productivity (Espindula et al., 2022). In the Amazon region, where annual rainfall exceeds 2,000 mm, irrigation remains necessary for cultivated genotypes. This is because the dry season (June–September), characterized by intense water deficit, coincides with flowering and early fruit development in robusta coffee. Additionally, high temperatures and significant increases in vapor pressure deficit (VPD) exacerbate plant stress (Ronchi & DaMatta, 2017; Silva et al., 2015). Furthermore, irrigation is used in the region as a water management technique to standardize crop flowering and, consequently, uniform grain maturation (Marsetti et al., 2013).

Water supplementation is a recognized strategy for reducing water deficit damage in coffee plants. Several studies have examined irrigation effects on *canephora* cultivation in the Amazon (Custódio et al., 2022; Espindula et al., 2022; Max et al., 2023; Solimões et al., 2023). However, understanding the physiological implications of soil water variations in Robustas Amazônicos requires further investigation. This study was conducted to evaluate physiological and biochemical parameters of robusta coffee cultivars under different soil water tensions. We hypothesize that: i) soil water tensions exceeding 60 kPa impair photosynthetic rate (A) via reduced stomatal conductance (gS) and consequently affect non-structural carbohydrate (NSC) production in coffee plants; ii) coffee plant photosynthetic productivity (A) is compensated by higher water use efficiency (WUE) under lower soil water availability, coupled with reduced sensitivity to vapor pressure deficit (VPD); and iii) greater water and solute retention in tissues can minimize cellular damage when soil water availability falls below crop evapotranspiration demand.

2 MATERIAL AND METHODS

2.1 Study site and plant material

A field experiment was conducted, dry season in the region during September in two consecutive years (2021 and

2022) at Embrapa Acre's experimental station in Rio Branco, Acre, Brazil (10°01'S, 67°42'W, 160 m altitude). According to the Köppen classification, the climate is AM (tropical monsoon type), with mean annual rainfall of 2,100 mm, maximum temperature of 30.9°C, minimum of 20.8°C, and relative humidity of 83% (Alvares et al., 2013). The region has a well-defined dry season from June to September, while the rainy season extends from October to May, with peak rainfall accumulation in the first quarter (AGRITEMPO, 2024). The soil was classified as a dystrophic Red-Yellow Argisol (Santos et al., 2018).

The study was conducted in a 29-month-old *Coffea canephora* Pierre ex A. Froehner plantation (established March 2019), comprising six different Robustas Amazônicos cultivars (clones). These genotypes originated from natural and controlled hybridization between Conilon (GS1) and Robusta (GS2) varieties from Embrapa Rondônia's Germplasm Bank. The materials were developed by Embrapa for cultivation in Western Brazilian Amazonia, selected for agronomic traits including yield, biotic stress resistance, inter-cultivar compatibility, and beverage quality (Teixeira et al., 2020). Planting density was 2,777.7 plants ha⁻¹, with 3 m x 1.2 m spacing. The coffee plants were evaluated at two distinct phenological stages. During the first assessment period, the 29-month-old plants were still in the juvenile phase, transitioning to the initial reproductive stage (flowering onset). In the second period, following the first commercial harvest and subsequent production pruning (standard crop management), the plants had already established a well-defined phenology for productive cycles.

2.2 Experimental design

The experiment was conducted using a split-plot design comprising five soil water tension treatments (20 kPa, 40 kPa, 60 kPa, 100 kPa, and non-irrigated control) as main plots and six robusta coffee cultivars (BRS 1216, BRS 2299, BRS 2314, BRS 3210, BRS 3213, and BRS 3220) as subplots. The experimental design followed a randomized complete block arrangement with three replications. Each experimental plot consisted of five plants, with data collected from the three central plants (useful plot area).

2.3 Irrigation management

Soil water tensions were managed using a pressure-compensating drip irrigation system. Emitters with a flow rate of 7.5 L h⁻¹ were installed at 30 cm spacing. The system was pressurized by an electric pump set, consisting of 75 mm (main), 50 mm (submain), and 16 mm (lateral) nominal diameter lines.

To determine the irrigation depth, undisturbed soil samples were collected at 30 cm depth and analyzed in the soil laboratory. Soil matric potential and moisture content were determined at tensions of 1, 3, 10, 30, 50, 100, and 1500

kPa, enabling the adjustment of the soil water retention curve according to the van Genuchten (1980) model. Field capacity (FC) was established at 10 kPa tension.

Irrigation was conducted from May to September. Soil water tension was monitored using electrical resistance sensors (0-199 kPa range) installed in the central experimental area at 30 cm depth and 10 cm from both the irrigation lateral and plant row, following Ferrão et al. (2017) recommendations. Irrigation was initiated when average sensor readings reached the predetermined treatment thresholds and continued until tension returned to FC. Irrigation duration was calculated based on the site-specific retention curve. Climatic data were obtained from an automated weather station located near the experimental farm (Figure 1).

The cumulative rainfall during the evaluation period (September) measured 49.4 mm and 67.5 mm in the first and second year, respectively, values that fall within the historical average range for the region. In the non-irrigated treatment, soil water tensions averaged 160 kPa.

2.4 Sampling and Evaluations

2.4.1 Gas Exchange Measurements

Gas exchange parameters were evaluated in one plant per plot using a portable open-flow infrared gas analyzer (IRGA Model LI-6400XT, LI-COR, Lincoln, NE, USA). Measurements were conducted under a photosynthetic photon flux density (PPFD) of $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$, a reference CO_2 concentration of $400 \mu\text{mol mol}^{-1}$, a fixed leaf chamber block temperature of 30°C , and ambient vapor pressure deficit (VPD) conditions. The following parameters were recorded: CO_2 assimilation rate (A ; $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), leaf transpiration rate (E ; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), stomatal conductance to water vapor (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), and intercellular CO_2 partial pressure (C_i ; Pa). Water use efficiency (WUE) was calculated as the A/E ratio ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$),

while instantaneous carboxylation efficiency (CE) was derived from the A/C_i ratio ($\mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$). All measurements were performed between 08:00 and 12:00 h on fully expanded, healthy leaves located in the upper third of the plant canopy.

2.4.2 Chlorophyll-a Fluorescence

Chlorophyll-a fluorescence data were acquired using a fluorometer (Model LI-6400-40, LI-COR, USA) integrated with the IRGA system. Measurements were performed concurrently with gas exchange analyses on leaves either light-adapted or dark-adapted for 30 minutes using aluminum foil wraps. Initial recordings included dark-adapted minimum fluorescence (F_0), followed by measurement of maximum fluorescence (F_m) after application of a saturating white light pulse ($8000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 0.7 s duration). For light-adapted leaves, a similar saturating pulse ($8000 \mu\text{mol m}^{-2} \text{s}^{-1}$; 0.7 s) was applied to determine light-acclimated maximum fluorescence (F_m'). Subsequently, steady-state fluorescence yield (F_s) was recorded, and following actinic light removal while maintaining far-red modulated measuring beams, light-adapted minimum fluorescence (F_0') was obtained. To maximize stomatal aperture, actinic light was provided through combined red and blue emitters, with blue light set at 10% intensity.

From these measurements, key photochemical parameters were derived: maximum quantum efficiency of PSII [$F_v/F_m = (F_m - F_0)/F_m$]; operational quantum efficiency of PSII [$\Phi_{PSII} = (F_m' - F_s)/F_m'$]; photochemical quenching coefficient [$qP = (F_m' - F_s)/(F_m' - F_0')$]; non-photochemical quenching coefficient [$NPQ = (F_m - F_m')/F_m'$]; and apparent electron transport rate through PSII [$ETR = (\Phi_{PSII} \times f \times I \times \alpha_{leaf})$, where f represents the fraction of absorbed quanta allocated to PSII (typically 0.5), I denotes incident photon flux density ($1200 \mu\text{mol m}^{-2} \text{s}^{-1}$), and α_{leaf} is the leaf light absorption constant (0.85). Additionally, the energy excess (ExC) was calculated from the ETR/A ratio ($\mu\text{mol e}^- \mu\text{mol}^{-1} \text{CO}_2$).

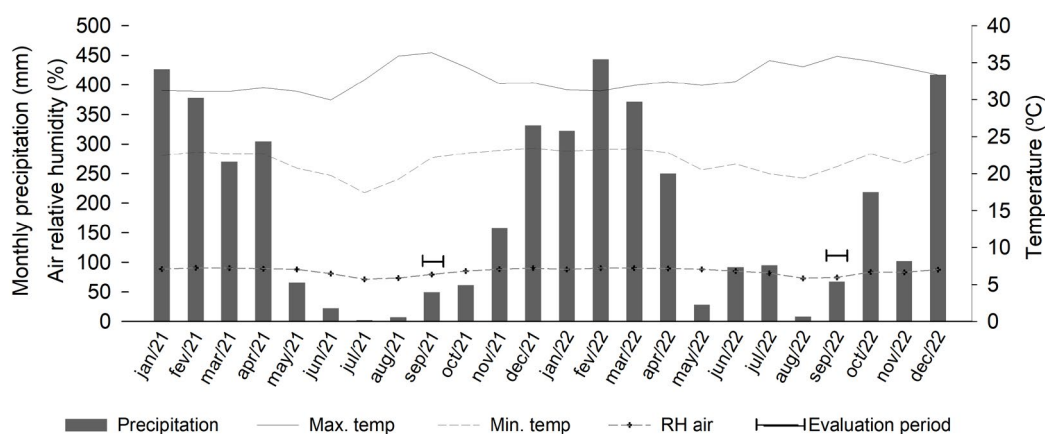


Figure 1: Monthly precipitation, air relative humidity (RH), and maximum/minimum temperatures between January 2021 and December 2022, near the experimental area.

(Climate station D4033 Rio Branco - Igarapé Judia - AC; National Meteorology Institute, 2024).

2.4.3 Photosynthetic Pigments

The contents of chlorophyll-a (*Chla*), chlorophyll-b (*Chlb*), total chlorophyll (*Chltotal*), and carotenoids (*Car*) were determined through extraction with 80% acetone, following the methodology described by Lichtenthaler and Welburn (1983). Approximately 0.05 g of leaf tissue fragments were placed in test tubes containing 5 mL of 80% acetone and protected from light. The samples were maintained for 72 hours at 25°C. Absorbance readings were obtained using a spectrophotometer at wavelengths of 663 nm and 647 nm for the determination of chlorophyll-a and chlorophyll-b, respectively, and at 470 nm for carotenoids. The concentrations (mg/g fresh weight) were calculated using the following equations: $Chltotal = (17.3 \times A_{647} + 7.18 \times A_{663})$; $Chla = (12.21 \times A_{663} - 2.81 \times A_{647})$; $Chlb = (20.13 \times A_{647} + 5.03 \times A_{663})$; and $Car = ([1000 \times A_{470}] - (3.27 \times [Chla]) - (104 \times [Chlb]))/229$.

2.4.4 Relative Water Content and Membrane Damage

The relative water content (*RWC*) was determined following the methodology proposed by Cairo (1995). Leaf discs (≈ 100 mg), collected from the same leaves used in previous analyses, were weighed using an analytical balance to determine fresh weight (FW). The discs were then placed in Petri dishes containing 10 mL of distilled water and maintained at 4°C for 48 h. After this period, the discs were removed, surface moisture was carefully blotted using filter paper, and the turgid weight (TW) was recorded. To obtain the dry weight (DW), leaf discs were oven-dried at 65°C in a forced-air circulation oven until constant weight was achieved. *RWC* was calculated using the equation: $RWC = [(FW-DW)/(TW-DW)] \times 100$.

Membrane damage (*MD*), indicative of oxidative stress, was estimated through electrolyte leakage. Leaf tissue samples (≈ 1 cm²), collected from the same leaves used for previous analyses, were placed in test tubes containing 10 mL of distilled water at 25°C for 48 h. The electrical conductivity of the solution (C1) was measured using a bench conductivity meter. Samples were then boiled in a water bath at 100°C for 1 h, and after cooling, a second conductivity reading (C2) was taken. *MD* was calculated according to the methodology proposed by Cavalcanti et al. (2004), where $MD (\%) = (C1/C2) \times 100$.

2.4.5 Metabolite Analysis

The aqueous extract was obtained using dried leaf fragments from the same leaves employed in previous analyses. After grinding, extraction was performed by heating in a water bath at 100°C for 1 h. The final extract was stored under refrigeration at 4°C. Total soluble amino acid (*TSAA*)

content was determined according to Yemm and Cocking (1955). To 650 μ L of citrate buffer (0.2 M, pH 5.0; NaOH 1.0 M) and 100 μ L of aqueous extract, 600 μ L of developer reagent (5% ninhydrin + 0.2 mM KCN) was added. After agitation, samples were maintained in a water bath at 100°C for 15 minutes, followed by cooling. Then, 650 μ L of 60% ethanol was added, and absorbance was measured at 570 nm using a spectrophotometer.

Free proline (*Pro*) content was determined by preparing a solution containing 1 mL of crude aqueous extract, 1 mL of acidic ninhydrin, and 1 mL of glacial acetic acid, which was then heated in a water bath at 100°C for 1 h. The reaction was stopped using an ice bath, followed by addition of 2 mL toluene. After agitation, the less dense phase was aspirated and absorbance was measured at 520 nm according to Bates (1973).

Total soluble carbohydrates (*TSC*) and starch contents were determined using the method of Dubois et al. (1956). For *TSC* quantification, 500 μ L of 5% phenol solution and 2.5 mL of concentrated sulfuric acid (H₂SO₄) were added to 500 μ L of crude aqueous extract. After agitation, samples were left to stand for 10 min before spectrophotometric reading at 490 nm. Starch determination involved acidic extraction of plant material, where 50 mg of ground material was immersed in 7 mL deionized water and maintained in a water bath at 100°C for 1 h. This extraction was performed twice, with supernatants discarded. Two additional extractions were then performed on the resulting residue using 2.5 mL of 30% HClO₄ for 30 min at 25°C. The two final supernatants were combined to obtain the final extract (5 mL), which was analyzed following the described methodology with spectrophotometric reading at 490 nm. The concentrations of carbohydrates, starch, proline, and free amino acids were calculated based on standard curves previously established, and were expressed in μ mol g⁻¹ of fresh weight (FW).

2.5 Statistical Analyses

The Shapiro-Wilk test ($p \leq 0.05$) was employed to assess data normality, followed by analysis of variance (ANOVA) ($p \leq 0.05$). Significant means were compared using Fisher's LSD test ($p \leq 0.05$) with Sisvar 5.6 software (Ferreira, 2014). Pearson correlation and principal component analysis (PCA) were performed using treatment combination means, processed in RStudio (R Core Team, 2021).

3 RESULTS

The analysis of variance summary shows the isolated effects of soil water tension treatments and robusta coffee clones (Tables S1 and S2 - supplementary material). No significant interaction was observed between water tensions $\times$ clones for any evaluated variable ($P < 0.05$).

3.1 Gas Exchange

In the first evaluation year, photosynthetic rate (A), stomatal conductance (gS), and transpiration (E) were significantly lower in non-irrigated (NI) plants (Figure 2). A slight reduction in gS and consequently in E was observed at 40 kPa tension. While soil water tensions did not affect water use efficiency (WUE) in the first year, the variations in A and C_i led to significantly lower carboxylation efficiency (CE) in NI plants. During the second year, treatments of 20 and 40 kPa showed higher A, gS, and E values, though A in these treatments did not differ statistically from NI.

Among cultivars, BRS 1216, 3210, and 3220 exhibited higher A coupled with greater gS, E, and C_i in the first year (Table 1), whereas BRS 2314 showed the lowest values. In the second year, variations in gS affected E and C_i but not A, with BRS 1216 maintaining the highest performance.

3.2 Chlorophyll Fluorescence

In 2021, irrigated plants (regardless of tension level) displayed higher F_v/F_m , $\Phi PSII$, qP, and ETR, while the ETR/A ratio (ExC) was elevated in NI plants (Figure 3). Cultivar BRS 3210 showed the highest F_v/F_m , whereas BRS 1216 had the lowest qP values (Table 1). In 2022, the 20 kPa treatment yielded the greatest F_v/F_m , while $\Phi PSII$, qP, and ETR peaked at 60 kPa (Figure 3). BRS 3220 exhibited the lowest F_v/F_m , whereas plants under 60 kPa tension showed significantly higher $\Phi PSII$, qP, ETR, and ExC. NPQ remained unaffected by soil water tensions (Table 1).

3.3 Photosynthetic Pigments

Chlorophyll-a, chlorophyll-b, and carotenoid contents were significantly lower in young coffee plants grown without irrigation, as observed in 2021 (Figure 4). Consequently, total chlorophyll content in these plants was also reduced. Conversely, the chlorophyll a/b ratio increased, indicating a more pronounced reduction in chlorophyll-b in coffee leaves under water deficit. Cultivar BRS 3220 exhibited the lowest chlorophyll-b content and consequently the highest chlorophyll a/b ratio (Table 2).

No significant differences were observed in pigment content across different soil water tension treatments during 2022 (Figure 4). The cultivar BRS 2299 exhibited significantly higher concentrations of Chl_a , Chl_b , total chlorophyll (Chl_{total}) and carotenoids (*Car*) compared to other cultivars (Table 2). Similarly, BRS 2314 showed elevated levels of Chl_b and Chl_{total} .

3.4 Relative Water Content and Membrane Damage

Leaf relative water content (RWC) remained unaffected by soil water tension variations during both

evaluation periods (Figure 5). However, younger coffee plants assessed in 2021 demonstrated increased membrane damage (MD) under lower soil water tensions. Among cultivars, BRS 1216 displayed the highest MD values under water deficit conditions (Table 2).

3.5 Metabolites

Significant variations in total soluble carbohydrate (TSC) content were observed across both evaluation periods (Figure 6). In the first year, non-irrigated coffee plants exhibited significantly higher TSC levels, whereas in the second year, irrigation favored leaf TSC accumulation regardless of the applied soil water tension. Leaf starch content varied only in 2022, showing higher concentrations under lower soil water tensions (60 and 100 kPa). Cultivars BRS 2299 and BRS 3210 displayed the lowest starch levels, while BRS 3220 showed the highest concentrations (Table 2). In 2021, total free amino acids (TFAA) and proline (Pro) contents were lower in non-irrigated plants, whereas no significant differences were observed among tension treatments in 2022 (Figure 6). Cultivar BRS 2299 consistently exhibited higher Pro levels in both evaluation periods (Table 2).

4 DISCUSSION

Under water stress, young coffee plants exhibited reduced gS, which in turn directly influenced other gas exchange parameters. Transpiration rates were entirely dependent on gS variations. Consequently, water use efficiency (WUE) values remained independent of different soil water tensions or cultivars. WUE reflects the psychological balance between carbon fixation (photosynthesis) and water loss (transpiration). Coffee plants are characterized by narrow stomatal conductance and efficient water status regulation (Martins et al., 2019; Semedo et al., 2021). Stomatal conductance proved to be a key adaptive mechanism in *C. canephora* plants under adverse conditions. This strategy likely contributed to maintaining leaf RWC in non-irrigated plants. According to Deuner et al. (2011), the reduction in gS while leaves remain turgid demonstrates active stomatal involvement in water conservation, initiating closure at the first signs of water restriction.

Beyond soil water availability, variations in gS and consequent photosynthetic parameters were further influenced by unfavorable climatic conditions for coffee cultivation. September, when evaluations were conducted, presents particularly challenging seasonal conditions in the region (Custódio et al., 2022; Rodrigues et al., 2018). This period combines low precipitation with high temperatures, intense solar radiation, and low air relative humidity (Figure 1), resulting in significantly increased vapor pressure deficit (VPD) (Custódio et al., 2022; Rodrigues et al., 2016). Plants

responded with markedly reduced conductance to minimize transpirational water losses. Thus, coffee stomatal sensitivity is jointly regulated by both soil and atmospheric water status (Deuner et al., 2011). The pronounced reduction in A in non-irrigated plants led to CO₂ accumulation in leaf intercellular spaces, indicating reduced carbon consumption. In this case substrate availability was insufficient to sustain photosynthetic rates, indicating that A in these plants was limited by non-stomatal factors, a hypothesis supported by the reduced CE. Notably, during the first evaluation cycle, plants experienced greater stress due to more severe water deficit conditions (Figure 1).

In the second year, variations in soil water tension appear to have less influence on the photosynthetic performance of the plants. Even under low conductance, A was increased in the treatment with the highest water stress. This shows the importance of coffee plant phenology on its physiological behavior, studied here under field conditions during two distinct phases: young and adult plants. Such responses could be supported by the hypothesis of greater root development in rainfed plants. In this case, after going through the stress period, young plants would have greater root system development with the reestablishment of rainfall. This may be the key point for greater tolerance. In the second

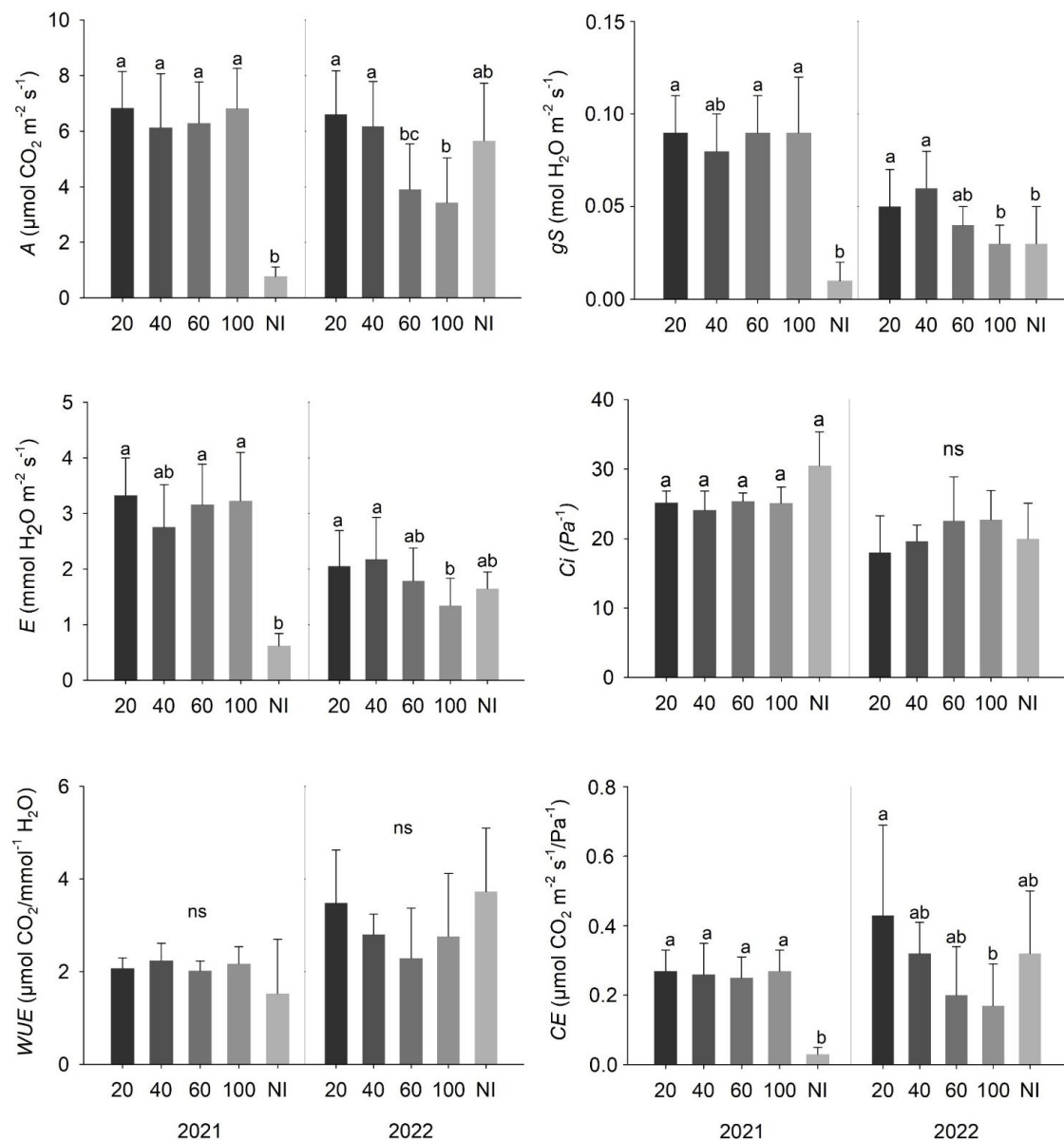


Figure 2: Photosynthetic rate (A), stomatal conductance (gS), transpiration (E), intercellular CO₂ concentration (Ci), water use efficiency (WUE), and carboxylation efficiency (CE) in *Coffea canephora* cultivars grown under soil water tensions of 20, 40, 60, and 100 kPa or non-irrigated (NI) in 2021 and 2022. Means followed by distinct letters within each year indicate significant differences according to Fisher's LSD test ($P < 0.05$).

Table 1: Mean values of photosynthesis (A), stomatal conductance (gS), transpiration (E), internal CO₂ concentration (Ci), water use efficiency (WUE), carboxylation efficiency (CE), maximum quantum yield of PSII (F_v/F_m), operational quantum yield of PSII ($\Phi PSII$), photochemical quenching coefficient (qP), non-photochemical quenching coefficient (NPQ), electron transport rate (ETR), and excess energy (ExC) in *Coffea canephora* cultivars during 2021 and 2022 growing seasons.

CULTIVAR	2021											
	A	gS	E	Ci	WUE	CE	F_v/F_m	$\Phi PSII$	qP	NPQ	ETR	ExC
BRS 1216	5.85ab	0.09a	3.03a	28.11a	1.94	0.22ab	0.78ab	0.095	0.15c	1.17	48.63	11.12
BRS 2299	5.12bc	0.06bc	2.48b	25.77bc	2.03	0.21abc	0.76bc	0.109	0.20a	1.38	54.33	13.13
BRS 2314	4.11c	0.04c	1.93c	24.36c	2.04	0.17c	0.77abc	0.106	0.20a	1.70	54.21	15.43
BRS 3210	6.16a	0.08ab	2.91ab	26.23ab	2.14	0.25a	0.80a	0.116	0.19ab	1.35	57.69	12.48
BRS 3213	5.04bc	0.07b	2.52ab	25.48bc	1.97	0.20bc	0.78abc	0.101	0.15bc	1.13	50.19	14.54
BRS 3220	5.90ab	0.07ab	2.82ab	26.16bc	1.91	0.24ab	0.75c	0.112	0.20a	1.48	57.18	15.15
CULTIVAR	2022											
	A	gS	E	Ci	WUE	CE	F_v/F_m	$\Phi PSII$	qP	NPQ	ETR	ExC
BRS 1216	5.73	0.062a	2.24a	22.75a	2.75	0.276	0.776a	0.079	0.134	1.08ab	40.10	8.12
BRS 2299	5.56	0.048b	1.92ab	21.11ab	3.11	0.294	0.782a	0.080	0.128	0.88b	40.70	8.50
BRS 2314	4.97	0.039b	1.68b	19.13bc	2.97	0.293	0.784a	0.078	1.138	1.34a	39.84	9.58
BRS 3210	5.05	0.040b	1.52b	18.37c	3.38	0.349	0.771a	0.075	0.133	1.47a	38.19	9.20
BRS 3213	4.81	0.036b	1.55b	21.26ab	3.12	0.267	0.787a	0.064	0.104	0.91b	32.68	8.29
BRS 3220	4.80	0.043b	1.88ab	20.86abc	2.74	0.262	0.733b	0.063	0.123	1.43a	32.26	8.06

Distinct lowercase letters in the column, within each year, indicate significant differences between means according to Fisher's t-test ($P < 0.05$).

year, after the first commercial harvest, the shoot/root ratio is reduced by production pruning (removal of plagiotropic branches), further contributing to the adjustment of the water relationship (less water loss associated with greater absorption capacity). According to Covre et al. (2015), non-irrigated conilon coffee plants have a larger surface area and root length and volume per volume of soil compared to irrigated plants. Therefore, plants that have suffered water stress have increased roots, and this root compensation promotes better absorption and utilization of water and nutrients (Solimões et al., 2023). This characteristic may be part of the set of mechanisms that enable better plant performance after cycles of water deficit (stress memory).

Coffee plantations not only withstand prolonged drought periods satisfactorily but also demonstrate rapid recovery post-stress (Covre et al., 2015; Martins et al., 2019). Coffee plants are notably characterized by post-drought compensatory growth, which is triggered upon release of water stress (Solimões et al., 2023). During recovery, Ronchi and DaMatta (2017) report a 20% higher growth rate in these plants. This phenomenon may contribute to the marked biennial bearing pattern observed in rainfed canephora cultivation. The root system of robusta coffee can also be affected by fruit load. These authors note that high-yield years lead to plant exhaustion and subsequent root system impairment (dieback), ultimately impacting the following harvest.

The photochemical performance of young coffee plants was significantly affected by water stress, with strong correlations observed between gas exchange variables (Figure

8). $\Phi PSII$ provides information about the absorbed energy directed to PSII photochemistry, and its decline may represent a mechanism adjusting PSII efficiency to photosynthetic photon flux density. $\Phi PSII$ also depends on energy-consuming biochemical reactions such as CO₂ assimilation (Guidi et al., 2019). In non-irrigated young coffee plants, reduced photosynthetic activity decreased energy utilization, necessitating adjustments to prevent potential damage from excess energy. The lower ETR values in these plants reflect this mechanism. The maximum energy dissipation capacity through fluorescence was also reduced under stress. F_v/F_m estimates the maximum photochemical efficiency of PSII, where absorbed light energy is used to reduce quinone. It also serves as a measure of PSII integrity. The decline of this ratio, normally ranging between 0.75-0.85, indicates PSII photoinhibition induced by stress factors (Guidi et al., 2019; Souza et al., 2020).

ExC values revealed that non-irrigated young coffee plants managed significantly higher energy relative to absorbed CO₂ compared to irrigated plants. This excess energy may negatively affect plants. Elevated ExC indicates increased oxidative pressure in chloroplasts that may lead to oxidative stress and photoinhibition. Excess electrons, instead of being used for NADPH⁺ reduction, are transferred to oxygen, resulting in reactive oxygen species (ROS) production (Guidi et al., 2019; Marçal et al., 2021). ExC showed negative correlations with all variables except NPQ (Figure 7). Potential oxidative pressure could theoretically

be mitigated through non-photochemical energy dissipation (NPQ) adjustments (Guidi et al., 2019), which did not occur in these plants. This suggests NPQ is not a primary strategy for *C. canephora* to dissipate excess energy and protect photosystems. The lower qP values under stress, combined with higher ExC and lower Fv/Fm and Φ PSII, provide evidence of photoinhibition. Alternatively, they may indicate photochemical phase adjustments responding to reduced CO₂ assimilation, though proper energy metabolism

remains crucial for oxidative pressure mitigation. In productive-phase plants under non-irrigation, despite high A values, photochemical efficiency remained lower, though without excess energy accumulation. Conversely, in 60 and 100 kPa treatments showing low A, higher ETR resulted in greater ExC. Overall, reduced fluorescence and PSII electron transport occurred in the second year compared to the juvenile phase, indicating higher photochemical activity during early growth stages.

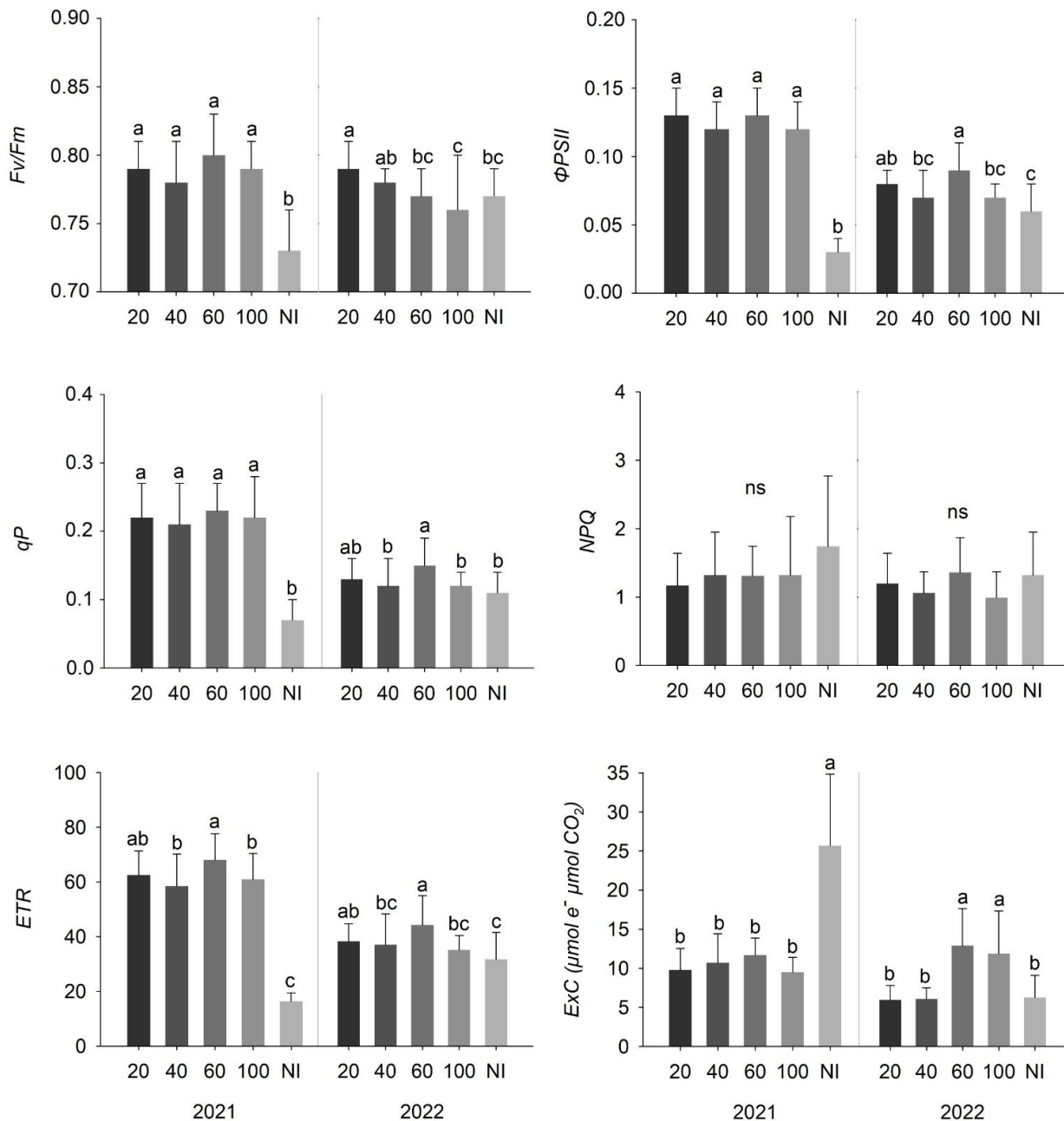


Figure 3: Photochemical parameters of *Coffea canephora* clones grown under different soil water tensions (20, 40, 60, 100 kPa, and non-irrigated control - NI) during 2021-2022: maximal PSII quantum yield (Fv/Fm), operational PSII efficiency (Φ PSII), photochemical quenching (qP), non-photochemical quenching (NPQ), electron transport rate (ETR), and excess excitation energy (ExC). Means followed by distinct lowercase letters, within each year, indicate significant differences according to Fisher's LSD test ($P < 0.05$).

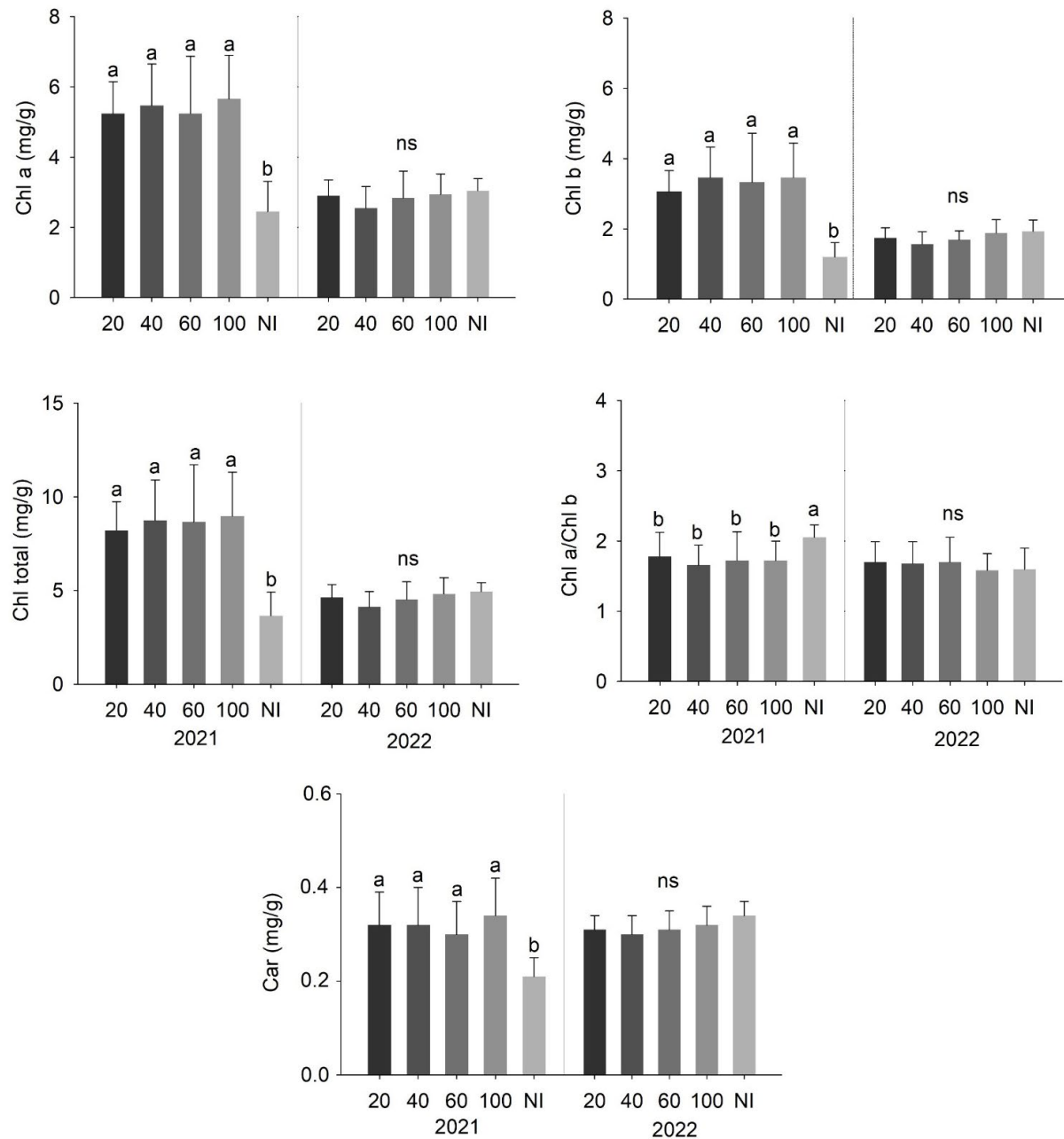


Figure 4: Chlorophyll-a (Chla), chlorophyll-b (Chlb), total chlorophyll (Chltotal), chlorophyll a/b ratio (Chl a/b), and carotenoid (Car) content in *Coffea canephora* clones grown under soil water tensions of 20, 40, 60, and 100 kPa and non-irrigated (NI) conditions during 2021 and 2022. Means followed by distinct lowercase letters, within each year, indicate significant differences according to Fisher's LSD test (P<0.05).

Photosynthetic pigments followed similar trends in productive-phase plants. While water treatments did not significantly affect pigment contents, lower chlorophyll concentrations were observed. Reduced chlorophyll suggests fewer light-harvesting antenna complexes. To decrease energy absorption and prevent chloroplast overload, plants may downregulate chlorophyll biosynthesis under moderate to severe water stress (DaMatta & Ramalho, 2006). Positive correlations between pigment contents, photosynthesis and

fluorescence support this hypothesis, particularly in the second year when A showed stronger relationships with chlorophyll variables. During the juvenile phase, only non-irrigated plants showed significant pigment reductions. The chlorophyll a/b ratio indicated proportionally greater reduction in chlorophyll b under water deficit. This demonstrates an adaptation and protection of the photosynthetic system by reducing the light-collecting antennae (PS II). Cultivar BRS 3220 exhibited generalized

leaf yellowing under low soil water availability (Espindula et al., 2022), consistent with its low foliar chlorophyll contents observed in our study.

Maintained RWC under water stress represents an interesting adaptation in these coffee plants. For juvenile plants, RWC showed no correlation with studied variables, while productive-phase plants demonstrated negative correlations, including with A and Φ PSII (Figure 7), showing it to be a more concerning factor at this stage. Studying young *C. arabica* plants, Catarino et al. (2021) observed that despite dramatic soil moisture reduction under water deficit, leaf water

potential decreased only after 40 days of water withholding (≈ -1.0 MPa), demonstrating the ability of these plants to maintain leaf moisture for an extended period (Martins et al., 2019; Semedo et al., 2021).

Soluble carbohydrate (TSC) accumulation under reduced water availability appears particularly important for juvenile coffee plants, which showed lower A. Reducing carbohydrates comprise readily mobilizable energy sources with high hygroscopicity and excellent osmoregulatory function (Sanches et al., 2017; Borgo et al., 2025). During the productive phase, despite maintained A, TSC content

Table 2: Mean values of chlorophyll-a (Chla), chlorophyll-b (Chlb), total chlorophyll (Chltotal), chlorophyll a/b ratio (Chl a/b), carotenoids (Car), membrane damage (MD), relative water content (RWC), total soluble carbohydrates (TSC), total free amino acids (TFAA), and proline (Pro) content in *Coffea canephora* clones during 2021 and 2022 growing seasons.

CULTIVAR	2021										
	<i>Chl_a</i>	<i>Chl_b</i>	<i>Chl_{total}</i>	<i>Chl a/b</i>	<i>Car</i>	<i>MD</i>	<i>RWC</i>	<i>TSC</i>	<i>Starch</i>	<i>TFAA</i>	<i>Pro</i>
BRS 1216	5.01	3.13a	7.83	1.82ab	0.28	34.20a	75.90	865.53	251.91	384.44	209.53c
BRS 2299	5.22	3.38a	8.61	1.73b	0.27	27.65b	79.99	764.89	260.85	499.30	280.38a
BRS 2314	4.65	2.82ab	7.47	1.76b	0.30	28.64ab	78.94	768.97	286.66	485.14	260.41ab
BRS 3210	4.73	2.83ab	7.39	1.71b	0.32	25.51b	78.54	824.71	274.39	469.58	210.76c
BRS 3213	5.06	3.13a	8.19	1.69b	0.32	26.66b	78.39	794.64	267.80	410.69	228.83bc
BRS 3220	4.20	2.10b	6.41	2.08a	0.26	25.17b	79.18	800.71	249.78	514.86	264.32ab
CULTIVAR	2022										
	<i>Chl_a</i>	<i>Chl_b</i>	<i>Chl_{total}</i>	<i>Chl a/b</i>	<i>Car</i>	<i>MD</i>	<i>RWC</i>	<i>TSC</i>	<i>Starch</i>	<i>TFAA</i>	<i>Pro</i>
BRS 1216	2.78bc	1.67b	4.45b	1.71	0.28d	18.63	72.08	970.92abc	371.74ab	782.22	99.50b
BRS 2299	3.40a	2.14a	5.53a	1.62	0.34a	15.57	76.53	922.34c	268.79c	882.85	116.11a
BRS 2314	3.12ab	2.06a	5.18a	1.58	0.33ab	17.59	72.07	1014.75a	376.35ab	780.76	111.20ab
BRS 3210	2.71bc	1.65b	4.35b	1.68	0.31bcd	15.57	76.84	1009.50a	290.67c	798.54	108.42ab
BRS 3213	2.77bc	1.70b	4.47b	1.62	0.32abc	16.43	72.79	948.65bc	364.11b	912.64	101.90b
BRS 3220	2.33c	1.36c	3.69c	1.71	0.30cd	17.41	74.53	998.87ab	435.46a	816.53	86.11c

Distinct lowercase letters in the column, within each year, indicate significant differences between means according to Fisher's t-test ($P < 0.05$).

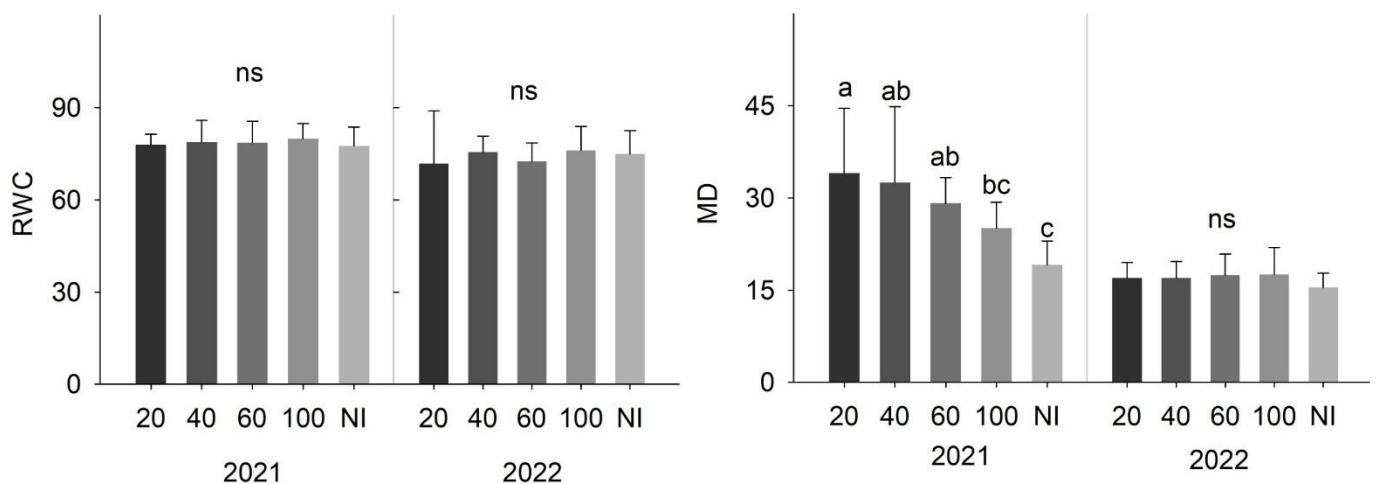


Figure 5: Relative water content (RWC) and membrane damage (MD) in *Coffea canephora* clones grown under soil water tensions of 20, 40, 60, and 100 kPa and non-irrigated (NI) conditions during 2021 and 2022. Means followed by distinct lowercase letters, within each year, indicate significant differences according to Fisher's LSD test ($P < 0.05$).

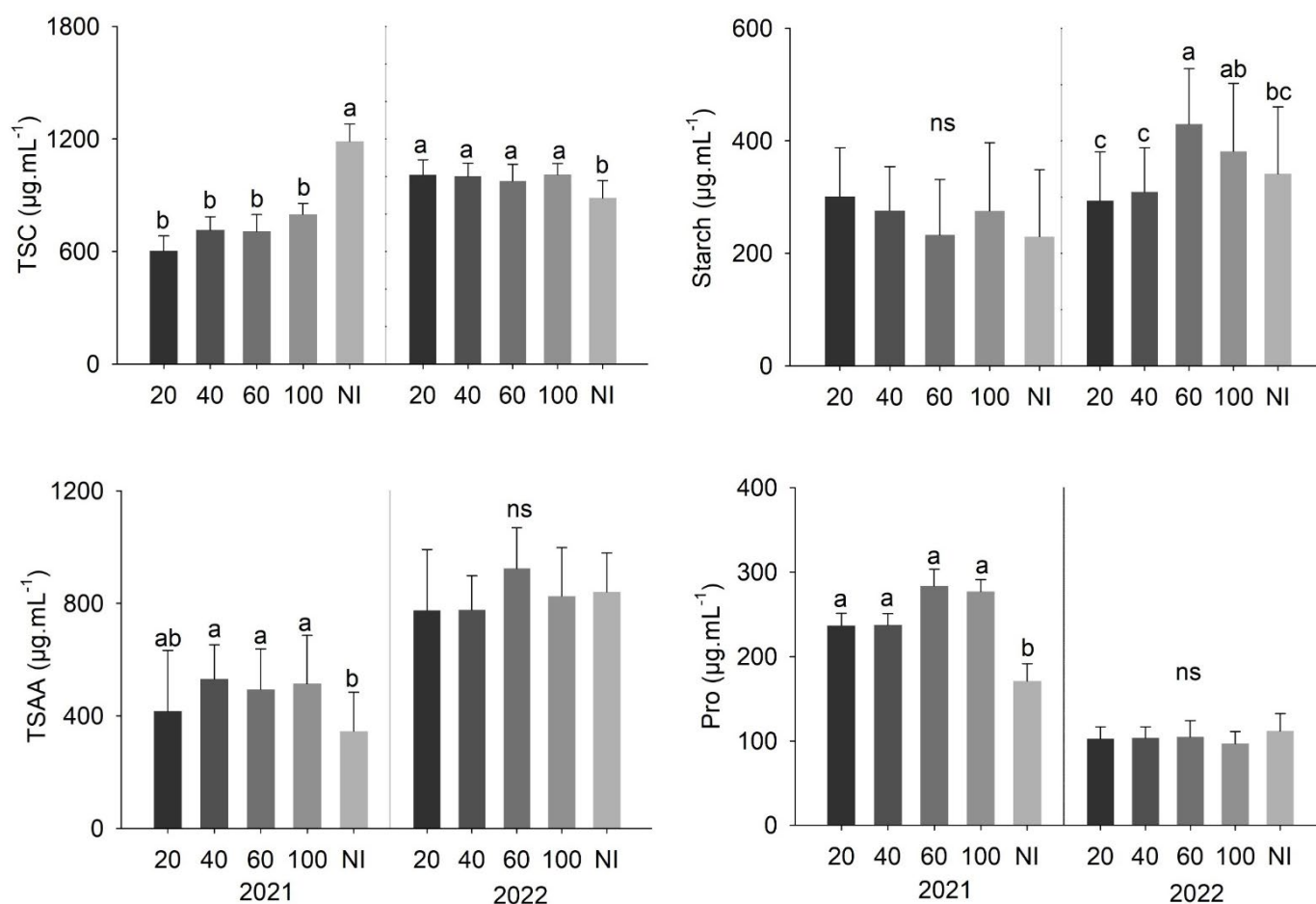


Figure 6: Contents of total soluble carbohydrates (TSC), starch, total free amino acids (TFAA), and proline (Pro) in *Coffea canephora* clones grown under soil water tensions of 20, 40, 60, and 100 kPa plus non-irrigated (NI) treatment during 2021 and 2022. Means followed by distinct lowercase letters, within each year, indicate significant differences according to Fisher's LSD test ($P < 0.05$).

decreased likely due to redirected for the fruits, which become the primary sinks in the plant (Bote & Jan, 2016; Covre et al., 2022). In this case, starch accumulation under moderate to severe deficit conditions would be of greater importance. The reduction in amino acid levels under water deficit conditions may indicate low osmoregulatory potential in plants, where this decrease is likely associated with the consumption of these compounds for maintaining vegetative and reproductive structures (Sanches et al., 2017).

Stress severity varied little among cultivars, particularly in adult plants where A showed no differences. Despite variations in gS, cultivars did not differ in WUE. The photosynthetic productivity of cultivar BRS 1216 correlated with its vegetative growth, standing out from others due to greater dry mass production and root volume (Araujo et al., 2021). Among cultivars, BRS 1216, BRS 3137, BRS 3193, BRS 3210, and BRS 3213 are characterized by higher productive potential under water-limited conditions, while BRS 2299, BRS 2314, BRS

2357, BRS 2336, and BRS 3220 show lower productivity (Espindula et al., 2019). In this study, during the juvenile phase, cultivars BRS 1216 and BRS 3210 exhibited superior performance with higher A and E, coupled with greater gS and C_i , along with higher F_v/F_m .

5 CONCLUSIONS

Stomatal regulation and PSII light absorption adjustments are key mechanisms in robusta coffee to mitigate water stress under field conditions. In juvenile plants, photosynthesis and transpiration are regulated by high stomatal sensitivity in non-irrigated conditions, while mature plants reduce pigment content to limit excess light and PSII oxidative stress. Cultivars BRS 1216 and BRS 3210 maintain higher photosynthetic productivity under stress. Robustas Amazônicos tolerate soil water tension up to 100 kPa and show enhanced stress responses in subsequent cycles after exposure.

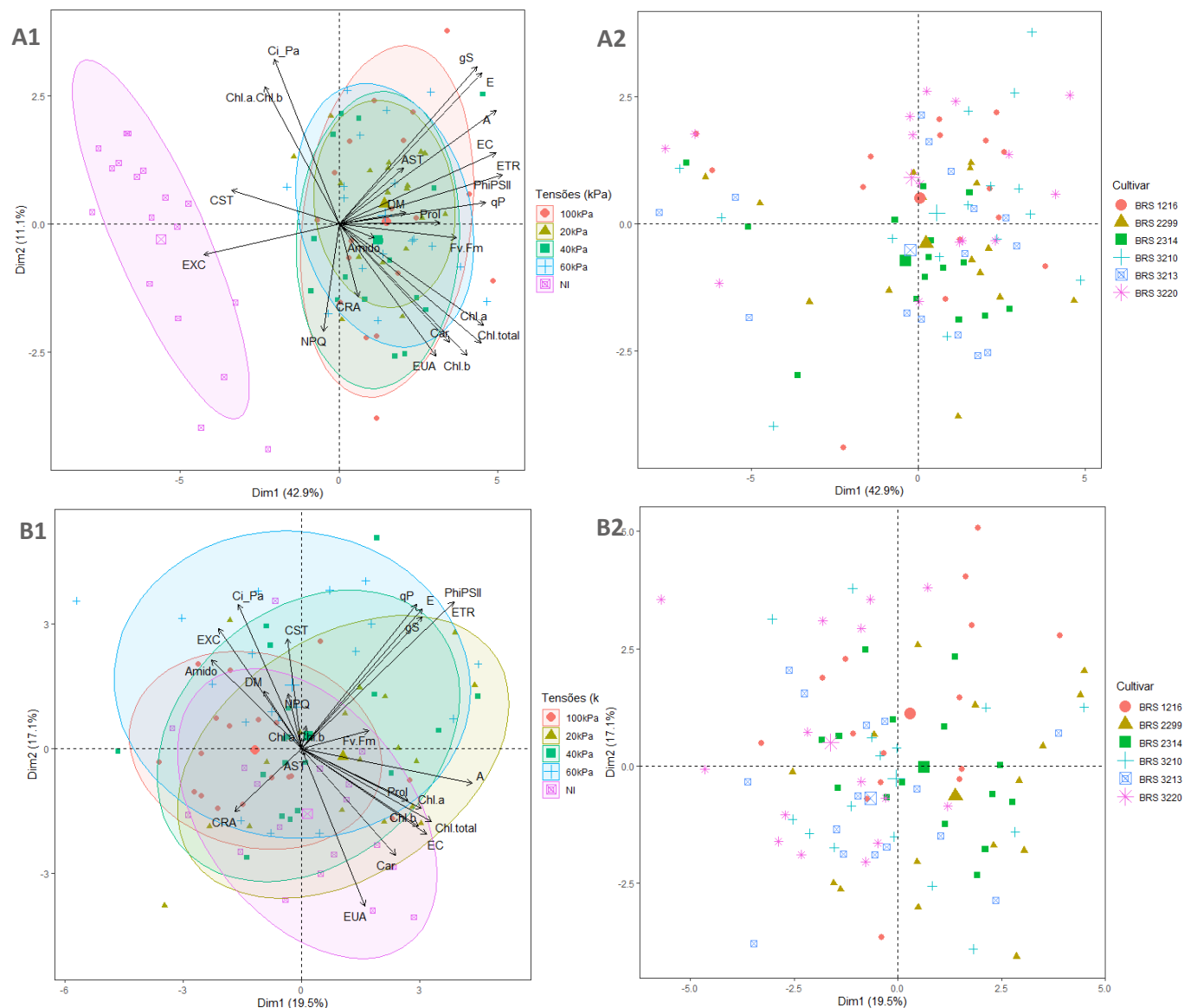


Figure 7: Principal Component Analysis of the relationship between gas exchange parameters, chlorophyll-a fluorescence, photosynthetic pigment content, and metabolites in *Coffea canephora* clones grown under different soil water tensions during 2021 (A1 and A2) and 2022 (B1 and B2).

Biplot displays mean scores of the first two principal components (PC1 and PC2). Cultivars: C1: BRS 1216; C2: BRS 2299; C3: BRS 2314; C4: BRS 3210; C5: BRS 3213; C6: BRS 3220. Parameters: A: photosynthetic rate; g_s : stomatal conductance; E: transpiration; C_i : internal CO_2 concentration; WUE: water use efficiency; CE: carboxylation efficiency; F_v/F_m : maximum quantum yield of PSII; $\Phi PSII$: operational quantum yield of PSII; qP: photochemical quenching coefficient; NPQ: non-photochemical quenching coefficient; ETR: electron transport rate; ExC: excess energy; Chl.a: chlorophyll-a content; Chl.b: chlorophyll-b content; Chl.a/b: chlorophyll a/b ratio; Car: carotenoids; MD: membrane damage; RWC: relative water content; TSC: total soluble carbohydrates; TSAA: total soluble amino acids; Pro: proline.

6 AUTHOR CONTRIBUTIONS

Conceptual Idea: Bravin, N.P.; Martins, M.O.; Methodology design: Bravin, N.P.; Martins, M.O.; Data collection: Bravin, N.B.; Sebim, J.P.M.; Feitosa, R.C.; Mota, B.B.; Martins, M.O. Data analysis and interpretation: Bravin, N.P.; Mota, B.B.; Martins, M.O., and Writing and editing: Bravin, N.P.; Mota, B.B.

7 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

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