



Can prolonged conditions of water deficit alter photosynthetic performance and water relations of coffee plants in central-west Colombia?



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ABSTRACT

In the coffee-growing areas of Colombia, the vulnerability of soils to conditions of water deficit has been determined and this limitation has been projected to increase its intensity and severity during extreme climatic scenarios, which constitute one of the factors limiting coffee production. The objective of this study was to evaluate the effect of prolonged water deficit conditions on the physiological performance of coffee plants cv. Castillo[®]. The evaluations were carried out in 13-month-old “Castillo[®]” variety plants established in a greenhouse which were subjected to two conditions: well-watered at field capacity and water deficit in the soil-applied through withholding the water supply for 40 days, using a completely randomized experimental design. Leaf water potential (Ψ_{pd} and Ψ_{md}), gas exchange, and chlorophyll fluorescence parameters (F_v/F_m and ETR) were measured. In the plants with water deficit, Ψ_{pd} and Ψ_{md} were decreased significantly. Five days after restricting the water supply, the g_s was significantly reduced to 95% at the end of the experiment compared to plants without stress. The F_v/F_m and ETR parameters showed no sensitivity under the water deficit conditions. Plants under water deficit decreased A_{max} by 81%, I_{sat} by 61%, and R_d by 50%, as compared to plants without stress. The transpiration and water-use efficiency decreased, while C_i had a greater concentration in plants under water deficit. These results indicate the physiological performance coffee cv. Castillo[®] plants were significantly affected by prolonged conditions of soil water deficit and that the reductions in photosynthetic processes were related to stomatal limitations.

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1. Introduction

Coffee (*Coffea arabica* L.) is one of the most commercialized agricultural products worldwide and supports around 70% of coffee production as a result of its higher quality beverages (DaMatta et al., 2019; Chemura et al., 2021). The distinctive characteristics of coffee quality have been attributed to climatic conditions (precipitation, solar radiation, temperature, and humidity) and topological and edaphic factors (soil depth, acidity/alkalinity, fertility) in the cultivable areas (Cheng et al., 2016; Chemura et al., 2021). *Coffea arabica* is grown mainly in the intertropical zone between latitude 20° N and 24° S in altitudinal ranges from 700 to 2000 m.a.s.l (Bunn et al., 2015; DaMatta et al., 2018). In this area, Latin American countries provide 60% of coffee production, with Brazil and Colombia being more prominent in terms of production volume and quality (ICO, 2020).

Colombia is the third-largest producer of *C. arabica* worldwide, after Brazil and Vietnam (Ceballos-Sierra and Dall'Erba, 2021), and is a leading producer of top-quality (Bastianin et al., 2018). Currently, the cultivated area includes 884 thousand hectares, and 540 thousand families depend economically on coffee production (FNC, 2020a). However, it has been reported that, in the coffee-growing areas of Colombia, the soil can be vulnerable to water deficit and this can affect the fruit load by 25% when it increases to 40 days (Ramírez et al., 2012). This situation has been projected to be more worrisome during the “El Niño” climate scenarios, since it increases the duration of the water deficit and therefore decreases the water availability (Poveda et al., 2001; Ramírez et al., 2012; 2014). Moreover, *C. arabica* is classified as sensitive to climate change, raising concerns about coffee yields in the coming decades, as well as the reduction of sustainable arable areas (Ovalle-Rivera et al., 2015; DaMatta et al., 2019; Ceballos-Sierra and Dall'Erba, 2021; Chemura et al., 2021).

Recently, climate change has generated increases in the intensity and severity of extreme scenarios, including prolonged drought,

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floods, and increasing heat waves (Fischer et al., 2016; Avila et al., 2020; Toral-Juárez et al., 2021). Drought events are the main climatic limitation for the *C. arabica* crop because they gradually reduce the availability of water in the soil (to generate a water deficit), affecting important physiological processes in the plants (Dias et al., 2007; Dubberstein et al., 2020; Souza et al., 2020; Almeida et al., 2021). This situation is concerning in areas cultivated with coffee, particularly in regions susceptible to drought, where the use of irrigation is limited due to the little availability of water (DaMatta et al., 2018; Avila et al., 2020). Therefore, coffee plants are expected to face more severe periods of water deficit, posing challenges for ensuring the sustainability of coffee production, both globally and in Colombia.

The prolonged conditions of soil water deficit is one of the most limiting factors for the morphological, physiological, and biochemical processes associated with the uptake of nutrients and water, as well as photosynthesis and carbon metabolism in the plants (Ramalho et al., 2018; Souza et al., 2020; Sun et al., 2020; Semedo et al., 2021). The main physiological response of plants under conditions of water deficit is a decrease in stomatal conductance (Brodribb and McAdam, 2017), which leads to low carbon supplies for ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), reducing the production of photoassimilates (Chaves et al., 2009; DaMatta et al., 2018; Dubberstein et al., 2020; Semedo et al., 2021). Furthermore, photosynthetic performance is impaired by photochemical and biochemical limitations that deteriorate the functioning of photosystems, the pooling of photosynthetic pigments, and the integrity of the membranes, which can trigger more oxidative stress related to an uncontrolled generation of reactive oxygen species-ROS (Zargar et al., 2017; Franck and Vaast, 2009; Souza et al., 2020; Dubberstein et al., 2020; Semedo et al., 2021). Therefore, the physiological alterations caused by the gradual reduction of water in the soil must be known because plants can trigger a series of responses that allow survival in extreme events such as drought.

The physiological responses of *C. arabica* plants under water deficit conditions have been well documented (Melke and Fetene, 2014; Tounekti et al., 2017; Avila et al., 2020; Souza et al., 2020; de Oliveira Santos et al., 2021; Semedo et al., 2021). *C. arabica* plants have developed a series of responses to drought events that are associated with stomatal control and efficiency in the absorption of water in the soil (DaMatta and Ramalho, 2006), as well as mechanisms of thermal dissipation, photoprotection as the activity of antioxidants and cyclical flow of electrons (Ramalho et al., 2018; Dubberstein et al., 2020). These responses have also been studied at the molecular level, where a greater expression of genes that increase the synthesis of aquaporins, abscisic acid-ABA, isoforms of ascorbate peroxidase, galactinol, and serine protease responsible for stomatal density and distribution have been reported (Miniussi et al., 2015; Avila et al., 2020; de Oliveira Santos et al., 2021). All these mechanisms promote better physiological performance in coffee plants, preserving the photosynthetic machinery, photochemical efficiency, and hydraulic conductance of plants under water deficit conditions. However, studies on the physiological alterations of Colombian varieties of *C. arabica* under water deficit are scarce.

In this study, we test the following hypotheses: (i) the stomatal activity and water status of the coffee plants are very sensitive at the prolonged conditions of soil water deficit; (ii) photosynthetic performance of coffee plants under prolonged conditions of soil water deficit are mainly affected by stomatal limitations. In this way, the objective of this research was to evaluate the effect of prolonged water deficit conditions on the physiological performance of Castillo® variety coffee plants, grown under semi-controlled conditions in the west-central coffee zone of Colombia. Alterations in the measurements of water relations in the plants, gas exchange, light response curves, and chlorophyll fluorescence parameters in plants subjected to water deficit were studied. This information is necessary to understand the diverse responses of plants under stress conditions for use

in the assisted selection and improvement of drought-tolerant cultivars in Colombia.

2. Materials and methods

2.1. Plant material and growing conditions

This experiment was carried out under semi-controlled conditions in a greenhouse with plants of the Castillo® variety, established at the Plan Alto headquarters of the “Centro Nacional de Investigaciones del Café-Cenicafé”, located in Manizales, Department of Caldas, Colombia (04° 59' 26.83' 'N; 75° 35' 27.09' 'W), over 1,384 m.a.s.l. During the study, the greenhouse had average climatic conditions of 23.5 °C, 71.59% relative humidity (Fig. 1), and a photosynthetically active radiation-PAR maximum of 768 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at noon. The environmental temperature and relative humidity were measured with an HMP60 sensor, and the PAR was determined with an SQ-110 sensor connected to a data transmission system automatically every five minutes.

The plant material used for this study was the Castillo® variety, resistant to coffee rust (*Hemileia vastatrix*) and together with other varieties make up to 80% of the cultivable area of coffee in Colombia (FNC, 2020b). The plants were established in January 2020 in 30 cm x 60 cm polyethylene bags, with a mixture of solarized soil and decomposed coffee pulp at a 3: 1 (v/v) ratio as reported by Sadeghian. (2008). After 13 months, they were transplanted to cylindrical stainless steel lysimeter containers with a 40 cm diameter and 40 cm height (one plant per container), with sandy-loam texture soil up to 30 cm deep, the physical-chemical characteristics of the soil used as substrate are shown in Table 1. The lime (120 g plant⁻¹) and fertilizers [N: 12 g plant⁻¹ (86 kg ha⁻¹); P: 6 g plant⁻¹ (43 kg ha⁻¹); K: 10 g plant⁻¹ (72 kg ha⁻¹)] were applied according to the recommendations reported by Sadeghian. (2014). Subsequently, in each container, MPS 6 and MPS 21 sensors (Teros 21, Decagon Devices, Inc, USA) were installed that were automatically adjusted to the data transmission system to measure the soil water potential (Ψ_s) to a soil depth between 20 to 25 cm, where the largest volume of roots is reported (Rendón et al., 2019). Then, the soil was added until reaching the maximum capacity of the container (50 L). Tensiometers (Irrrometer) were also installed at the same depth as described above to monitor soil moisture and determine the irrigation timing.

2.2. Experiment design and treatments

This experiment was conducted in February 2021, which coincides with the greatest leaf formation (Valencia, 1999). After 20 days of adaptation, the transplanted plants had developed at least a couple of leaves (Arcila-Pulgarín et al., 2002), and the evaluation of the two treatments began: T1) plants control (without stress), which were irrigated daily for maintained moisture at field capacity, between 0 and 0.033 MPa, and T2) plants under prolonged soil water deficit, in which the water supply was restricted for 40 days, conditions reported in the cultivable areas of coffee in Colombia (Ramírez et al., 2012). A completely randomized design with six repetitions per treatment was used; each repetition corresponded to one plant. The measurements were taken every five days after applying the treatments.

2.3. Physiological measurements

2.3.1. Leaf water potential

The water potential was measured at pre-dawn- Ψ_{pd} (4:00- 5:00 h) and at midday- Ψ_{md} (12:00 – 13:00 h), using a Scholander chamber (model 600, PMS Instruments Company, USA) on fully developed leaves, selected from the third or fourth pair of leaves from the apex of a plagiotropic branch. The leaves were bevel cut from the petiole

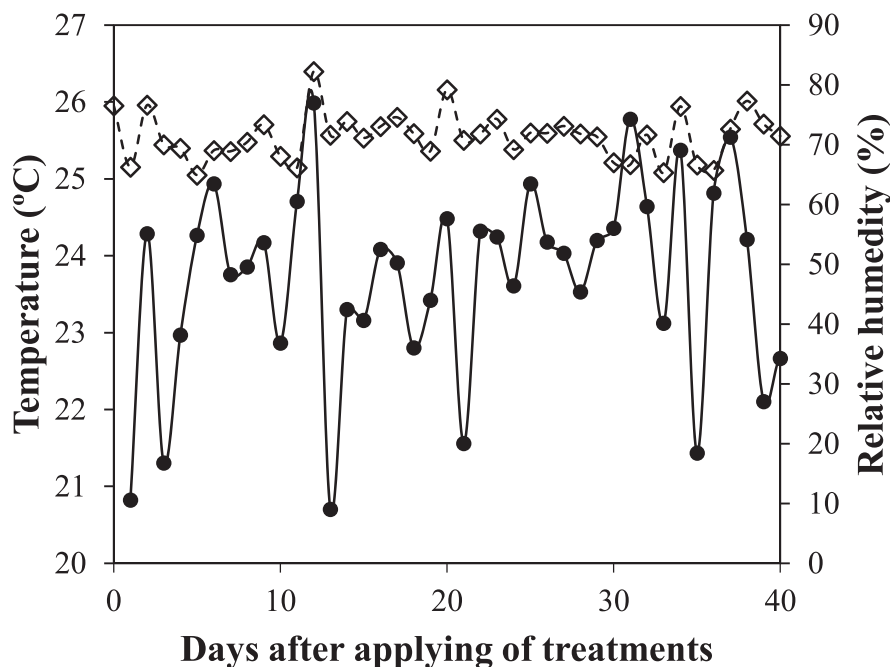


Fig. 1. Variation of temperature (• solid symbols) and relative humidity (◊ open symbols) according to days after withholding water in with well-watered and under water deficit coffee plants.

Table 1

Physical-Chemical characteristics of the soil used as substrate in the cylindrical containers of the without and with water deficit coffee plants.

pH	Texture	Organic Matter	Clay	Sands	Silt	N	K	Ca	Mg	Al	CEC*
			%					Cmol (+) kg ⁻¹			
4.0	Sandy loam	9.2	12	54	35	0.38	0.53	1.01	0.23	1.1	25

* Cationic Exchange Capacity

using a knife, and the surface was cleaned with deionized water and filter paper to remove cellular debris. The leaves were stored in zip-lock bags containing wet paper towels. The leaves were introduced into the Scholander chamber with the petiole cut exposed to atmospheric pressure, and compressed nitrogen was slowly applied until the xylem sap bubbled up, and the pressure was recorded.

2.3.2. Stomatal conductance

Stomatal conductance (g_s) was measured from 9:30 to 10:30 h, when the highest g_s values have been reported for coffee (Ghini et al., 2015; DaMatta et al., 2016), using a steady-state diffusion leaf porometer (model SC-1, Decagon Devices, INC, USA). Measurements were taken in the middle part of the adaxial surface on the leaf blade, on fully developed leaves, selected from the third or fourth pair of leaves from the apex of a plagiotropic branch.

2.3.3. Chlorophyll "a" fluorescence parameters

The parameters were measured with a Fluorpen modulated fluorometer (FFP 100-MAX-LM, Photon Systems Instruments, Czechia) using a one-light saturation pulse of 3000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and one actinic pulse of 350 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ times 60 s^{-1} . Measurements were taken from 4:30 to 5:00 h, when the leaves were adapted to darkness. Measurements were taken in the middle part of the adaxial surface on the leaf blade, on fully developed leaves, selected from the third or fourth pair of leaves from the apex of a plagiotropic branch. The ratio of variable fluorescence/maximum fluorescence (F_v/F_m) was calculated, which has been used as the potential photochemical efficiency of PSII (Φ PSII). The relative rate of

transport of the electrons of the PSII-ETR was calculated with equation [1] proposed by Genty et al. (1989).

$$ETR = 0.5 * 0.8 * PAR * \Phi \text{ PSII} \quad (1)$$

Where: 0.5 is a factor that assumes equal distribution of energy between the two photosystems, with a 0.8 coefficient of light absorbance, and PAR of the fluorometer.

2.3.4. Photosynthetic light response curves

The response curves to photosynthetic light on the net carbon assimilation rate (A/PFFD) were measured with an infrared $\text{CO}_2/\text{H}_2\text{O}$ gas analyzer (CIRAS-3, PP Systems, USA). Measurements were taken 40 days after applying the treatments from 8:30 a.m. to 10:30 a.m. on the middle part of the leaf blade in three fully developed leaves (for each treatment), selected from the third or fourth pair of leaves from the apex of a plagiotropic branch, located in the upper part of the canopy, preserving the natural angle of the leaves. These measurements were taken by increasing the photosynthetic photon flux density - PFFD by 13 levels (0, 20, 50, 80, 100, 200, 300, 400, 500, 700, 800, 1000, 1300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), keeping the leaf temperature stable at 25°C and the RH between 50 and 70%. The maximum photosynthetic rate (A_{max}), quantum yield (ϕ), saturation point (I_{sat}), light compensation (I_{comp}), and respiration (R_d) were determined from these curves (A/PFFD). Nonlinear regression techniques were used to estimate these parameters, as proposed by Lobo et al. (2013). The influence of PFFD on the transpiration rate (E), the internal carbon concentration (C_i), and the efficient water use -WUE (A/E) was also measured.

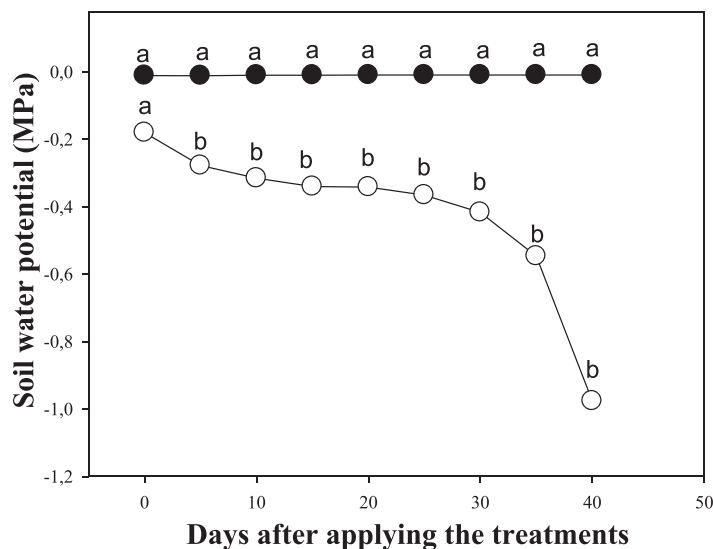


Fig. 2. Changes in soil water potential according to days after withholding water in with well-watered (solid symbols) and under water deficit (open symbols) coffee plants. Different letters denote significant differences among means within each time according to the Student-t test ($p < 0.05$).

2.4. Statistical analysis

The Ψ_{pd} , Ψ_{md} , g_s , F_v/F_m , and ETR data were statistically analyzed using the t-Student test to detect significant differences ($p < 0.05$) in each measurement and between plants without stress and plants under water deficit after fulfilling the assumptions of normality (Shapiro-Wilk) and homogeneity of variances (Levene's test).

For the A , E , C_i , and WUE curves as a function of the PFD in plants with and without water deficit, non-linear regression techniques were carried out, and the function was selected based on the coefficient of determination- R^2 , the mean square error-MSE and the significance ($p < 0.05$) of the parameters of each equation. The A/PFD model was a rectangular hyperbolic, as proposed by Michaelis-Menten [2] and used by Causton and Dale (1990).

$$A = \frac{A_{max} * PFD}{K + PFD} - Rd \quad (2)$$

Where: A is net photosynthetic rate, A_{max} is maximum photosynthetic rate, PFD is photosynthetic photon flux density, K is the saturation constant below half the saturating photon flux density, and Rd is dark respiration. To calculate I_{comp} , the equation [3] used by Rodríguez et al. (2019) was employed. The ϕ was based on the slope of the linear regression corresponding to the number of absorbed photons.

$$I_{comp} = \frac{(K * Rd)}{(A_{max} - Rd)} \quad (3)$$

All statistical analyses were performed with SAS version 9.4 (SAS Institute, 2013).

3. Results

3.1. Plant water relations

Restricting the water supply in the coffee plants generated gradual changes in Ψ_s , changing from -0.18 MPa at the beginning of the experiment to -1.0 MPa at the last measurement at 40 days after restricting the water supply in the soil. In the plants without stress, the variation in Ψ_s was very slight (between -0.010 to -0.008 MPa). In plants with stress, a decrease of the Ψ_s (-0.034 MPa) was observed after 15 days; subsequently, there was a gradual decrease -0.34 to

-1.0 MPa until reaching a reduction of 90%, in comparison with plants without water deficit (Fig. 2).

The water potential of the leaves in pre-dawn (Ψ_{pd}) and at mid-day (Ψ_{md}) were significantly lower in the stressed plants. Mean values of Ψ_{pd} and Ψ_{md} of -0.38 and -1.12 MPa were recorded in the plants without stress, as compared to -2.29 and -2.82 in the plants under water deficit, respectively. After 15 days of restricting the water supply in the stressed plants, statistically significant differences were observed, with a reduction in the Ψ_{pd} of -1.24 MPa for the plants without stress (Ψ_{pd} of -0.28 MPa). This indicated that, at that time, the plants were under conditions of moderate water deficit, which gradually became more severe, from Ψ_{pd} -2.53 to -4.0 MPa from 20 to 40 days after applying the treatments. Meanwhile, Ψ_{md} was more negative than Ψ_{pd} and had a similar progressive decrease after 15 days of restricting water supply in the stressed plants (Fig. 3).

3.2. Stomatal conductance (g_s)

In the plants under water deficit, there was a significant reduction of g_s , compared to plants without stress. This reduction was from 252.98 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ at the beginning of the experiment to 15.04 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ when the experiment ended. The plants began to partially reduce their g_s (151.40 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$, $p < 0.05$) five days after restricting water supply in the soil, as compared to the plants without stress (217.56 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$). However, the drastic reduction in g_s (70.78 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) started to be observed 15 days after applying the treatments, coinciding with the reduction of Ψ_{pd} , with a great loss of turgor in the guard cells, indicating stomata closure. This situation is more critical at 40 days because the g_s decreased considerably, up to 95% (15.04 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), in the plants without stress (Fig. 4).

3.3. Chlorophyll fluorescence parameters

In the plants under water deficit, F_v/F_m values between 0.82 to 0.76 and ETR values between 120 to 111 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ were recorded throughout the experiment. In the plants without water deficit, the F_v/F_m values were 0.82 to 0.80, and the ETR values were 121 to 118 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$. After 10 days of restricting the water supply, the plants under stress showed a significant reduction in both F_v/F_m and ETR, as compared to the plants without stress. At 15 days, these results were statistically similar both for plants with

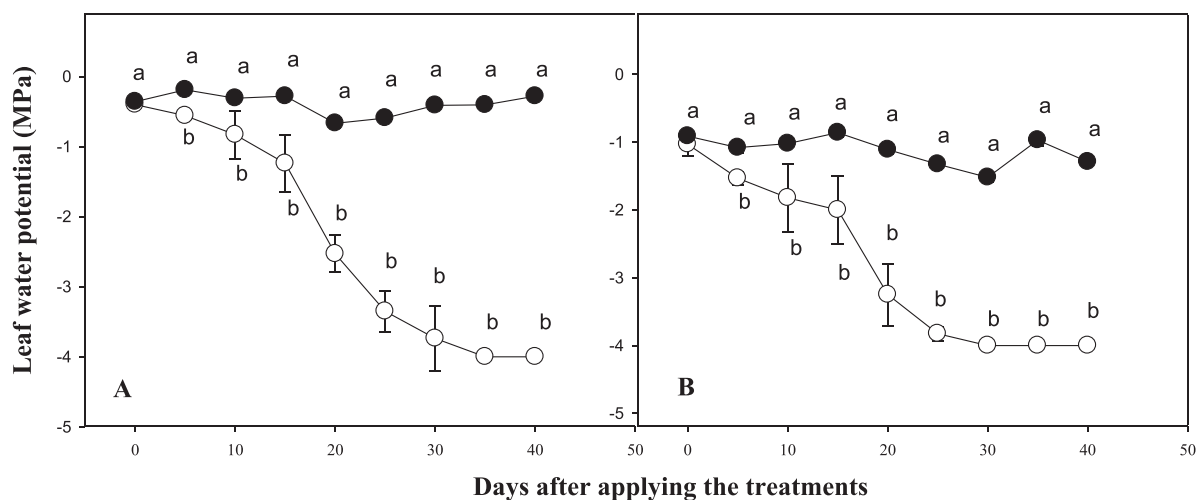


Fig. 3. Leaf water potential coffee plants under water deficit. Ψ_{pd} (A) and Ψ_{md} (B) were measured in leaves of well-watered (solid symbols) and with water deficit (open symbols) plants. Each point represents the mean of six replicates \pm standard error. Different letters denote significant differences among means within each time according to the Student-*t* test ($p < 0.05$).

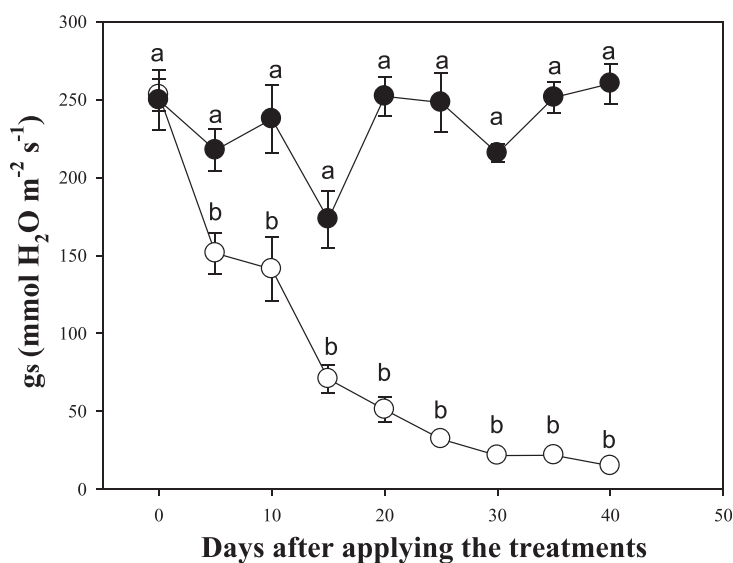


Fig. 4. Stomatal conductance coffee plants. Measurements were made in leaves of well-watered (solid symbols) and with water deficit (open symbols) plants. Each point represents the mean of six replicates \pm standard error. Different letters denote significant differences among means within each time according to the Student-*t* test ($p < 0.05$).

and without water deficit. Up to 30 days after applying the treatments, a significant reduction was observed in the plants under stress in F_v/F_m , with a value of 0.79, while ETR was reduced at 35 days to values of $113 \mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$, as compared with plants without stress (F_v/F_m 0.81 and ETR $119 \mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$). However, after 40 days, both F_v/F_m and ETR decreased in the plants under water deficit considerably (Fig. 5).

3.4. Photosynthetic light response curves

The response curve in the plants with water deficit showed that A was considerably lower than in the control plants, without significant variations, with increases in PPFD from $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. In the plants without stress, increases in A were recorded when PPFD intensified (Fig. 6). In the plants under water deficit (Fig. 3b), the decrease was 81% for A_{max} , 61% for I_{sat} , and 50% for R_d , as compared to plants without water deficit. In contrast to the values of φ and I_{comp} , the differences were slight for the plants without stress; however, the efficiency in the use of quantum (φ : $0.01 \mu\text{mol photon}^{-1}$) for the

fixation of $1 \mu\text{mol}$ of CO_2 was lower for the stressed plants (Table 2). On the other hand, the differences in A were mainly associated with limitations in g_s in the plants under water deficit (Fig. 8).

The other gas exchange parameters [E , C_i , and WUE] as a function of the PPFD showed different behaviors in the plants subjected to water deficit (Fig. 7). By restricting the water supply in the soil, the E was considerably lower in the plants under stress, with a cubic type regression as a function of PPFD. In the plants without stress, it increased logarithmically from 0.9 to $2.4 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ as a function of the PPFD increments (Fig. 7a). For C_i , this parameter had the same exponential behavior for the plants with stress and without water deficit, where the C_i concentrations decreased according to the increase in PPFD, without significant differences between the plants with and without water deficit. There was a marked reduction of up to $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, with C_i values of 303 and $335 \mu\text{mol CO}_2$ for the plants without stress and with water deficit, respectively (Fig. 7b). Between 80 to $1,300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, the stressed plants presented higher C_i values. The opposite case was seen for WUE , where the stressed plants presented values that changed from

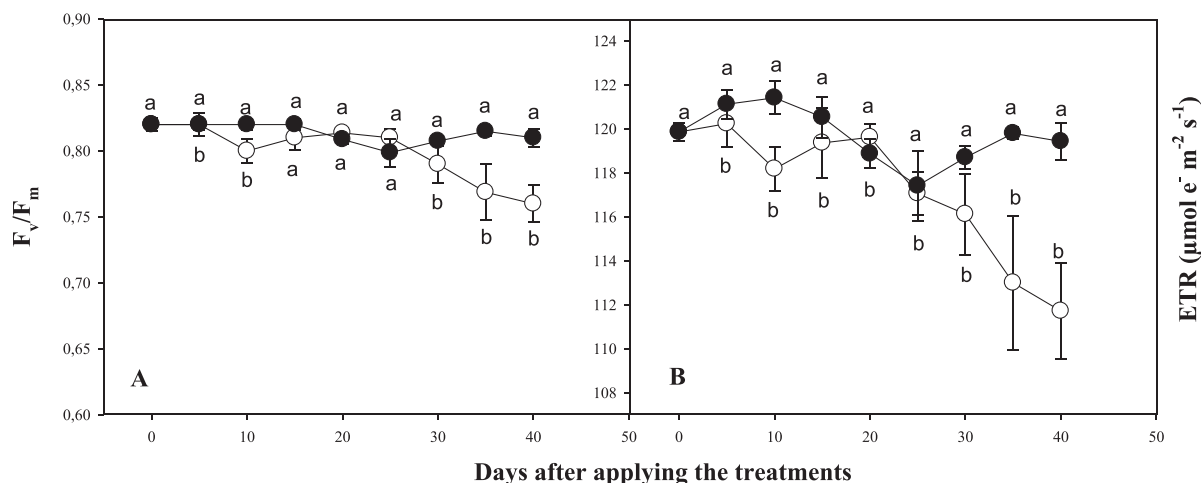


Fig. 5. Chlorophyll fluorescence parameters coffee plants under water deficit. The F_v/F_m (A) and ETR (B) were measured in dark-adapted leaves of well-watered (solid symbols) and with water deficit (open symbols) plants. Each point represents the mean of six replicates \pm standard error. Different letters denote significant differences among means within each time according to the Student-t test ($p < 0.05$).

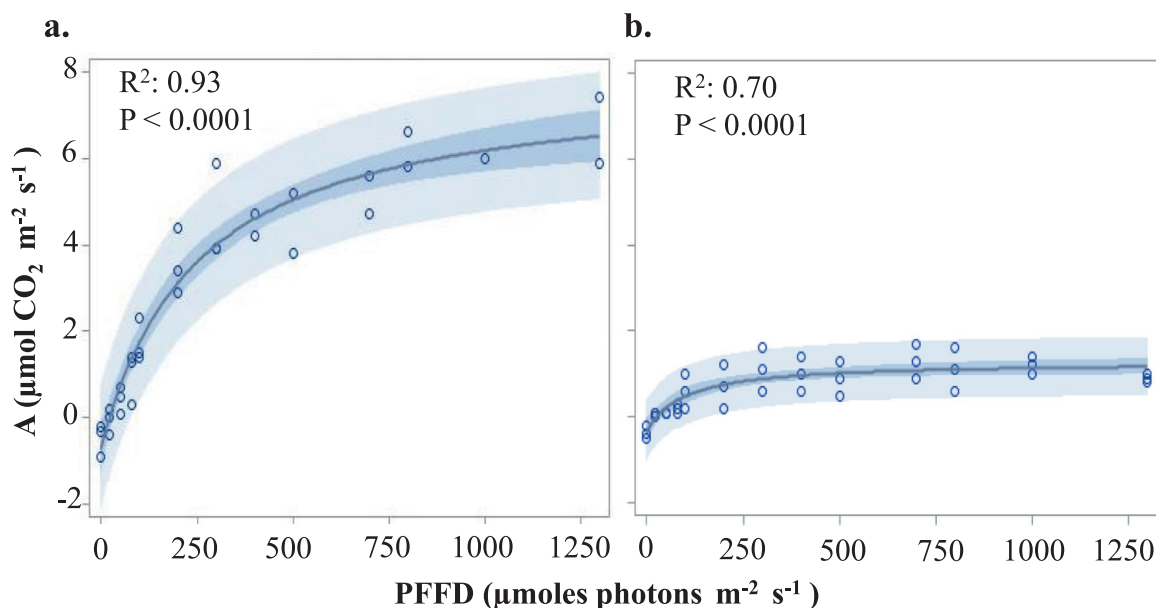


Fig. 6. Photosynthetic light-response curves as a function of PFFD in coffee plants. Values A were measured between 8:30 and 10:30 h at the end of the experiment on fully expanded leaves of well-watered (a) and with water deficit (b) coffee plants. The dark color indicates the 95% confidence limit and the light color the prediction limits.

Table 2

The parameters of the net photosynthetic light-response curve in without and with water deficit coffee plants. [A_{max} – maximum photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); φ – quantum yield ($\mu\text{mol CO}_2 \mu\text{mol photon}^{-1}$); I_{sat} – saturation point beyond which there is no significant change in A ($\mu\text{mol photon}^{-1} \text{ m}^{-2} \text{ s}^{-1}$); I_{comp} – light compensation point ($\mu\text{mol photon m}^{-2} \text{ s}^{-1}$); and Rd – dark respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)].

Parameters	Without stress	With stress
A_{max}	8.59	1.62
φ	0.02	0.01
I_{comp}	22.56	25.19
I_{sat}	489.80	189.70
Rd	0.72	0.34

0.25 to 1.97 $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ with increases in PFFD, but these values were much lower than those obtained in well-watered plants, where the recorded values changed from 0.40 to 3.62 $\text{CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ (Fig. 7c) although the same exponential behavior was found in the two treatments.

4. Discussion

The water deficit in this study affected the physiological performance of the *C. arabica* variety Castillo® plants, altering the net photosynthetic rates (Fig. 6), g_s (Fig. 4), and the measurements of E , C_i , and WUE (Fig. 7). The study results allow us to appreciate the different physiological mechanisms expressed by the Castillo® variety coffee plants to face the prolonged conditions of water deficit in the soil reported in the coffee-growing areas of Colombia under field conditions (Poveda et al., 2001; Ramirez et al., 2012; Molina et al., 2016). This is important information to have tools in assisted selection in breeding programs or to seek crop management strategies that can reduce the harmful effects of water deficit.

In our study, the intensity and duration of stress significantly altered the Ψ_{pd} and Ψ_{md} , key parameters for predicting the water status of coffee plants (Costa et al., 2018; DaMatta et al., 2018). On the one hand, the gradual changes of Ψ_s between -0.27 and -1.0 MPa (Fig. 2) decreased the Ψ_{pd} in the leaves, indicating that, as the water content in the soil was reduced, the water relations of the plants

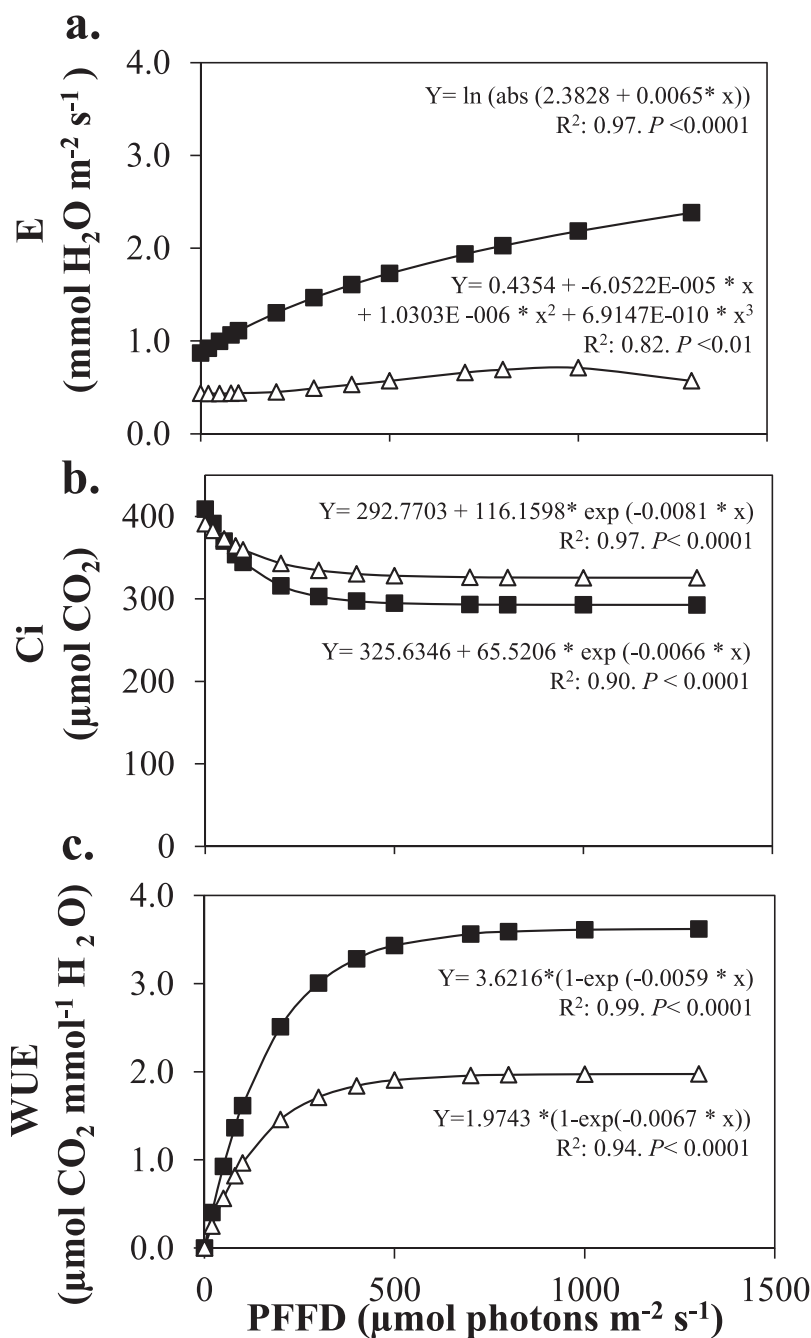


Fig. 7. Relationship between gas exchange parameters. The E (a), C_i (b), and WUE (c) as a function of PPFD in well-watered (\square solid symbols) and with water deficit (Δ open symbols) coffee plants. Each point represents the mean of three replicates ($n=3$).

were affected since Ψ_s and Ψ_{pd} have a direct relationship (Tounekti et al. 2018). The decrease in the value of Ψ_{pd} -2.29 MPa reported in this study (Fig. 3a) showed that *C. arabica* plants may trigger a low elasticity of the cell wall and losses of turgor, as previously have been reported in the study of the authors Tounekti et al. (2018) and Souza et al. (2020) in *C. arabica* plants with similar values Ψ_{pd} between -1.5 and -2.5 MPa. Likewise, with decreases in Ψ_{pd} from -1.4 MPa, the decrease in leaf water content and hydraulic conductance of the plant have been confirmed, evidencing the detrimental effect of water deficit on the water absorption process in *C. arabica* plants (Dias et al., 2007; Miniussi et al., 2015; Tounekti et al., 2018).

On the other hand, in the plants under stress the mean values of Ψ_{pd} -2.29 MPa and Ψ_{md} -2.82 MPa reported throughout the study showed that the *C. arabica* “Castillo®” variety plants preserve the

rehydration process from midday stressful conditions (Fig. 3). This must be since the Ψ_s is stable until 30 days after the application of the treatments and also may be to an osmotic adjustment at the cellular level according to the decrease Ψ_{pd} (DaMatta and Ramalho, 2006), specifically an increase in the accumulation of proline to maintain the turgor of the leaf cells, as reported in the studies by Tounekti et al. (2018) and Maestri et al. (1995) in *C. arabica* plants under water deficit conditions. However, in this study, after 25 days of restricting water supply, the plants began to lose their ability to rehydrate in the leaves after the stressful conditions of midday, as evidenced by mean values of Ψ_{pd} (-3.40 MPa) and Ψ_{md} (-3.83 MPa) that were statistically similar ($p > 0.05$) (data not shown) (Fig. 3). Although the Ψ_{pd} and Ψ_{md} (-4.0 MPa) values recorded at the end of this study did not exceed the reference water potential values (-7.0

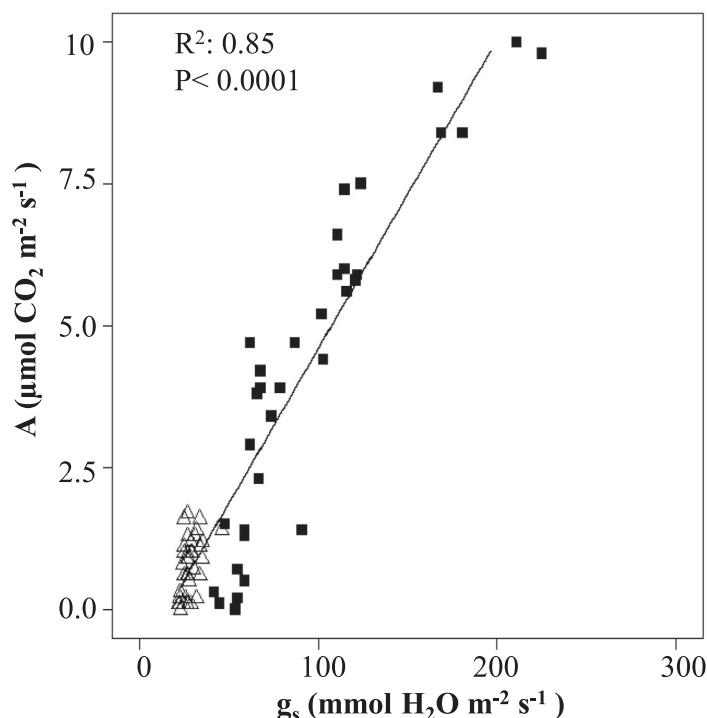


Fig. 8. Relationship between rate of net carbon assimilation (A) and stomatal conductance (g_s) in coffee plants. Values of A and g_s were measured between 8:30 and 10:30 h at the end of the experiment on fully expanded leaves of well-watered (\square solid symbols) and with water deficit (Δ open symbols) coffee plants.

to -8.0 MPa), where total wilting or death of *C. arabica* plants occur (Pereira et al., 2016; Costa et al., 2018; DaMatta et al., 2018), however, this stress can restrict essential physiological processes in the *C. arabica* plants, such as photosynthesis (Melke and Fetene, 2014; Sun et al., 2020).

As expected, the significant early decreases in g_s (Fig. 4) were the first physiological response of *C. arabica* plants subjected to water deficit conditions, as reported by Tounekti et al. (2018) and Souza et al. (2020). In the present study, these decreases were observed from five days after having restricted the water supply in the soil (Fig. 4), indicating that coffee plants have a higher degree of stomatal sensitivity to a limited supply of water in the soil or changes in the water potential of the leaves, as compared to air temperatures, atmospheric CO_2 concentrations or atmospheric vapor pressure deficits (DaMatta and Ramalho, 2006; Melke and Fetene, 2014; Landsberg and Waring, 2017). It has been reported that g_s decreases curvilinearly with reductions in leaf water potential (DaMatta et al., 2018). Furthermore, this g_s reduction response reported in this study confirms that *C. arabica* plants have efficient stomatal control (DaMatta and Ramalho, 2006), and this mechanism involves plants sensing stress early and, as a response, generating partial closure of stomata to avoid losses through transpiration; thus, they conserve water to prevent the cessation of metabolic and physiological activities. The responses to the closure of the stomata in coffee have been related to the biosynthesis and signaling of ABA in leaves, highlighting the role that this hormone plays in the tolerance to water deficits in the soil (Silva et al., 2018; de Oliveira Santos et al., 2021; Almeida et al., 2021). However, the 95% decrease in g_s reported 40 days after restricting the water supply (Fig. 4) may lead to limitations in the net assimilation of CO_2 in *C. arabica* plants (DaMatta et al., 2002; Frank and Vaast, 2009; Almeida et al., 2021).

In the plants with water deficit evaluated in this study, the A (Fig. 6b) was considerably affected, as compared to the plants without stress (Fig. 6a), with decreases of 81% in the A_{max} ($1.62 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), as well as reductions of 61% and 50% in the photosynthetic parameters I_{sat} ($189.70 \mu\text{mol photon}^{-1} \text{ m}^{-2} \text{ s}^{-1}$) and R_d ($0.32 \mu\text{mol}$

$\text{CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (Table 2), evidencing the strong detrimental effect of the intensity of water deficit on the photosynthetic performance of *C. arabica* variety Castillo® plants. These limitations in A caused by water deficit have been confirmed in the studies by DaMatta et al. (1998), Tounekti et al. (2018) and Souza et al. (2020). On the other hand, the A values reported in the plants without stress were within the reference range (4 to $11 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) reported for *C. arabica* (DaMatta et al., 2018). In this study, decreases in A were related to limitations in g_s in the plants under stress (Fig. 8), which was mainly due to the effect of the soil water deficit on the loss of plant water status, leading to decreases in g_s , limitations in the diffusion of CO_2 and reduction in the activity of RuBisCo (Almeida et al., 2021). This result has been reported for *C. arabica* in the studies by Dias et al. (2007), Frank and Vaast. (2009) and Almeida et al. (2021).

The significant decreases of g_s in the plants with water deficit also altered the measurement of gas exchange as a function of PPFD (Fig. 7), as evidenced by the marked decreases of E in the plants with water deficit, as compared to the plants without stress. (Fig. 7a), indicating the high sensitivity of stomata to the water deficit; however, this mechanism regulates water loss through transpiration, avoiding dehydration of tissue and cessation of essential metabolic activities in *C. arabica* plants (DaMatta and Ramalho, 2006; Tounekti et al., 2018; Almeida et al., 2021). On the other hand, there was a higher C_i in the plants with water deficit, from $80 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, as compared to the plants without stress (Fig. 7b). This possibly indicated that there was a reduction in both carboxylation activity and regeneration of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo). These results were confirmed in the studies by Souza et al. (2020) and Almeida et al. (2021). The WUE was low for the plants with stress (Fig. 6c), which was mainly due to the representative reductions of A and E (Souza et al., 2020), indicating that the amount of CO_2 fixed with the loss of water in the atmosphere was lower in these plants than in the plants without water deficit.

Under prolonged conditions of water deficit, it has been reported that the photosynthetic processes of coffee plants are impaired by

photochemical and biochemical limitations in the photosynthetic apparatus (Tounekti et al., 2018; Dubberstein et al., 2020; de Oliveira Santos et al., 2021). In our study, *C. arabica* variety Castillo[®] showed that the parameters F_v/F_m and ETR were not sensitive to the water deficit conditions that the plants were subjected (Fig. 5), and these results have also been reported by DaMatta et al. (2002), Menezes-Silva et al. (2017) and Dubberstein et al. (2020). The values of 0.82 to 0.76 for F_v/F_m have been reported for coffee plants subjected to water deficit (Menezes-Silva et al., 2017; Dubberstein et al., 2020), and these results did not exceed the range from 0.85 to 0.75 observed in unstressed plants (Björkman and Demmig, 1987), which indicates that the PSII reaction centers remained open and active to continue the transfer flow of photosynthetic electrons; therefore, there was no photoinhibition damage in the photosynthetic machinery (Dubberstein et al., 2020). As compared with the study by Tounekti et al. (2018), where *C. arabica* plants showed damage from photoinhibition after 21 days of water deficit.

The values of 120 to 111 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ for ETR have also been reported in coffee plants under water deficit (DaMatta et al., 2016; Menezes-Silva et al., 2017). Although there were slight and significant decreases after 35 days of restricting the water supply in the soil, these results showed that the energy absorbed by the PSII continued to regulate the ETR uninterrupted, adjusting the production of ATP and NADPH for the reactions of carboxylation in the tricarboxylic acid cycle. Therefore, there are no photochemical and biochemical limitations that harm the photosynthetic processes of coffee plants (Dubberstein et al., 2020; Souza et al., 2020).

Recently, it has been reported that *C. arabica* plants can regulate the effects of water deficit caused on the photochemical efficiency of photosynthetic devices. On the one hand, the expression of genes related to the synthesis of antioxidant enzymes and oligosaccharides are mechanisms that protect plants from oxidative damage at the photosystem level (Menezes-Silva et al., 2017; de Oliveira Santos et al., 2021). Likewise, the cyclical flow of electrons in *C. arabica* plants subjected to severe conditions of water deficit acts as a protection mechanism through an increase in the abundance of proteins that influence the transport of electrons at the photosystem level (Dubberstein et al., 2020), along with increases in the non-photochemical quenching parameters q_N and NPQ, which protect the PSII reaction centers by dissipating excess energy in the form of heat (Tounekti et al., 2018; Souza et al., 2020; de Oliveira Santos et al., 2021). *C. arabica* plants have developed various photoprotection mechanisms that play key roles in promoting tolerance to stressful conditions from water deficits in the soil.

5. Conclusions

These results suggest that *C. arabica* variety Castillo[®] plants are significantly affected by the prolonged conditions of soil water deficit. The plants water relations are slowly affected until 20 days after applying the treatments, although under the prolonged condition of soil water deficit for 40 days showed an important decrease. The first physiological response of the coffee plants under water deficit was early decreases in g_s , reflecting an efficient control of the stomata as a response to avoid the loss of water through transpiration. However, these significant reductions in g_s led to limiting the assimilation rates of CO_2 . Likewise, up to 40 days after restricting the water supply in the soil in this study, there were no photochemical limitations in the photosynthetic apparatus, confirming that decreases in photosynthesis are caused by stomatal limitations. It is possible to have damage from photoinhibition in the photosynthetic apparatus. However, this will require more research that can corroborate photochemical limitations under water deficit conditions that are longer than those evaluated in this study.

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.

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