ROOT GROWTH, WATER RELATIONS AND PHOTOSYNTHETIC EFFICIENCY IN CLONES OF *Coffea canephora*

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I dedicate

it to my parents Maria Aparecida and Pedro and to my sister Daiane, who never spared any efforts so that I could achieve my dreams.

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SUMMARY

ABSTRACT

BARONI; Danilo Força; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro; April 2022; Root growth, water relations and photosynthetic efficiency in clones of *Coffea canephora*; Professor Advisor: Eliemar Campostrini

In the process of selecting canéfora coffee genotypes with greater capacity to acclimatize to supra and infra-optimal environmental factors, notably soil and air water limitation, the knowledge of morphophysiological and anatomical responses from the leaf to the root is of great importance. The objective was to study the relationships between root system depth growth, midday photosynthesis depression, water relationships and shoot growth in Coffea canephora clones. Two experiments were carried out, the first being related to the depression of photosynthesis at noon (MDP) and the relationship with root system growth under conditions of good soil water availability, and the second being associated with morphophysiological and anatomical responses from root to leaf in clones of Coffea canephora contrasting with the growth in depth of the root system, when the plants were submitted to two consecutive events of water deficit submitted to consecutive events of soil water deficit and rehydration. Both experiments were carried out in a greenhouse, at Universidade Estadual do Norte Fluminense –

Darcy Ribeiro, with clones of Coffea canephora Pierre ex Froehner (clones 'A1' and '19' in the first experiment and clones '3V' and 'A1' in the second). In order to carry out more detailed analyzes of the root system, the plants were grown in PVC tubes measuring 0.2 m in diameter x 1.0 m in height. In the first study, under conditions of good soil water availability, a lower MDP was observed associated with the increase in the maintenance cost of the photosynthesis, not converting the greater assimilation of CO2 at the time of higher temperature and DPVar in greater plant growth. In the second study, after the withdrawal of irrigation water, in both water deficit events, root growth in deeper soil layers seems to be more advantageous at the beginning of the period (moderate water stress) and in the plant recovery after the rehydration, once the quick recovery time of photosynthetic rate and stomatal conductance was associated with deeper root system and greater plant growth. However, the combined hydraulic, anatomical, photosynthetic and photochemical adjustments seem to be more determinant as an acclimatization response to severe water deficit than a deep root system.

RESUMO

BARONI; Danilo Força; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro; Abril de 2022; Crescimento radicular, relações hídricas e eficiência fotossintética em clones de *Coffea canephora*; Professor Orientador: Eliemar Campostrini

No processo de seleção de genótipos do cafeeiro canéfora com maior capacidade de aclimatação aos fatores ambientais supra e infraótimos, notadamente a limitação hídrica do solo e do ar, o conhecimento de respostas morfofisiológicas e anatômicas a partir da folha até a raiz é de grande importância. O objetivo foi estudar as relações entre o crescimento em profunduidade do sistema radicular, a depressão da fotossíntese ao meio-dia, as relações hídricas e o crescimento da parte aérea em clones de *Coffea canephora.* Foram realizados dois experimentos, sendo o primeiro, relacionado à depressão da fotossíntese ao meio-dia (DFM) e as relações com o crescimento do sitema radicular em condições de boa disponibilidade hídrica do solo, e o segundo, foi associado às respostas morfofisiológicas e anatômicas da raiz à folha em clones de *Coffea canephora* constrastantes ao crescimento em profundidade do sistema radicular, quando as plantas foram submetidas a dois eventos consecutivos de déficit hídrico submetidos a eventos consecutivos de déficit hídrico do solo e reidratação.

Ambos os experimentos foram realizados em casa de vegetação, na Universidade Estadual do Norte Fluminense – Darcy Ribeiro, com clones de *Coffea canephora* Pierre ex Froehner (clones ‘A1’ e ‘19’ no primeiro experimento e clones ‘3V’ e ‘A1’ no segundo). Para a realização de análises mais detalhadas do sistema radicular, as plantas foram cultivadas em tubos de PVC com dimensão de 0,2 m de diâmetro x 1,0 m de altura. No primeiro estudo, em condições de boa disponibilidade hídrica do solo, uma menor DFM esteve associada ao aumento do custo de manutenção Da fotossíntese, não convertendo a maior assimilação de CO_2 no horário de maior temperatura e DPV_{ar} em maior crescimento da planta. Já no segundo estudo, em ambos os eventos de déficit hídrico, o crescimento radicular em camadas mais profundas do solo parece ser mais vantajoso no início do período (estresse hídrico moderado) e na recuperação após a reidratação, uma vez que a rápida recuperação da taxa fotossintética e da condutância estomática foi associada ao sistema radicular mais profundo e maior crescimento das plantas. No entanto, os ajustes hidráulico, anatômico, fotossintético e fotoquímico combinados parecem ser mais determinantes como resposta de aclimatação ao déficit hídrico severo, do que um sistema radicular profundo.

1. INTRODUCTION

Coffee production is one of the main responsible for Brazil's economic growth. Two species of coffee trees, *Coffea arabica and C. canephora*, are cultivated in several Brazilian states, and the production of fruits of these species drives internal and external trade, which promotes increased income for farmers and the entire chain around this important commodity, through direct and indirect jobs. Coffee growing is one of the main activities of Brazilian agribusiness, and is responsible for generating more than 8 million jobs in the country (MAPA, 2018).

Coffee production, as other crops, could be negatively and intensely affected by future climate change. These climate changes are associated with changes in the frequency and severity of extreme events, including increased heat waves, floods and prolonged drought episodes (DaMatta et al., 2018).

Although increasing atmospheric CO_2 concentration can mitigate the effect of high temperatures on *C. arabica and C. canephora* (Rodrigues et al., 2016), these benefits can be progressively eliminated as the drought increase (Gray et al., 2016). In extreme events of rainfall and drought, associated with global climate variability, the predicted increases can bring great uncertainties as to how these environmental factors may affect productivity, coffee yield and beverage quality

(DaMatta et al., 2018), being this is an extremely harmful scenario for the growth and development of these species (Bunn et al., 2015).

In the context of climate change, it is important to evaluate the adaptation and mitigation strategies of coffee trees, with the aim of improving the sustainability of crops (DaMatta et al., 2018). Among these strategies, there is the selection of superior genotypes in terms of tolerance/resistance to soil and air water limitation.

Drought-adapted plants are often characterized by having vigorous (DaMatta, 2004) and deep (Pinheiro et al., 2004) root systems. However, detailed studies on the characteristics of the root system of coffee plants are still scarce in terms of growth (size, volume and depth) in various environments (Carvalho et al., 2008; Andrade Júnior, 2013), as well as the relationship between root depth and photosynthetic capacity and anatomy of young coffee plants. Even more complex, under water limitation, is the quantification of how much the growth or reduction of the root system can interfere with the photosynthetic capacity and vice versa.

The relative conditions of water deficit can also reduce the water transport capacity of the leaf, inducing a loss of turgor and thus a reduction in the growth of this organ (Scoffoni et al., 2014; 2017; Trifilo et al., 2016). These effects of water limitation on the leaf can interfere with thermoregulation. As well as roots, leaves are key components in the hydraulic system of plants, as they play an important role in gas exchange and in determining plant growth (Sack and Holbrook, 2006; Brodribb et al., 2007). Studies of the root and leaves hydraulic traits provide insights from the cellular scale, leaf/root scale to the whole plant scale (Sack and Scoffoni, 2012).

The search for morphological, physiological and anatomical responses in the scale of organ and tissues from roots to leaves can bring information of great importance to the process of selecting genotypes with greater capacity of climatic conditions acclimation, notably the soil and air water limitation. We objective was to study the relationships between root system growth in soil depth, midday depression of photosynthesis (associated with increased atmospheric demand and solar radiation), percentage of photoinhibition, water relations and shoot growth in clones of *C. canephora*.

2. REVIEW OF LITERATURE

2.1. Taxonomic classification, origin and characterization of the species

The coffee plant belongs to the Rubiaceae family and the Coffea genus (Charrier and Berthaud, 1985) and has around 130 species (Davis and Rakotonasolo, 2021). However, world trade is supported only by the species *C. arabica* L. and *C. canephora* Pierre ex A. Froehner, which are responsible for almost 99% of coffee production (Partelli et al., 2011; Davis et al., 2012).

The species *C. arabica* is popularly known as 'Arabica' and *C. canephora* Pierre as 'Robusta'. *C. canephora* is diploid (2n = 22 chromosomes), self-incompatible and, therefore, cross-fertilized. Such incompatibility is of the gametophytic type and is linked to the S1, S2, S3 and S4 alleles (Conagin and Mendes, 1961; Berthaud, 1980; Mishra and Slater, 2012).

C. canephora is native to the African rainforest and originates from a large area stretching from West Africa, including Cameroon, Central African Republic, Congo, Democratic Republic of Congo, Uganda and northern Tanzania to northern Angola (Musoli et al., 2009). The origins of the robusta/conilon coffee tree are located at altitudes of up to 1,200 meters, with an average annual temperature between 23 and 26 °C, with small variations, and annual rainfall can exceed 2000 mm, distributed over 9 to 10 months (Coste and Cambrony, 1992; Davis et al., 2006). Thus, canephora coffee is expected to develop better under an annual average temperature of 22 to 26 °C (DaMatta and Ramalho, 2006; Matiello et al., 2010).

The reproductive traits and the existence of “Conilon” and “Robusta” variety within the species *C. canephora* allow a great heterogeneity between the genotypes. This statement was corroborated by Ferrão et al. (2009), in a study developed with several clones belonging to the “Conilon” and “Robusta” varieties, which found great divergence in plant architecture, leaf size and shape, disease response, drought tolerance/resistance and grain shape.

According to Ferrão et al. (2017), the “Conilon” variety is characterized by plants with shrub growth, early flowering, branched stems, elongated leaves, greater susceptibility to diseases and greater tolerance to water deficit when compared to the “Robusta” variety. According to the same authors, these two varieties also present differences between the aroma and flavor of the drink, with “Robusta” having the highest proportion of classification as premium coffee.

2.2. Economic importance

According to Carvalho et al. (2017), coffee production and marketing are related to about 20 to 25 million families in more than 50 countries. For the 2017/18 harvest, global production of this commodity was estimated at 159.66 million bags, 1.2% higher than in 2016/17. Of this total, there was a 12.1% increase in the production of “Robusta” cultivars, largely due to increases in production in Vietnam, Indonesia and Brazil.

Brazil is the largest exporter and producer of coffee and the second largest consumer of the beverage in the world. In the 2016/2017 harvest, production was 44.97 million of Arabica and Robusta coffee processed bags. In the production of both species, the estimate for the 2018 harvest was an increase of 29.1% (CONAB, 2018).

In 2017, conilon coffee production was 10.721 million of bags, with Espírito Santo being the largest producer, with about 5.915 million bags (CONAB, 2018). Despite being the largest producer, Espírito Santo has had a decrease in conilon coffee production in recent years (CONAB, 2017). This may be related to unfavorable weather conditions and increased production cost (Baitelle et al., 2017).

The coffee production associated with robusta/conilon has great economic importance, since, in the roasting and grinding industries, it is raw material for the soluble coffee industry and acts as a component in the composition of blends with *C. arabica*. Thus, due to the higher industrial yield, higher content of soluble solids and lower average prices in the commercialization, this process associated with the robusta/conilon industry provides the final product with great ability to compete in the market (Ivoglo et al., 2008).

2.3. Climate change and the influence on coffee culture

Since pre-industrial times, the CO_2 atmospheric concentration has increased. Currently has reached values above 418 ppm (NOAA, 2022). The global average surface temperature in 2021 was 0.84 °C above the 20th-century average. Is predicted the double of the actual atmospheric CO_2 concentration for 2060, in parallel with an increase in temperature of up to 4.8 °C (Sherwood et al., 2020). These climate changes will have a great effect on the species, and specifically in *C. arabica* and *C. canephora*. For 2050, *C. arabica* could lose 56% of areas currently suitable for cultivation, particularly in Brazil, East Africa and Madagascar, and gain only 9% of new suitable areas (Magrach and Ghazoul, 2015).

According to developed models*, C. canephora* could lose 55% of the currently suitable areas, mainly in West Africa and Brazil. On the other hand, the suitable future area could double in size. However, of the future area suitable for the cultivation of *C. canephora*, 65% is under forest cover and 24% is currently under other crops growing (Magrach and Ghazoul, 2015).

Although there are suggestions that the area suitable for growing Arabica and Robusta will be sufficient to meet expected future demand (Magrach and Ghazoul, 2015), there will be regional changes in the global suitability of coffee (Bunn et al., 2014). The areas suitable for future coffee cultivation in Brazil, currently the world's largest producer, are in decline (Magrach and Ghazoul, 2015). This result is driven by changes in precipitation and temperatures, along with increasing seasonality, where extremes are increasingly stronger, i.e. dry seasons, increasingly drier and wet seasons, increasingly wetter and warmer. (Haggar and Schepp, 2012) and vice versa.

In C3 species, the higher CO_2 concentration can increase the net photosynthetic rate, due to the higher ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) carboxylation rate and photorespiration decrease, which simultaneously results in increases in the availability of substrate and competitive inhibition by O_2 (Long et al., 2004; Kirschbaum, 2011). However, in the long term, this increase in photosynthetic rate can result in the photoassimilates accumulation, due to the inability to use them. It may reduce the photosynthetic potential due to the “negative feedback” effect of photosynthesis (Drake et al., 1999; Ainsworth and Rogers, 2007; Kirschbaum, 2011).

Ramalho et al. (2013) studied *C. arabica* (cv. Icatú and IPR108.) and *C. canephora* (cv. Conilon Clone 153 - EMCAPA 8113), grown over a year under controlled environmental conditions. They found that both species showed an increase in photosynthesis when the CO_2 concentration was increased from 380 to 700 ppm, without any negative regulation of photosynthesis. This response was linked to the consumption of photoassimilates and the regeneration of ribulose 1,5-bisphosphate (RuBP) and inorganic phosphorus - Pi (through the use of triose phosphate), associated with the continuous production of vegetative and reproductive structures during the experiment (Ramalho et al., 2013).

The increase in atmospheric CO_2 concentration can attenuate the effect of high temperatures in *C. arabica* and *C. canephora* under adequate conditions of water availability, since in the photosynthetic carbon assimilation, the photochemical and biochemical activities of the plants are improved under enrichment of CO_2 (Rodrigues et al., 2016). However, in soybeans, the benefits of increasing CO_2 concentration may be progressively diminished as drought increase (Gray et al., 2016). These authors found that gas exchange, biological nitrogen fixation and yield of several soybean cultivars were negatively influenced when high CO_2 concentration occurs in association with soil water restriction.

Additionally, water stress can intensify the impacts of high temperature (Brown et al., 2016). According to DaMatta et al. (2006), coffee plants exposed to higher temperatures tend to reduce net carbon gain by increasing respiration at a higher rate than photosynthesis. The magnitude of these impacts varies between plant species, however, there is indeed a consistent negative impact for all plants (Hatfield and Prueger, 2015).

Although there is evidence suggesting that in the absence of stressful conditions, C3 species are likely to be more productive with increasing atmospheric CO_2 concentration than C4 species, there are great uncertainties about food production in a future scenario with general global warming and altered regional rainfall patterns (DaMatta et al., 2010).

2.4. Abiotic factors (water, light and temperature): effects on leaf gas exchange

As the plants coordinate the response to water deficit under drought conditions or high evaporative air demand strongly influences productivity and survival in different environments (Boyer, 1982). Stomatal control is at the heart of this process, responding quickly to small environmental changes to optimize the exchange of water for carbon (Farquhar and Sharkey, 1982). The stomata regulate the water status of the plant, controlling the rate of water loss to the atmosphere, corresponding to the capacity of the soil-plant hydraulic system to supply water to the leaves (Attia et al., 2015). As a result, stomatal control is used as an indicator of water stress and is an important factor in photosynthesis studies (McDermitt, 1990).

The stomatal response to the increase in water demand is driven by two hypothetical mechanisms: the hydropassive mechanism, which occurs through changes in the turgor of the guard cells as a result of the variation of the leaf apoplastic water potential, and the hydroactive mechanism, which occurs through adjustment of the guard cell turgor through ion uptake or release, or synthesis of organic solutes in guard cells (Sussmilch et al., 2019).

Both hypotheses have been advanced to explain stomatal closure as a function of increased air vapor pressure deficit (DPV_{ar}). The first hypothesis assumes that guard cells are hydraulically distal to epidermal cells in the transpiration flow – that is, the total hydraulic resistance of soil to guard cells is greater than that of soil to epidermis – so that a drop in air humidity, associated with high air temperature, causes a greater drop in water potential in guard cells than in epidermal cells (Farquhar, 1978; Maier-Maercker, 1983; Dewar, 1995), causing a hydropassive reduction of the opening stomata. The other hypothesis assumes that guard cells “hydroactively” release osmotic solutes in response to a drop in water status, as well as actively regulating solute content in response to other environmental factors such as light and CO_2 (Darwin, 1898; Stalfelt, 1929; Meidner, 1986; Buckley, 2005).

In both hypothetical mechanisms, the epidermal mechanical advantage is outweighed by a disproportionate drop in guard cell turgor – this is caused by a disproportionate decline in the water potential of the guard cell in the first hypothesis, or in the osmotic pressure of the guard cell in the second hypothesis (Buckley, 2019).

Recent studies have shown that abscisic acid (ABA), which is known to close stomata by inducing solute loss from guard cells, is quickly synthesized de novo within leaves in response to reduced air humidity (Xie et al., 2006; Bauer et al., 2013; McAdam et al., 2016b). A study developed by Ikegami et al. (2009) showed that when leaves and roots of Arabidopsis were isolated from each other and subjected to water stress, ABA concentrations greatly increased in leaves but not in roots. Much of the ABA present in roots may in fact originate in leaves (Ikegami et al., 2009; McAdam et al., 2016a; Castro et al., 2019), and ABA synthesis in roots may require transported precursors leaves (Ren et al., 2006; Manzi et al., 2015; Zhang et al., 2018).

Solar radiation on the leaf and the state of hydration of the same directly interfere in the process of opening and closing of the stomata, which in turn influence the productivity of the crop (Costa and Marenco, 2007). On sunny days, and around midday, higher levels of irradiance on the leaf increase the evaporative demand on the air (high VPD_{ar}) and between the leaf and air ($VPD_{leaf-air}$) and induce stomatal closure in response to excessive loss of water through transpiration. In this condition, even with adequate availability of water in the soil, if the water demand of air and leaf-air is high, the hydraulic resistances may not allow adequate access of water in the leaf, and thus the leaf water deficit is observed through the stomatal closure (Kitano and Eguchi, 1993).

The high stomatal sensitivity of coffee plants is associated with environmental factors such as light, temperature and VPD_{ar}, in addition to $VPD_{leaf-air}$ (Kanechi et al., 1996). Temperature, VPD_{ar} and $DPV_{leaf-air}$ are shown to be the most important in stomatal control when the soil is at field capacity (Nunes, 1988). Thus, the high VPD_{ar} and $VPD_{leaf-air}$, which usually occur between midday and 3 p.m., in a cloudless sky condition, influence the water balance of the plant, which can affect photosynthesis and plant growth (Devi and Redd, 2018). This MDP has been frequently observed in various cultures (Hirasawa et al., 1989; Hu et al., 2009). As photosynthesis is an important biochemical process for the growth and productivity of crops, MDP can be a limiting factor for the productivity of crops of agronomic and forestry interest (Yokoyama et al., 2019).

It has been reported that stomatal limitation is a major cause of MDP under mild to moderate water stress (Flexas et al., 2004; Grassi and Magnani, 2005). As a consequence, there is a reduction in the CO_2 imput into the leaves, and therefore, the assimilation of CO_2 is reduced.

According to Larcher (2000), gas exchange changes during the plant development cycle and depends on the annual and daily course of environmental fluctuations around the plant. Thus, photosynthetic capacity is an intrinsic characteristic of each plant species, depending on phenophase and external conditions. Under saturating light conditions, it has been shown that, in coffee trees, the maximum values of photosynthetic rates are around 7 to 12 μmol of CO_2 m^{-2} s^{-1} (Rena et al., 1994). However, under conditions of high temperature and water-limited soil and air, this rate may be reduced because of an increase in the rate of photorespiration due to stomatal closure. Therefore, photosynthesis is extremely sensitive to the effects of water deficit (Lisar et al., 2012) and other environmental stresses.

2.5. Abiotic factors (water, light and temperature): effects on leaf photochemistry

Plants are sessile organisms subjected to daily abiotic stresses that determine harmful effects on the photosynthetic apparatus (Guidi et al., 2019). In C3 species, when stomata close in response to stresses such as drought or high temperature, CO_2 assimilation is reduced, photosynthetic reduction of O_2 via photorespiration increases and serves as a sink for excess excitation energy in the photosynthetic apparatus (Cornic and Briantais, 1991). However, increases in the photorespiratory reduction rate of O_2 are not sufficient to dissipate excess excitation energy in photosystem II (PSII) antennas (Guo et al., 2009). Thus, the leaf may become unable to increase the dissipation of this energy, and photodamage to the PSII reaction centers may occur. In plants grown outdoors, and under high solar radiation and high temperature, the midday depression of the photochemical efficiency of PSII, evaluated mainly through the emission of chlorophyll fluorescence, is reported by several authors (Horton et al., 1996; Pons and Welschen, 2003). In the leaf, supra-optimal light and temperature stress can limit plant productivity, although there is no tissue damage (Long et al., 1994). Non-stomatal limitations may be the primary cause of MDP in *Myrica rubra* plants, and stomatal closure at midday may accelerate photodamage and D1 protein degradation (Guo et al., 2009).

Under field conditions, and throughout the day, plants are exposed to different intensities of solar radiation. When the plant canopy absorbs more photons than can be used by photosynthesis, photoinhibition occurs, evaluated through a decrease in the efficiency of the energy conversion of photons into ATP and NDPH, and consequently, into photoassimilates (Demmig-Adams and Adams, 1992). Photoinhibition can be reversible or irreversible and is related to the large flux of photons when electron transport to acceptors is limited (Melis, 1999). The degree of photoinhibition depends on the balance between photodamage and PSII core repair via D1 polypeptide (Demmig-Adams et al., 2012).

Light-induced inhibition of PSII activity is increased under water stress conditions. Although light energy is the primary source of energy for the photosynthesis process, excess light energy generates reactive oxygen species (ROS), mainly by excessive reduction of photosynthetic electron transport components, i.e. quinone A (QA), subsequently reducing molecular oxygen to produce ROS (Parkash and Singh, 2020). ROS production is also associated with supraoptimal temperatures (Todorov et al., 2003; Guo et al., 2006), since high temperatures can cause photosynthetic pigment degradation (Rodrigues et al., 2018). The increase in temperature accelerates kinetic energy and the movement of molecules across membranes, accelerating chemical reactions, weakening chemical bonds, and making the lipid matrix membrane more fluid, and protein denaturation and degradation may occur (Wahid et al., 2007). Due to their high reactive capacity, ROS are highly harmful and, by inhibiting the synthesis of proteins necessary for PSII repair, they can inactivate PSII (Murata et al., 2012).

2.6. Leaf hydraulics: importance in water transport and tolerance to water deficit

The rate of water loss from leaves or leaf transpiration (E) is mainly determined by the diffusion of water vapor from within the mesophyll to the air and evaluated using the $VPD_{leaf-to-air}$ (Xiong and Nadal, 2020). Although the distances that water must travel within the leaves are small in relation to the entire soil-plant-atmosphere continuous, the interior of the leaves constitutes a resistance for the water transport (Sack and Holbrook, 2006), contributing significantly to the plant hydraulics resistance (Xiong and Nadal, 2020).

In studies of leaf hydraulic dynamics, Martins et al. (2014) reported a great restriction in maximizing gas exchange of coffee leaves imposed by the hydraulic architecture of the leaves. According to Ribeiro et al. (2009), Nardini et al. (2014) and Mesquita et al. (2016), there is a correlation between gas exchange and leaf hydraulic conductance (K_L), since the decrease in gas exchange is associated with a lower K_L. Sinclair et al. (2007) found that in soybean genotypes with different levels of water deficit tolerance, the leaf hydraulic traits can influence the water relations of plants under conditions of higher evaporative demand (i.e. high VPD_{ar}).

The loss of cellular turgor is arguably the most recognized classic indicator of plant water stress, having impacts on cellular structural integrity, metabolism, and whole plant performance (Kramer and Boyer 1995; McDowell 2011). Consequently, leaf water potential (Ψ_{leaf}) at the point of turgidity loss (Ψ_{TLP}, MPa units) has been used to assess physiological drought tolerance for decades (Bartlett et al., 2012; Zhu et al., 2018).

Ψ_{TLP} is classically measured in drought tolerance assessments, as one of the six main leaf indices related to cellular water relationships, and structural properties typically calculated from a plot of Ψ_{leaf} versus leaf water content in dehydration, known as the pressure-volume curve (P-V curve) (Bartlett et al., 2012).

Plants with low Ψ_{TLP} tend to maintain stomatal conductance, hydraulic conductance, photosynthetic rate and growth even under reduced soil water potential, which is important in drought situations during the growing season (Abrams and Kubiske 1990; Sack et al., 2003; Baltzer et al., 2008; Mitchell et al., 2008; Blackman et al., 2010). Therefore, Ψ_{TLP} is a trait that quantifies the ability to 'tolerate' drought rather than 'avoid' drought by ceasing gas exchange and surviving on stored water (Chaves et al., 2002; Brodribb and Holbrook, 2005; Ogburn and Edwards, 2010). Other indices of the P-V curve, such as the fraction of apoplastic water, modulus of elasticity (ε), osmotic potential at total hydration (Ψ_o) and tissue capacitance (*C*) were also correlated with various aspects of drought tolerance (Niinemets, 2001; Brodribb and Holbrook, 2003; Lenz et al., 2006; Baltzer et al., 2008).

Plants of certain species improve drought tolerance by making the Ψ_{TLP} more negative, and this can be done by solutes accumulation (decreasing Ψ_o), reducing the symplastic water content, and redistributing more water outside the cell walls and/or increasing the flexibility of the cell wall (decreasing ε) (Bartlett et al., 2012). While a more negative Ψ_{TLP} is thought to benefit drought tolerance, a less negative Ψ_{TLP} may be beneficial as it allows leaves to quickly lose turgor and close stomata as Ψ_{leaf} declines and thus maintain a high relative content of water at the turgor loss point- RWC_{TLP} (Walter and Stadelmann, 1968; Read et al., 2006). In fact, maintenance of cellular hydration is more important than turgor, as dehydration can induce shrinkage, causing structural damage to the wall, and potentially osmotic stress to the cell, due to an increase in ion concentration, which can disrupt metabolic processes (Bartlett et al., 2012), as an example the high concentration of Mg^{2+} in the stroma causing inhibition of ATP production. The synthesis of RuBP and several portains can also be inhibited by the high concentration of ions in the plant cell (Lawlor and Cornic, 2002).

The relationship between a high ε, Ψ_{TLP} and drought tolerance has been called one of the oldest controversies in ecology (Lamont et al., 2002), giving rise to numerous hypotheses, such as: (1) as leaves dehydrate, a high ε causes the Ψl_{eaf} to decrease quickly allowing sustained uptake of water in dry soil (Bowman and Roberts 1985); (2) a high ε is related to a lower Ψ_{TLP} (Lenz et al., 2006); (3) a high ε contributes to a less negative Ψ_{TLP}, allowing stomata to close quickly with loss of turgor, maintaining a high RWC_{TLP} (Walter and Stadelmann, 1968; Read et al., 2006); (4) a high ε is necessary to provide mechanical support for cells with very negative Ψ_o and Ψ_{TLP}, preventing disruption due to excessive turgor pressure when cells are fully hydrated (Jones, 1992); (5) a high ε would mechanically restrict the shrinkage of cells with highly negative Ψ_o and Ψ_{TLP}, allowing RWC_{TLP} to remain high (Cheung et al., 1975; Jones, 1992) and (6) a high ε may not play a direct role in drought tolerance and instead improve the carbon and/or nutrient balance, contributing to longer leaf life (Grubb, 1986; Sack 2004; Markesteijn et al., 2011).

A high Ɛ would bring additional benefits to plants in arid areas through characteristics that are not directly linked to water relations, for example, through a high specific leaf mass and high leaf density, which can confer greater leaf longevity (Loveless, 1961; Groom and Lamont, 1999; Chaves et al., 2002; Wright and Westoby, 2002). On the other hand, in dry habitat plants with flaccid leaves and tissues with greater water storage, flexible cell walls may contribute even more to a greater water storage capacity after stomatal closure, being characterized by an impermeable cuticle (Ogburn and Edwards, 2010).

Although leaf and whole-plant drought tolerance is generally coordinated, some species with relatively tolerant leaves may be sensitive to drought at the whole-plant level, as they have a superficial root system (Scoffoni et al., 2011). On the other hand, species with drought-sensitive leaves may have excellent drought survival, especially succulent species, when Ψ_{TLP} is likely to be less important than the capacitance of water storage cells (Chaves et al., 2002; Ogburn and Edwards, 2010).

2.7. Leaf anatomy and xylem vessel characteristics: importance in plant hydraulics

Leaf anatomy is well documented as one of the intrinsic factors that determine the photosynthetic performance and plant water balance (Terashima et al., 2011). Several studies (Patakas et al., 2003; Tholen et al., 2008; Hassiotou et al., 2010; Scafaro et al., 2011) suggest that anatomical characteristics of leaves affect mesophilic conductance and thus cause the change in leaf net photosynthetic rate (A_{net}). Long-distance water transport in plants requires the coordination of all organs along the water transport pathway, and the hydraulic resilience of a species in a given environment is determined by a set of functional traits and the integration of these traits in a given environment organism level (Meinzer and McCulloh, 2013; McCulloh et al., 2014).

The thickness of the photosynthetic cells of the palisade parenchyma is an important trait to rule the ability to resist water deficit (Ennajeh et al., 2010). Often, in drought-tolerant cultivars, thicker spongy parenchyma with large intercellular space is observed, which facilitates CO_2 diffusion (Hajnajari et al., 2019). The deposition of epicuticular waxes on the leaf surface provides protection against water loss. Likewise, the presence of trichomes or papillae can increase the leaf boundary air layer and reduce *E*, which confers greater drought tolerance (Baker and Myhre, 1969).

The vessel element, the xylem unit, is the fundamental component in stem xylem for water transport (Hacke et al., 2017). The xylem hydraulic conductivity is proportional to the fourth power of the vessel lumen diameter, indicating that a vessel with a wider lumen provides greater conductivity (Tyree and Zimmermam, 2002). However, vessels with a wider lumen are more prone to embolism, leading to water transport failure and tree death under water deficit conditions (Tyree and Sperry, 1988; Anderegg et al., 2012). Thus, a trade-off between hydraulic efficiency and cavitation resistance has been widely found in several tree species (Hacke et al., 2006; Worbes et al., 2013; Eilmann et al., 2014; Zhu et al., 2017).

Regarding embolism caused by drought, frost, herbivory or mechanical damage to the xylem, several experiments have shown that vessel elements with a smaller diameter, smaller size and greater frequency are more resistant than large vessel elements (Sperry and Sullivan, 1992; Cavender-Bares et al., 2005; Bauerle et al., 2011; Cao et al., 2018). In environments limited by water, and for plants to have shoot growth, these adaptive characteristics mentioned above and related to the xylem anatomy are important to reduce the risk of embolism, and thus maintain the water transport capacity relatively stable in the plant (Boughalleb et al., 2015). According to Cao et al. (2018), leaf thickness, hydraulic conductivity and vessel element length are representative predictors associated with isotopic discrimination of δ13C carbon, when this variable is used in the selection of drought-tolerant genotypes.

Therefore, measurement of hydraulic characteristics provides crucial information about the plant's ability to transport water to photosynthetic and growing tissues, which may postulate a sensitivity to abiotic stress factors and negatively affect species distribution (Brodribb, 2009).

2.8. Root system: importance in maintaining the water status of the plant

When grown in environments with limited soil water, plants can adopt strategies to “avoid” drought or “tolerate” drought. Dehydration prevention is associated with a variety of adaptive traits to minimize water loss and maximize water uptake. Both can be caused by a combination of morphological, anatomical and physiological adaptations. In contrast, drought tolerance refers to ability of the plant to maintain normal functions during periods of low tissue water potential (Ennajeh et al., 2010). Therefore, root traits associated with drought tolerance are important for plant drought resistance mechanisms (Ma et al., 2013).

The communication signaling between the root and the shoot can be divided into non-hydraulic signals originated by the root (chemical signals) and hydraulic signals. Under progressive soil drought conditions, chemical signals (mainly ABA) are first produced, which are transported through the transpiration pathway to the shoot, with negative effects on leaf expansion rate and stomatal opening (Blackman and Davies, 1985; Croker et al., 1998; Gutschick and Simonneau, 2002; Lorena and Ernesto, 2005). Chemical signals can substantially reduce water loss from the stomata, where water deficit in the shoot is sometimes not detectable. This action is considered the first defense against a possible drought (Blackman and Davies, 1985; Davies et al., 1994; Xiong et al., 2007). However, continuous drought establishes a hydraulic gradient between the leaf and the dry soil, which accelerates the development of leaf water deficit, through the reduction of leaf turgor pressure (Ψ_P) and consequently the Ψ_{leaf} (Croker et al., 1998). The reduction in Ψ_{leaf} can decrease stomatal conductance (g_s), reducing leaf gas exchange, and consequently delaying plant growth (Ma et al., 2013). Thus, under water stress conditions, the roots induce a signaling cascade to the branches, via the xylem, causing physiological changes that determine the level of adaptation to stress (Anjum et al., 2011).

Soil drying can also change the allocation of dry matter between root and shoot (Ma et al., 2013), so that plants grown in dry soil tend to allocate more photoassimilates to build a deeper root system, since, for a plant to acquire water from the soil, a deep root system is generally more advantageous than a root system closer to the soil surface (Kramer, 1969).

Morphology, as well as root growth, plays an essential role in maintaining water for the plant, so drought adaptation is related to deep, cheap, and vigorous root systems (Blum, 2005). For example, efficient root development (quick growth in depth) is essential for the survival of seedlings in soils that are subjected to quick drying from the surface to the interior of the soil, since moisture remains available in the deeper layers of the soil (Wasaya et al., 2018).

Plants with greater root diameter are more capable of exploiting compact soil (Bao et al., 2014). Other traits such as root tissue density, specific surface area and specific root length are correlated with increased crop productivity under drought conditions (Vadez et al., 2013).

For water uptake from deeper soil layers under water stress, and in various crops such as sorghum, pulses, rice, corn and wheat, several studies report the importance of a deep root system, with low energy cost (larger air space), and steep (greater angle to the ground surface) (Eissenstat et al., 1992; Krishnamurthy et al., 1999; Kashiwagi et al., 2006; Reynolds et al., 2007; Hammer et al., 2009; Manschadi et al., 2010; Wasson et al., 2012; Steele et al., 2013), since in drought conditions, the ability of the plant to extract water from depth has great relevance in the balance of the water relationship, as well as in carbon assimilation (Wasaya et al., 2018). It is important to report that a greater hydraulic conductivity of the roots can contribute to a better absorption of water in soils with water limitation. Therefore, a good understanding of plant responses to abiotic stresses can be useful in selecting more resistant crop varieties (Den Herder et al., 2010).

3. CHAPTER 1

CONTRASTING *Coffea canephora* CLONES FOR MIDDAY DEPRESSION OF PHOTOSYNTHESIS: THE IMPACT IN THE GROWTH TRAITS

ABSTRACT

We study aimed at evaluating the underling mechanisms related to midday depression of photosynthesis (MDP) and its impact on the vegetative growth of *Coffea canephora* Pierre ex Froehner. The experiment was carried using two clones of *C. canephora* with contrasting MDP (clones ‘A1’ and ‘19’), maintaining the soil always well-watered. The growth analyzes, as leaf and plagiotropic branch number, were carried out in the end of the experiment, whilst the leaf gas exchanges, chlorophyll *a* fluorescence and leaf water potential, were carried out in a representative period, where the temperatures, VPD_{air} and PPFD achieved elevated values around midday. The volume pressure curve was performed immediately after the previous cited analyzes and in the final of experiment the dry mass of each segmentation of the plant was also measured. The leaf stomatal conductance (g_s) was highly sensitive to changes in $VPD_{leaf\text{-}air}$, even under well-watered conditions, mainly in ‘A1’, that was more sensitive to midday VPD increase than ‘19’, due higher midday depression of g_s and net photosynthesis. This response was highly associated to the leaf cell hydraulic traits. The ‘19’ showed a better elastic adjustment, that triggered less midday stomatal closing than the A1’. The ‘A1’ photochemical apparatus was less damaged over the daily period, manly at midday than in ‘19’, as observed through parameters related to energy specific fluxes, quantum yield and photosynthetic performance index. The high leaf hydraulic traits sensibility to dehydration in ‘A1’, enabled a leaf cell turgor loss, a higher stomatal closing to midday and hence a higher leaf wilt than in ‘19’, reducing leaf exposition to sunlight and a lower photochemical damage. Then, a considerable reason for ‘19’ had not showed a higher shoot growth may be by the energetical cost requested in the PSII repair mechanisms. As found in our study, specifically for ‘A1’, the MDP can be a result of mechanism that indirectly protects plants against later damage. Furthermore, lower MDP could be associated to the increase of the maintenance costs of this process, and would not be converted in higher plant growth, as observed in ‘19’.

Key words: *Coffea canephora*, photochemical apparatus, root growth depth, stomatal conductance.

RESUMO

Nosso objetivo foi avaliar os mecanismos subjacentes relacionados à depressão da fotossíntese ao meio-dia (MDP) e seu impacto no crescimento vegetativo de *Coffea canephora* Pierre ex Froehner. O experimento foi realizado utilizando dois clones de *C. canephora* com MDP contrastante (clones ‘A1’ e ‘19’), mantendo o solo sempre bem irrigado. As análises de crescimento, como número de folhas e ramos plagiotrópicos, foram realizadas no final do experimento, enquanto as trocas gasosas foliares, clorofila a fluorescência e potencial hídrico foliar, foram realizadas em um período representativo, onde as temperaturas, VPD_{air} e PPFD atingiram valores elevados por volta do meio-dia. A curva de pressão de volume foi realizada imediatamente após as análises citadas anteriormente e no final do experimento também foi medida a massa seca de cada segmentação da planta. A condutância estomática foliar (g_s) foi altamente sensível às mudanças no $VPD_{folha\text{-}ar}$, mesmo em condições de boa irrigação, principalmente em 'A1', que foi mais sensível ao aumento do VPD ao meio-dia do que '19', devido à maior depressão do gs ao meio-dia e fotossíntese líquida. Esta resposta foi altamente associada às características hidráulicas das células foliares. O ‘19’ apresentou melhor ajuste elástico, que desencadeou menos fechamento estomático ao meio-dia do que o A1’. O aparato fotoquímico ‘A1’ foi menos danificado durante o período diário, principalmente ao meio-dia do que em ‘19’, conforme observado através de parâmetros relacionados a fluxos específicos de energia, rendimento quântico e índice de desempenho fotossintético. A alta sensibilidade das características hidráulicas foliares à desidratação em ‘A1’, possibilitou uma perda de turgescência das células foliares, um maior fechamento estomático ao meio-dia e, consequentemente, uma maior murcha foliar do que em ‘19’, reduzindo a exposição foliar à luz solar e um menor dano fotoquímico. Então, uma razão considerável para '19' não ter apresentado um maior crescimento de brotos pode ser pelo custo energético solicitado nos mecanismos de reparo do PSII. Conforme encontrado em nosso estudo, especificamente para ‘A1’, o MDP pode ser resultado de mecanismo que protege indiretamente as plantas contra danos posteriores. Além disso, menor MDP poderia estar associado ao aumento dos custos de manutenção desse processo, e não se converteria em maior crescimento da planta, como observado em ‘19’.

Palavras-chave: Aparelho fotoquímico, *Coffea canephora*, condutância estomática, profundidade do crescimento radicular.

INTRODUCTION

Coffee is one of the most traded agricultural commodities in the world. About 99% of coffee production worldwide is represented by two species, *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner (DaMatta et al., 2019). Espírito Santo State, Southeast Brazil, is responsible for *ca.* 15% of the world's C. *canephora* production (Venancio et al., 2020). This brasilian producing region is characterized by dry climate, with high air temperatures, low relative air humidity, elevated air vapor pressure deficit (VPD_{air}), and excessive irradiance (Venancio et al., 2020; Machado Filho et al., 2021). These environment conditions have the most elevated values at midday, and are responsible for decreasing plant C-assimilation, a functional response known as midday depression of photosynthesis - MDP (Xu and Shen, 1996).

MDP is related to stomatal and/or nonstomatal effects (Xu and Shen, 1996; Miao et al., 2021). Stomata are usually closing under low air humidity at midday, related to plant's inability to properly supply the leaves with water due to hydraulic resistance, occurring in roots and leaves, which reduced the CO_2 availability at active sites of Rubisco (Brodribb et al., 2015). Reduced water transport capacity through the plant under such dry and high light environmental conditions, drastically decreases leaf water potential, which triggers the stomatal closure through hydroactive stomatal responses, *i.e.*, abscisic acid (ABA)-mediated responses (McAdam and Brodribb, 2014; McAdam and Brodribb, 2015; Merilo et al., 2018; Buckley, 2019) or hydropassive stomatal response (El-Sharkawy et al., 1985).

Nonstomatal effects of MDP can occur due to decrease in the leaf water content impaired the photochemical and biochemical process, including impacts in photosynthetic pigment pools, photosystems system performance, enzyme activities (e.g., RuBisCO) and membrane integrity (Chaves et al., 2003, Muller et al., 2011, Ramalho et al., 2014; 2018; Fahad et al., 2017). Usually, nonstomatal effects of MPD are related to photoinhibition phenomenon, that is exacerbated in conditions of the strong light, soil water stress and high temperature at midday (Ma et al., 2021). In such conditions, the incoming light energy exceeds the maximum utilization capacity of the photosynthetic apparatus and heat stress leads to a reduction in light use efficiency (Prasad et al., 2009; Dwivedi et al., 2019; Ma et al., 2021), resulting in reactive oxygen species synthesis (Dwivedi et al., 2019).

Singlet oxygen is synthetized due to excessive releasing of electrons in the electron transport chain, higher than consuming capacity of the Calvin cycle (Gururani et al., 2015). This can inhibit the translation of plastid PsbA mRNA, resulting in inactivation the photosystem II (PSII) repairing (Nishiyama et al., 2006; Takahashi and Murata, 2008; Nishiyama et al., 2011; Nishiyama and Murata, 2014).

Measurements of chlorophyll *a* fluorescence is one elegant and practical way to evaluate the photochemical machinery functionality, and to obtain insight into efficiencies of energy transfer and heat dissipation (Ripoll et al., 2016), as well as to characterize the effects of stress on adaptive mechanisms (Misra et al., 2012; Kalaji et al., 2014; 2016). The JIP-test is based in chlorophyll *a* fluorescence measurements, able to provide information about structure and function of PSII, to detecte the type of stress, and to differ the specific responses for a given type of stress (Ripoll et al., 2016).

Overall, stomata close when leaves begin to dehydrate. However, under specific environmental conditions, modification in root water uptake capacity plays a more important role than stomatal closure in avoiding stress-induced growth reduction (Matsuo et al., 2009). For example, increased root length density and deeper root systems are often viewed as desirable traits for drought adaptation (Valdez, 2014), due to increased uptake water capacity throughout the deep soil profiles. On the other hand, a robust root system, with both great water uptake capacity and transport could be less useful in an agricultural context whether the leaves have a high hydraulic resistance and a high stomatal sensibility.

Both atmospheric water demand under high airvapor pressure deficit (VPD_{air}) and soil water content are responsible in stomatal conductance (g_s) regulation, through of processes that result in changes in the turgor pressure of the guard cells (Buckley, 2005), suggesting that g_s is a key factor limiting both crop transpiration and yield (Roche, 2015). In fact, high stomatal sensitivity to VPD_{air} could be disadvantageous under adequate water availability, or even during short periods of water deficit (Machado Filho et al., 2021) to maximize the productivity, because the maximized CO_2 assimilation becomes more important than decreased transpiration (DaMatta, 2003).

The water relations in leaves can be described mainly through leaf water potential (Ψ_{leaf}), leaf water potential at turgor loss (Ψ_{TLP}), relative water content at turgor loss (RWC_{TLP}), osmotic potential at full turgor (Ψo), and bulk modulus of elasticity (€) (Bartlett et al., 2012; Xiong and Nadal, 2019). Ψ_{TLP} indicates the capacity of a plant to maintain cell turgor pressure under drought stress, so that species with less negative Ψ_{TLP} tend to have wider hydraulic safety margins (Zhu et al., 2018; Liu et al., 2019). However, this can be one disadvantage in agriculture context, once a small change in water availability, whether in the soil and/or in atmosphere, could trigger a loss of the leaf cell turgescence and decrease g_s. Therefore, plants with more negative Ψ_{TLP} can resist leaf dehydration, thereby sustaining g_s, photosynthesis and growth under decreased water availability (Tognetti et al., 2000; Baltzer et al., 2008; Blackman et al., 2010; Zhu et al., 2018).

MDP could be a limiting factor for crop production even under non-soil water stress conditions (Yokoyama et al., 2019), mainly for coffee crop, that naturally show large limitations to CO_2 diffusion from the atmosphere to the chloroplasts, due intrinsically low g_s and mesophyll conductance (Batista et al., 2012; DaMatta et al., 2019). In this context, selection of clones with lower MDP, a frequent factor in field-grown coffee (Batista et al., 2012; Santos et al., 2012; Martins et al., 2019) could maximize both plant growth and yield.

Elevated correlation between photosynthetic capacity and photosynthate demands (growth, reproduction, and storage) is expected (Demmig-Adams et al., 2017). However, the rate of photoassimilates supplying of sink organ depends on combined capacities of leaf photosynthesis, phloem loading and transport, and sink strength (Patrik and Colyvas, 2014). It is therefore not surprising that not always a higher photosynthesis will be translated into higher plant growth and/or yield, once a set of factors is underling to this process. In this sense, coffee plants (a perennial crop) did not show a down-regulation of photosynthesis when grown under elevated CO_2 concentration due, among other things, to both high allocation of resources to continuous vegetative growth of leaves and branches (which can be reinforced by regular pruning) and to the growth of reproductive structures (Ghini et al., 2015; Rodrigues e al., 2016; Ramalho et al., 2018; Rakocevic et al., 2020). Therefore, screening responses of *C. canephora* clones to MPD can explain differences in clone specific reactions when cultivated without soil water deficit.

Herein, some questions about *C. canephora* were addressed: (1) Would a lowering in MDP result in elevated carbon gain over the day, and hence increased vegetative growth? (2) Can the stomatal and non-stomatal effects that lead to MDP be different among coffee clones? (3) Which effect (stomatal or non-stomatal) is more limiting for coffee growth? (4) Can the root growth depth impact on stomatal responses through the leaf water relations (especially Ψ_{TLP}) at midday in a well-watered soil?

We hypothesized that: (1) the maintenance of leaf photosynthesis intensity around midday can contribute to higher net carbon gain over the day, resulting in higher plant growth, and (2) that root depth plays important role in maintenance of stomatal aperture associated with lower Ψ_{TLP} at midday even in a well-watered soil. This study aimed at evaluating the underling mechanisms related to MDP and its impact on the vegetative growth of *C. canephora* clones in a well-watered soil.

MATERIAL AND METHODS

Local of experiment, specie characterization and others useful information

The experiment was carried out in the greenhouse at the State University of Northern Fluminense, Campos dos Goytacazes (21°44’47” S and 41°18’24” W at 10 m altitude), Brazil, using two clones of the *C. canephora*, differing in values of MDP measured from May 2019 to April 2020. On May 2019, five-months-old seedlings produced from cuttings, were transplanted to PVC tubes of 1.0 x 0.2 m (height and diameter), containing sand + soil substrate in the 1:1 proportion. Substrate chemical analysis was performed to determine the soil fertility and ensure the correct fertilization in a function of crop demands.

Plants of two clones (clones ‘A1’ and ‘19’) were distributed in seven blocks, every one which represented one repetition (n=7) in completely random design. During the experimental period, the plants were irrigated two times over the day (early morning and evening), maintaining the soil always well-watered.

The micrometeorological variables as temperature (°C), relative humidity (%), air vapor pressure deficit (VPD_{air}, MPa) and photosynthetic photons fluxes density (PPFD, µmol m^{-2} s^{-1}) were recorded each 60 minutes, using a data logger (model 2000 Weather Stations, Spectrum Technologies, Plain-field, Illinois, USA).

The leaf gas exchanges, chlorophyll *a* fluorescence and leaf water potential were measureds seven months after transplanting in four representative days (December 13, 16, 17 and 18), where the temperatures, VPD_{air} and PPFD achieved elevated values around midday. The volume pressure curve was performed immediately after the previously cited analyzes and in the final of experiment the dry mass of each segmentation of the plant was also measured. This measure will be detailed, as described below.

Leaf gas exchange

Net leaf photosynthetic rate (A_{net}), stomatal conductance (g_s) and transpiration rate (E) were measured using a portable open-system IRGA (Li-Cor 6400xt, LI-COR, Lincoln, USA), with an external CO_2 supply of 400 µL L^{-1}, and *ca.* 1500 µmol m^{-2} s^{-1} of PPFD. The measurements were performed at four representative days (December 13, 16, 17 and 18), in two daily periods, morning (between 8-9 a.m.) and to midday (between 12-1 p.m.), on completely recently developed leaves (usually 4-5th pared from apex) of fully sun exposed plagiotropic branches. The results were expressed as the four days medium values relative to each daily period (morning and midday).

The relation between the morning ($A_{net\ morning}$) and midday ($A_{net\ midday}$) net photosynthetic rates enabled to calculate percentage (%) of net photosynthetic rate variation, that was defined as midday depression of photosynthesis (MDP). The equation 1 was applied:

$$MDP = \left(1 - \frac{A_{net} midday}{A_{net} morning}\right) x\ 100 \qquad \text{(Eq. 1)}$$

The similar equations to the Eq. 1 were used to calculate percentage (%) of variations in g_s and E between morning and midday daily periods.

Chlorophyll a fluorescence

Chlorophyll (Chl) *a* fluorescence was measured in the same leaves used for the gas exchange measurements, on the same days, six times over the day (5 a.m., 7 a.m., 10 a.m., 1 p.m., 4 p.m. and 7 p.m) using a Pocket PEA fluorometer (Hansatech, King‟s Lynn, UK). The sampled leaves were dark-adapted for 30-40 minutes using leaf clips (Hansatech, King‟s Lynn, UK) to turn the reaction centers into an "open" (oxidised Q_A) state (Bolhár Nordenkampf et al., 1989). The results were expressed as the four days medium values relative to each daily period (morning and midday).

From the rapid kinetics of fluorescence emission over time, some variables were obtained using JIPtest (Strasser and Tsimilli-Michael, 2001; Strasserf et al., 1995; 2004), such as those related to: (1) energy specific flux (absorption energy flux per active reaction center (ABS/RC), total energy dissipated per reaction center (DI_0/RC), flux of excitation energy trapped per active reaction center (TR_0/RC), electron flux transferred per active reaction center (ET_0/RC), (2) quantum yield (maximum quantum yield of primary photochemical reactions (φPo), probability of electron transport beyond Q_A (ΨEo), quantum efficiency of electron transfer from Q_A to electron transport chain beyond (φEo), (3) performance index of photosynthetic apparatus (PI_{ABS}), and (4) density of active PSII reaction centers (RC/CS_0).

Leaf water potential

Leaf water potential at predawn (Ψ_{pd}) and at midday (Ψ_{md}) was measured in the third pair of leaves from apex of the plagiotropic branch formed top third of the plant, immediately after leaf excision, using a pressure chamber (model 3000, Soil Moisture Corp., Santa Barbara, CA), according to Schölander et al. (1965). Measurements Ψ_{md} were performed on the same days when the gas-exchanges and chlorophyll *a* fluorescence were measured. The equation applied to MDP also was used to calculate the midday depression of Ψ_{leaf}. The results were expressed as the four days medium values relative to each daily period (morning and midday).

Pressure-volume curve (P-V curves)

P–V curves were determined one week after gas exchange measurements, in the same leaves used for the gas exchange measurements and chlrophyll fluorescence, measuring one block for day, leading seven days for full analysis. Ten hours before the analyses, the plagiotropic branches containing between five to eight pair of leaves were carefully cut and submerged in water until full rehydration. The branches were maintained covered with dark plastic bag, to ensure the full stomatal close and then full rehydration. Sampled leaves were excised from rehydrated branches under water and dehydrated slowly on the bench top.

P–V curves were determined using a Scholander pressure chamber (Scholander et al. 1965), followed by the free-transpiration method described in previous studies (Talbot et al. 1975; Hinckley et al. 1980; Dreyer et al. 1990). The leaf mass and water potential were measured at short intervals at the beginning and at higher intervals with time passing until attained the values close to –3 MPa. Individual leaf area was measured using a leaf area meter (Li-3100, Li-Cor, Lincoln, NE, USA). Individual leaf dry mass was determined after desiccation in a forced-air oven at 65 °C for 72 h (Silva and Queiroz, 2006). The parameters were obtained from Sack and Pasquet-Kok (2010) protocol. Briefly, the turgor loss point (Ψ_{TLP}) was estimated as the point of transition between curvilinear and linear portions of the graph, plotting the inverse of Ψ_{leaf} against leaf relative water content (RWC). Osmotic potential (Ψ_o) was estimated by extrapolating the straight-line section to 100% RWC and the bulk modulus of elasticity (ε) from the slope of the pressure potential between full turgor and turgor loss point (Sack and Kok, 2010).

Plant growth traits

The total leaf area (TLA), leaf number (LN) and plagiotropic branch number (PBN). Leaf (LN) and plagiotropic branch number (PBN) were measured in the end of the experiment. Posteriorly, leaves, branchs and roots were separated and later dried in a forced-air oven at 65 °C for 72 h (Silva and Queiroz, 2006), to determine the leaf, branchs and root dry mass. Roots were separated in four parts according soil profile (0-0.25; 0.25-0.50, 0.50-0.75 and 0.75-1.0m) to determine the root distribution in the soil depth. Before dried root immersion was performed, to estimate root volume (RV), applying the equation 2:

$$RV = FV_{PVC} - IV_{PVC} \qquad \text{(Eq. 2)}$$

where,

FV_{PVC} was final volume of the tube (after immersion of root), and
IV_{PVC} was initial volume of the tube (before immersion of root).

The TLA per plant was measured using a leaf area meter (Li-3100, Li-Cor, Lincoln, NE, USA), while specific leaf mass (SLM, g. m^{-2}) was obtained from a ratio between leaf dry mass and total leaf area.

Experimental design and statistical analysis

The experiment was conducted in a randomized design, with two clones (‘A1’ and ‘19’) and seven replicates (plants). The leaf gas exchanges, Chl *a* fluorescence parameters, leaf water potential and root distribution were measured in five replicates and submitted to a two-way ANOVA (two factors - clone and daily period). When ANOVA *p*-value was significant ($p \leq 0.05$), the Tukey test was applied to compare medium values. The plant growth traits variables were measured in seven replicates and submitted to *t* test ($p \leq 0.05$) to compare the clones, whilst P-V curve parameters were measured in five replicates and submitted to *t* test ($p \leq 0.05$) to compare the clones. Linear correlation analyses between leaf hydraulic traits, leaf gas exchange parameters at midday, chlorophyll *a* fluoresce parameters at midday, plant growth traits and absolute root dry mass distribution were applied. To facilitate the correlations among variables, all studied variables were classified into five groups: (i) leaf hydraulic parameters, (ii) leaf gas exchange parameters at midday, (iii) chlorophyll a fluorescence parameters at midday, (iv) plant growth parameters, and (v) absolute root dry mass distribution. All statistical analyses were performed using a R software (R Core Team, 2020).

RESULTS

Climatic variables and plant conditions

The photosynthetic photon flux density (PPFD) increased from 5 a.m., reaching the maximum values at 12 p.m., and posteriorly decreasing until 6 p.m. during the whole period of the experiment, as well as in the representative days (Figure S1A and 1A). The maximum values of T (°C) were found between 10 a.m.

and 3 p.m. (Figure S1B and 1B). In observed growth period, about 65% days showed T > 30 °C in a daily interval from 11 a.m. to 3 p.m. As expected, the relative humidity (RH, %) showed the opposite behavior to T, reaching the minimum values between 10 a.m. and 3 p.m. (Figure S1C and 1C). During the night, from 6 p.m. to 5 a.m., the values of the air vapor pressure deficit (VPD_{air}) were maintained between 0 to 1 MPa (Figure S1D and 1D). The increase of T and decrease of RH, which started from 5 a.m., resulted in increased VPD_{air}, reaching the maximum values near of the midday (between 11 a.m. and 2 p.m.). More than 50% of the days from May 2019 to April 2020, showed VPD_{air} higher than 2.5 MPa in the midday daily period, as found in the representative days (Figure 1D).

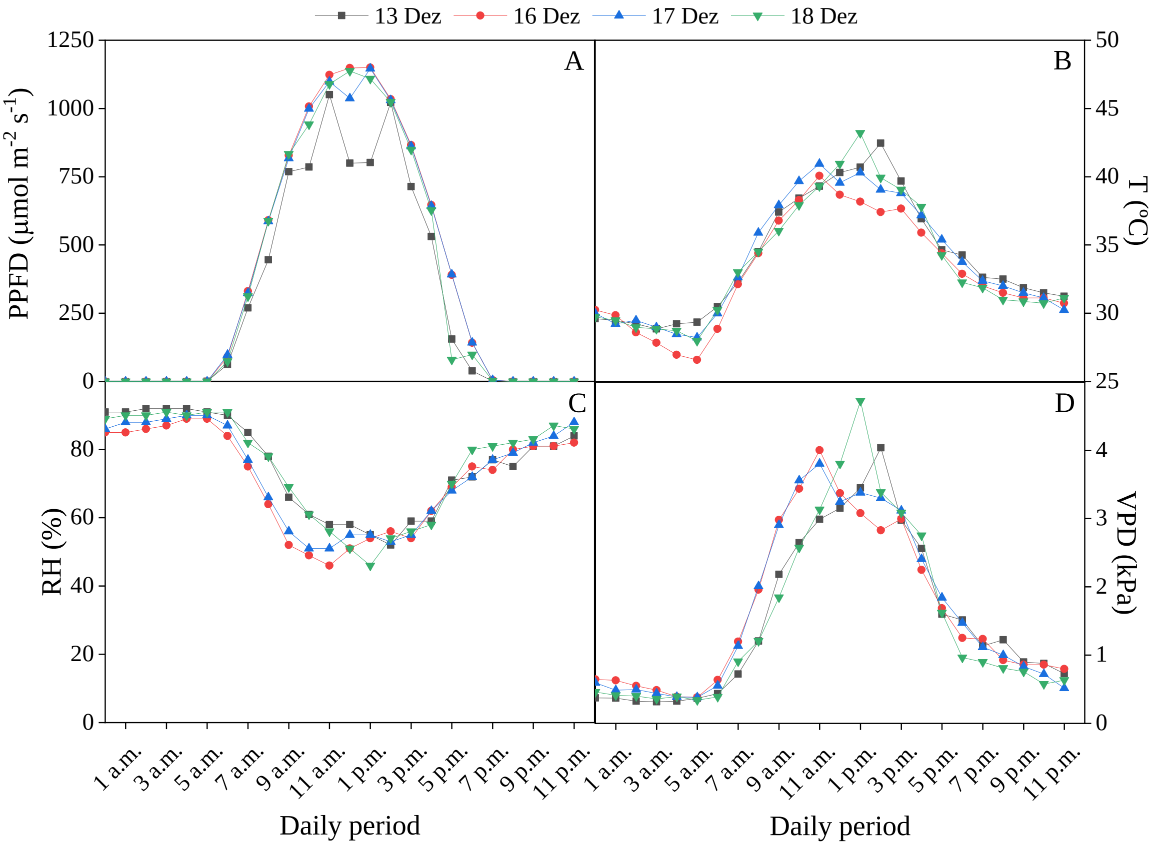

Figure 1. Photosynthetic photon flux density (PPFD, A), air temperature (T, B), relative humidity (RH, C), air vapor pressure deficit (VPD_{air}, D) during the days (December 13, 16, 17, and 18) of gas exchange, chlorophyll *a* fluorescence, and leaf water potencial measurements.

Physiological traits: gas exchange, hydraulic traits, and chlorophyll a fluorescence

The net photosynthetic rate (A_{net}), stomatal conductance (g_s) and transpiration rate (E) was higher in clone ‘19’ than in ‘A1’. No difference between clones was found in the internal CO_2 concentration (C_i). The A_{net} and g_s decreased in both clones in midday compared to the morning period, whilst E and C_i increased (Figure 2). The midday depression of A_{net} (MDP) was higher in ‘A1’ (42.42%) than in ‘19’ (24.85). The midday depression of g_s was like A_{net} MDP, where ‘A1’ decreased 27.57% whilst ‘19’ decreased 5.16%. On the other hand, the midday depression of E was lower in ‘A1’ (13.69%) than in ‘19’ (38.61%). The C_i increased in midday for 17.55% and 14.57% in ‘A1’and ‘19’, respectively, not differing among the two clones.

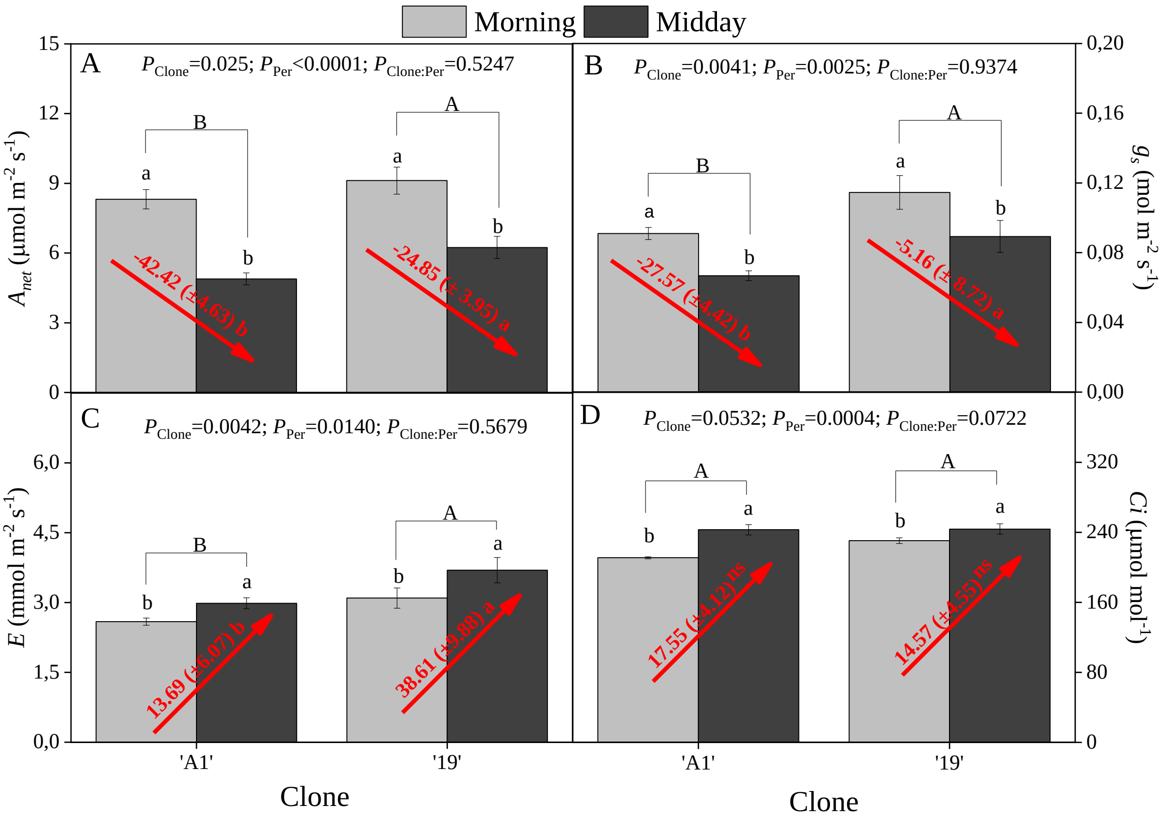

Figure 2. Leaf gas exchange parameters in conilon coffee clones (‘A1’ and ‘19’), net photosynthetic rate (A_{net}, A), stomatal conductance (g_s, B), transpiration rate (E, C) and internal CO_2 concentration (C_i, D) measured in two two daily periods (morning and midday). For each parameter, the medium values ± statistical error (n = 5) followed by different minor letters express significant differences between the daily periods, separately for each clone (a, b), or between clones, independently of period (A, B) at $p \leq 0.05$ using the Tukey test. Red arrows represent the relative variation of the leaf gas exchange parameters from morning to midday for each clone.

The leaf water potential (Ψ_w) decreased at midday, not differing among the two clones (Table 2).

Table 2. Leaf water potential measured in two daily periods (predawn and midday) in conilon coffee clones (‘A1’ and ‘19’). The medium values ± SE (n = 5) followed by different letters express significant differences between the daily periods, (a, b) at $p \leq 0.05$ using the Tukey test. ns no significative.

Clone	**Leaf water potential (MPa)**	
	Predawn	**Midday**
‘A1’	-0.129 ± 0.009^{ns} (100%)	-0.820 ± 0.083^{ns} (-635%)*
‘19’	-0.160 ± 0.016 (100%)	-0.950 ± 0.097 (-594%)
Medium	-0.144 ± 0.053 a (100%)	-0.885 ± 0.053 b (-615%)

* Percentage of leaf water potential decrease from predawn to midday.

The pressure volume curve (P–V curves) parameters [saturated water content (SWC) and cell elasticity modulus (Ɛ)] were statistically different between ‘A1’ and ‘19’ (Table 3). The SWC was 16% higher in ‘A1’ than ‘19’, whilst Ɛ was 22% lower. No difference was found in the osmotic potential (Ψ_o), relative water content at the turgor loss point (RWC_{TLP}), water potential at turgor loss point (Ψ_{TLP}) and leaf capacitance at full turgor (C) between the two clones.

Table 3. P–V curves parameters, saturated water content (SWC), osmotic potential (Ψ_o), relative water content at turgor loss point (RWC_{TLP}), cell elasticity modulus (Ɛ), water potential at turgor loss point (Ψ_{TLP}) and leaf capacitance at full turgor (C) measured in conilon coffee clones (‘A1’ and ‘19’). The medium values ±

SE (n = 5) followed by different letters express significant differences between clones, (a, b) at $p \leq 0.05$ using the *t* test.

Clone	**SWC**	**Ψo (MPa)**	**RWC_{TLP}**	**ε (MPa)**	**Ψ_{TLP} (MPa)**	***C* (MPa^{-1})**
‘A1’	2.08 ± 0.13 a	-1.30 ± 0.08	92.97 ± 0.59	17.35 ± 1.52 b	-1.54 ± 0.08	0.049 ± 0.004
‘19’	1.79 ± 0.08 b	-1.44 ± 0.10	93.99 ± 0.57	22.26 ± 1.12 a	-1.62 ± 0.09	0.041 ± 0.002
P_{value} (≤0.05)	0.015	0.170^{ns*}	0.230^{ns}	0.015	0.346^{ns}	0.154^{ns}

* ns, no significative.

The specific energy fluxes parameters were statistically different for both clones over the daily period (Figure 3). The absorption energy flux per active reaction center (ABS/RC) (Figure 3A), total energy dissipated per reaction center (DI_0/RC) (Figure 3B) and flux of excitation energy trapped per active reaction center (TR_0/RC) (Figure 3C) increased from predawn (5 a.m.) until 10 a.m. After this daily time, ABS/RC, DI_0/RC and TR_0/RC for both clones decreased until achieved the minimum values at 7 p.m. At midday period (13 p.m.), ABS/RC and DI_0/RC reduced quicker in ‘A1’ than in ‘19’ (Figure 3A). Furthermore, ABS/RC increased 27% from 5 a.m. to 1 p.m. in ‘A1’, whilst in ‘19’ the increase was significatively higher (57%). DI_0/RC increased 50% from 5 a.m. to 1 p.m. in ‘A1’, whilst in ‘19’ increased 120%.

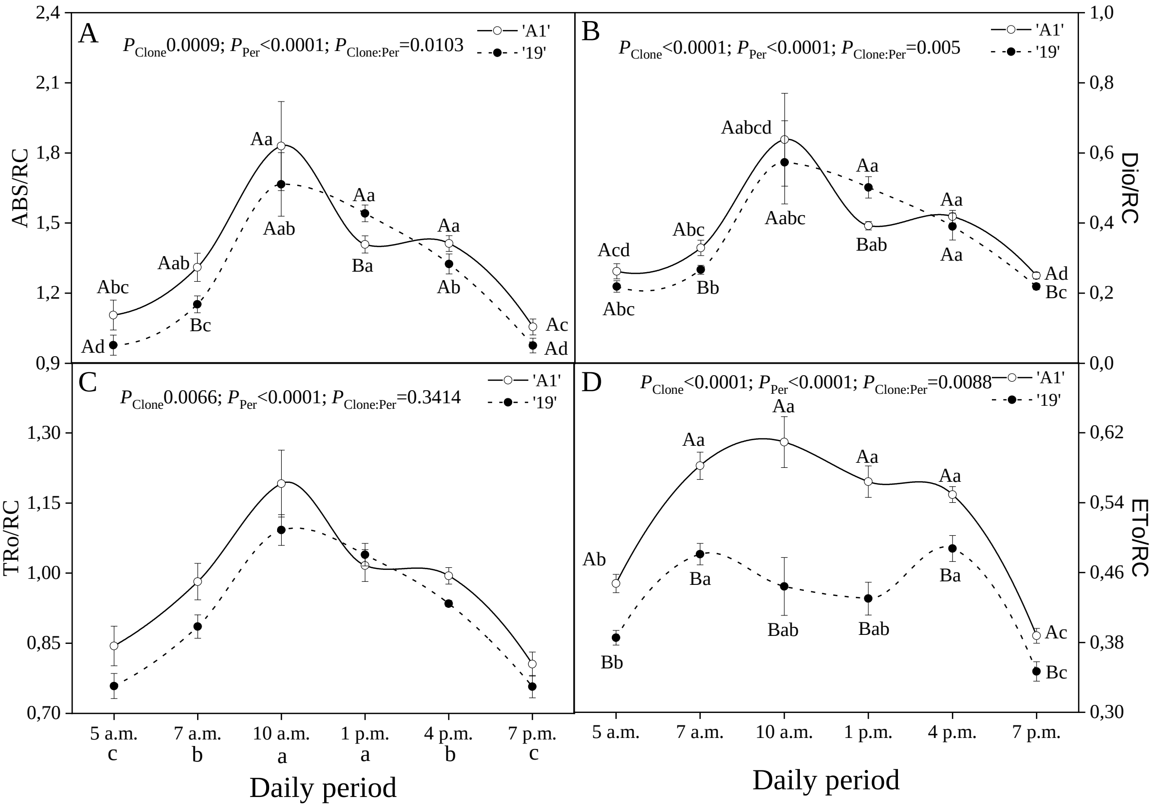

Figure 3. Leaf chlorophyll *a* fluorescence parameters associated to energy specific fluxes in conilon coffee clones (‘A1’ and ‘19’) measured in six daily periods (5, 7, 10 a.m., 1, 4, 7 p.m.). Absorption energy flux per active reaction center (ABS/RC) (A), total energy dissipated per reaction center (DI_0/RC) (B), flux of excitation energy trapped per active reaction center (TR_0/RC) (C) and electron flux transferred per active reaction center (ET_0/RC) (D). For each parameter, the medium values ± SE (n = 5) followed by different letters express significant differences between the daily periods, separately for each clone (a, b, c, d), or between clones for each period (A, B) at $p \leq 0.05$ using the Tukey test.

No difference was found in the flux of excitation energy trapped per active reaction center (TR_0/RC) between the clones over the daily period (Figure 3C), only for the period of the day. On the other hand, the electron flux transferred per active reaction center (ET_0/RC) was higher in ‘A1’ in all daily period than ‘19’ (Figure 3D). The higher difference between clones was found at 1 p.m., where ‘A1’ showed ET_0/RC 30% higher than ‘19’.

The parameters related to *PSII* quantum yield were statistically different for both clones over the daily period (Figure 4). The maximum quantum yield of primary photochemical reactions (φ_{Po}) decreased from 5 a.m. to 1 p.m. in the two clones, achieving minimum values between 10 a.m. and 1 p.m. However, the recovery of φ_{Po} was quick from 10 a.m. to 4 p.m. in ‘A1’, whilst in ‘19’ was slowly (Figure 4A). The probability of electron transport beyond Q_{A-} (Ψ_{Eo}) and quantum efficiency of electron transfer from Q_{A-} to electron transport chain beyond (φ_{Eo}) were affected during 10 a.m. and 1 p.m. daily period in the ‘19’, a decrease of 16 and 25% in relation 5 a.m., respectively (Figure 4B and 4C). On the other hand, no significant decrease in Ψ_{Eo} and φ_{Eo} was found in ‘A1’ from 10 a.m. to 1 p.m.

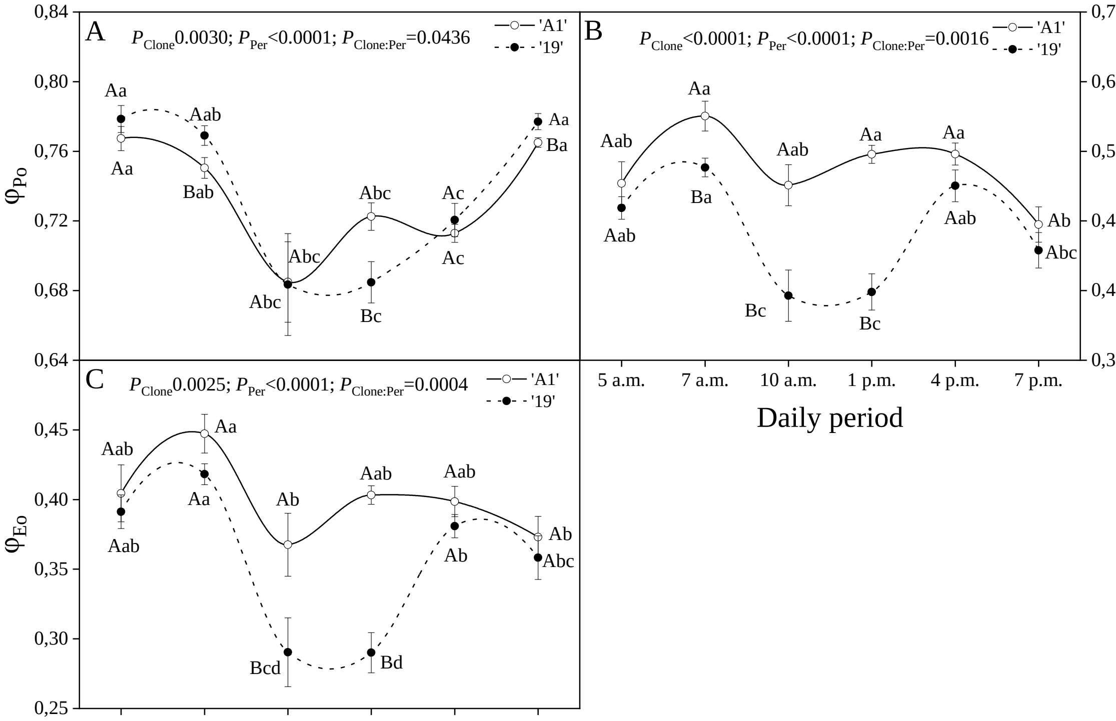

Figure 4. Leaf chlorophyll *a* fluorescence parameters associated to PSII quantum yield in conilon coffee clones (‘A1’ and ‘19’) measured over the daily period (5, 7, 10 a.m., 1, 4, 7 p.m.). Maximum quantum yield of primary photochemical reactions (φ_{Po}) (A), probability of electron transport beyond Q_{A-} (Ψ_{Eo}) (B), and quantum efficiency of electron transfer from Q_{A-} to electron transport chain beyond (φ_{Eo}) (C). For each parameter, the medium values ± SE (n = 5) followed by different letters express significant differences between the daily periods, separately for each clone (a, b, c, d), or between clones for each period (A, B) at $p \leq 0.05$ using the Tukey test.

The photosynthetic performance index (PI_{ABS}) decreased from 5 a.m. to 1 p.m in ‘19’ (67%), whilst was unchanged in ‘A1’ over the daily period. The density of active PSII reaction centers (RC/CS) also decreased from 5 a.m. to 10 a.m., without difference between the clones.

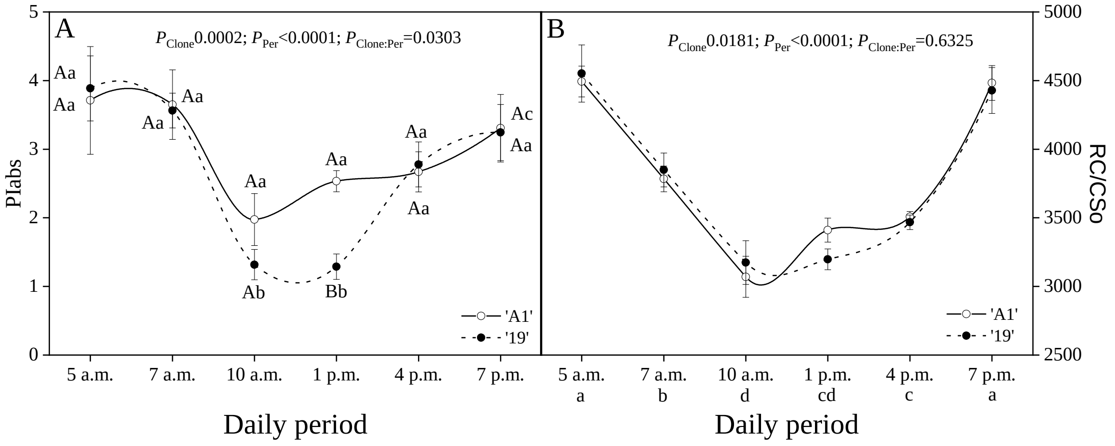

Figure 5. Leaf chlorophyll *a* fluorescence parameters associated to photosynthetic performance index (PI_{ABS}) (A) and density of active PSII reaction centers (RC/CS_0) (B) in conilon coffee clones (G-A1 and G-19) over the daily period (5, 7, 10 a.m., 1, 4, 7 p.m.). For each parameter, the medium values ± SE (n = 5) followed by different letters express significant differences between the daily periods, separately for each clone (a, b, c), or between clones for each period (A, B) at $p \leq 0.05$ using the Tukey test.

Morphological traits: shoots and roots

The branch dry mass (BDM) and plagiotropic branch number (PBN) were statistically different between the clones (Table 4). ‘A1’ showed 40% and 33% higher BDM and PBN than ’19', respectively. No statistical difference was found in the root volume (RV), root dry mass (RDM), shoot dry mass (SDM), leaf fry mass (LDM), leaf area (LA) and leaf number (LN).

Table 4. Root and shoot morphological traits in conilon coffee clones (‘A1’ and ‘19’), root volume (RV), root dry mass (RDM), shoot dry mass (SDM), branch dry mass (BDM), leaf dry mass (LDM), leaf area (LA), leaf number (LN), and plagiotropic branch number (PBN).

Variables	Clone		*P*-value ($\leq$0.05)
	‘A1’	**‘19’**	
RV (m^3)^{ns}	0.00070 (± 0.00004)	0.00064 (± 0.00006)	0.4072
RDM (Kg)^{ns}	0.121 (± 0.009)	0.108 (± 0.012)	0.3254
SDM (Kg)^{ns}	0.227 (± 0.01)	0.209 (± 0.01)	0.0707
BDM (Kg)*	0.107 (± 0.006)	0.091 (± 0.005)	**0.0194**
LDM (Kg)^{ns}	0.120 (± 0.004)	0.118 (± 0.008)	0.7671
LA (m^2)^{ns}	1.35 (± 0.06)	1.25 (± 0.09)	0.268
LN^{ns}	410.29 (± 32.71)	342 (± 44.45)	0.0846
PBN*	38.71 (± 2.51)	29.14 (± 3.28)	**0.0405**

For each parameter, the medium values ± SE (n = 7) followed by different letters express significant differences between clones at $p \leq 0.05$ (when significant marked in bold) using the *t* test. ns, no significative.

Statistical difference was found in the absolute root dry mass distribution between the clones when the analyze was performed over the soil depth (Figure 5). Both clones showed the highest concentration of the root dry mass in the superficial soil layer of 0-0.25m. The root dry mass was higher in ‘A1’ than in ‘19’ until 0.5m of the soil depth. In the deepest soil layer, from 0.75 to 1.0 m, the root dry mass was higher in ‘19’ than in A1’. No difference was found in the absolute root dry mass from 0.25 to 1.0m in ‘19’, whilst in ‘A1’ the absolute root dry mass decreased over the soil depth (0.25-0.50 > 0.50-0.75 = 0.75-1.0m).

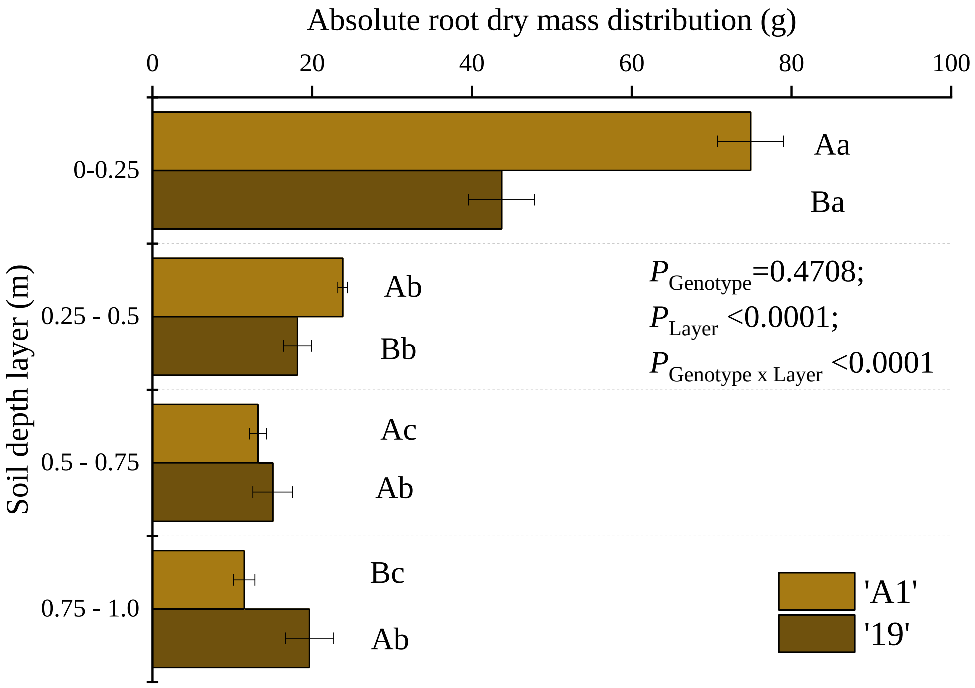

Figure 5. Absolute root dry mass distribution over the soil depth in conilon coffee clones (A1 and 19) cultivated in PVC tubes from May of 2019 to April of 2020. The medium values ± SE (n = 5) followed by different letters express significant differences between clones, separately for each soil depth (A, B), or between soil depth for each clone (a, b) at $p \leq 0.05$ using the Tukey test.

The studied variables from six groups were intercorrelated (Figure 6). The leaf hydraulic parameters showed a few correlations with other groups of parameters. SWC was negatively correlated to Ɛ and positively to *C*, whilst Ψ_o was positively correlated to Ψ_{TLP} and RV. Ψ_{TLP} was positively correlated to RV and RDM. The main correlations of the leaf hydraulic parameters were obtained for the Ɛ and *C*, which were negatively correlated, as expected. Interestingly the Ɛ was positively correlated to A_{net} and negatively to root dry mass from 0 to 0.25m ($RDM_{0-0.25m}$). On the other hand, *C* was negatively correlated to A_{net}, g_s and *E*.

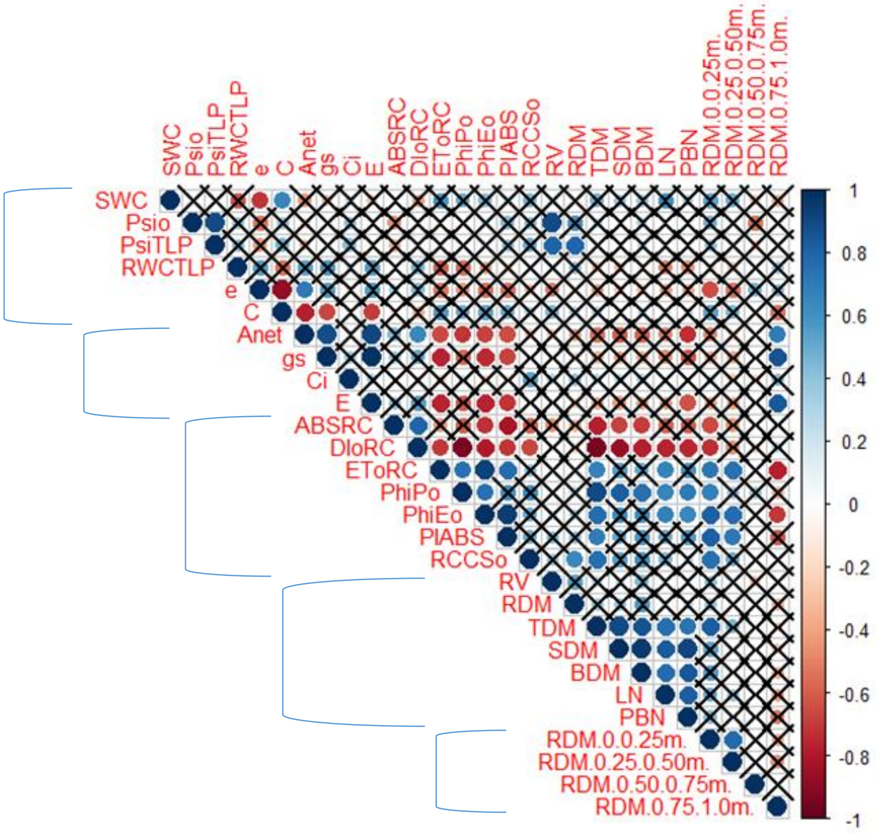

Figure 6. Graphical presentation of coefficients (values corresponding to circle size and color intensities) and p-values (<0.05 when not crossed) for correlations among **leaf hydraulic parameters** [saturated water content (SWC), osmotic potential (Psio), water potential at turgor loss point (Psi_{TLP}), relative water content at turgor loss point (RWC_{TLP}) and cell elasticity modulus (e)], **leaf gas exchange parameters at midday** [net photosynthetic rate (A_{net}), stomatal conductance (g_s), transpiration rate (*E*) and internal CO_2 concentration (C_i)], **chlorophyll a fluorescence parameters at midday** [absorption energy flux per active reaction center (ABS/RC), total energy dissipated per reaction center (DI_0/RC), electron flux transferred per active reaction center (ET_0/RC), maximum quantum yield of primary photochemical reactions (φ_{Po}), quantum efficiency of electron transfer from Q_{A-} to electron transport chain beyond (φ_{Eo}), photosynthetic performance index (PI_{ABS}) and density of active PSII reaction centers (RC/CS_0)], **plant growth parameters** [root volume (RV), root dry mass (RDM), total dry mass (LDM), shoot dry mass (SDM), branch dry mass (BDM), leaf number (LN) and plagiotropic branch number (PBN)] **and absolute root dry mass distribution** [root dry mass from 0 to 0.25m ($RDM_{0-0.25m}$), root dry mass from 0.25 to 0.50m ($RDM_{0.25-0.50m}$), root dry mass from 0.50 to 0.75m ($RDM_{0.50-0.75m}$) and root dry mass from 0.75 to 1.0m ($RDM_{0.75-1.0m}$) in *Coffea canephora*.

The leaf gas exchange parameters at midday (A_{net}, g_s and E) were intercorrelated, as expected (Figure 6). The A_{net} was positively correlated to DIo/RC and root dry mass from 0.75 to 1.0m ($RDM_{0.75-1.0m}$), and negatively correlated to ETo/RC, φ_{Po}, φ_{Eo}, PI_{ABS} and PNB. The g_s and E also showed a positive correlation with the $RDM_{0.75-1.0m}$, whilst the correlation between g_s or E with ETo/RC, φ_{Eo}, PI_{ABS} was negative.

The chlorophyll *a* fluorescence parameters at midday showed high correlations with other groups of parameters (Figure 6). The ABS/RC was positively intercorrelated to DIo/RC and negatively to φ_{Eo} and $PI_{ABS,}$ as well as was negatively correlated to total dry mass (TDM), SDM, BDM and $RDM_{0-0.25m}$. The DI_0/RC was negatively correlated to all chlorophyll *a* fluorescence parameters at midday (ET_0/RC, φ_{Po}, φ_{Eo}, PI_{ABS} and RC/CS_0), except to ABS/RC. Interestingly, the DI_0/RC was negatively correlated to TDM, SDM, BDM, LN, PBN and $RDM_{0-0.25m}$, whilst φ_{Po} showed a positive correlation to theses parameters. The ETo/RC was intercorrelated to φ_{Po}, φ_{Eo} and PI_{ABS}, but also positively correlated to TDM, LN, $RDM_{0-0.25m}$ and root dry mass from 0.25 to 0.50m ($RDM_{0.25-0.50m}$). Interestingly, ETo/RC and φ_{Eo} were negatively correlated to $RDM_{0.75-1.0m}$. On the other hand, the PI_{ABS} was positively correlated to TDM, $RDM_{0-0.25m}$ and $RDM_{0.25-0.50m}$, whilst RC/CSo was positively correlated to RDM, TDM and $RDM_{0-0.25m}$.

The plant growth parameters (TDM, SDM, BDM, LN and PBN) were intercorrelated, as expected. Finally, a positive correlation between TDM and $RDM_{0-0.25m}$ was found, evidencing that the roots in the first 0.25m of the soil profile imply directly in the TDM.

DISCUSSION

Mechanisms of midday depression of photosynthesis: stomatal, hydraulic, and photochemical limitations

A_{net} is determined by the characteristics of the photosynthetic apparatus, that change the capacity during plant growth and under the impact of environment, *i.e.* irradiance, temperature and mineral nutrient supply (Guo et al., 2009). Then, the midday depression of leaf gas exchange [(A_{net} and stomatal conductance (g_s)] have been suggested to be caused by an increase in leaf-to-air VPD ($VPD_{leaf\text{-}air}$) (Roessler and Monson 1985; Raschke and Resemann 1986; Tenhunen et al. 1987), as well as, by long periods of high photosynthetic photon flux density (PPFD) (Kuppers et al., 1986; Chaves et al., 1987; Correia et al., 1990), as found in our work.

The g_s of coffee leaves is highly sensitive to changes in $VPD_{leaf\text{-}air}$, even under well-watered conditions, and decrease drastically with VPD_{air} increase (Barros et al., 1997; Silva et al., 2004; Ronquim et al., 2006; Chaves et al., 2008; Franck and Vaast, 2009), mainly in 'A1'. The 'A1' was more sensitive to midday VPD_{air} increase than '19', reducing the g_s and consequently, the A_{net}. The stringency of stomatal regulation differs considerably in different tree species (Klein, 2014; Meinzer et al., 2017), as well as, to the same species, as observed in 'A1' and '19'.

The stomatal regulation could be related to the difference in the cell elasticity modulus (Ɛ) between the two studied clones. The elastic and osmotic adjustment may represent an alternative of plants to maintain cell turgor to reduce or avoid desiccation-induced tension (Kramer and Boyer, 1995; Pallardy, 2008; Blum, 2016). An increased Ɛ, as found in '19', leads to stiffer cell walls, which results in a greater water potential drop upon a unit of cellular water lost and thus may lead to greater soil-to-leaf water potential gradients (Cheung et al., 1975; Kramer and Boyer, 1995), improving the root water uptake. Yet, the '19' showed a higher capacity to uptake the deeper water in the soil, due the higher $RDM_{0.75\text{-}1.0m}$, positively correlated to Ɛ. Furthermore, the higher Ɛ in '19' could be linked to greater foliar tissue density (Niklas, 1991; Niinemets, 2001), in our work expressed as specific leaf mass. On the other hand, the photochemical apparatus in 'A1' was less damaged over the daily period than in '19', manly at midday, as observed through parameters related to energy specific fluxes, quantum yield and photosynthetic performance index.

The ABS/RC increase was accompanied by increase in the TR_0/RC and hence DI_0/RC for both clones from predawn to midday, as reflex of sunlight radiation increase and reaction centers inactivation (RC/CS_0 decrease). However, the extension of photoinhibition depends on the balance between photodamage and repair mechanisms of photosystem II center (Demmig-Adams et al., 2012), that was more effective and/or less demanded in ‘A1’ than in ‘19’.

The ’A1’ maintained a high ET_0/RC over the daily period and a quick decrease in ABS/RC, TR_0/RC, and DI_0/RC, when compared to ‘19’. Hence, there were lower decreases in φ_{Po}, Ψ_{Eo}, φ_{Eo} and PI_{ABS} at midday period in ‘A1’ than in ‘19’. The light-harvesting function of the leaf is compromised at lower water status than those cause a significative hydraulic function loss (Trueba et al., 2019) in ‘A1’, but not in ‘19’, because the late maintained higher stomatal aperture at midday.

The maintenance of a higher A_{net} at midday in ‘19’ than in ‘A1’ was clearly influenced by the higher stomatal aperture. But the high PPFD intensity in the midday triggered a higher photochemical damage in the photosynthetic apparatus, demanding of the higher activity of the PSII repair mechanisms. Clearly, the maintenance of a higher A_{net} in ‘19’ was a disadvantage, looking from a photochemical view, due the negative correlation between A_{net} and Chl *a* fluorescence parameters (ETo/RC, φ_{Po}, φ_{Eo}, PI_{ABS}) at midday. This mean that ‘A1’ showed higher efficiency and/or a lower demand to PSII repair mechanisms at the period of high sunlight radiation than ‘19’, although both clones showed a reversible photochemical damage until the beginning of the evening, by the complete recovery of the chlorophyll *a* fluorescence parameters (Figures 3, 4, and 5).

Midday depression of photosynthesis: adaptative trait to environment conditions?

As discussed in the previous topic, the two clones showed clear difference in the stomatal sensibility, which triggered the MDP response. However, no difference was found in the shoot growth between the two clones (Table 4), which induced the question: Does the MDP is an adaptative trait, or one specific environmental sensibility?

The higher stomatal sensibility in ‘A1’, enabled a leaf cell turgor loss, a higher stomatal closing at midday and hence a higher leaf wilt, when compared to ‘19’. This cascade of responses decreased the photochemical damages of leaf exposition to sunlight. This could be related to leaf angle more perpendicular to sun rays in midday in 19’ than in ‘A1’, as the opposite strategy in avoiding MDP. On the other hand, even in well-watered soil condition, the combination of leaf hydraulic trait (cell elasticity modulus) and deep rooting (down of 0.75m) enabled a higher A_{net} at midday in ‘19’ than in ‘A1’ (Figure 6), indicating that deep rooting can improve the water absorption even in well-watered soil conditions. But the negative correlation of A_{net} to Chl *a* fluorescence parameters at midday (ETo/RC, φ_{Po}, φ_{Eo}, PI_{ABS}) indicated that a higher energetical cost in the PSII repair mechanisms was requested in ‘19’ than in ‘A1’.

The higher MDP can result in a lower carbon assimilation during the midday period in ‘A1’, but a lower photochemical damage and hence lower energetic cost for PSII repair, whilst a opposite response was found in ’19. That is, the clones showed a different acclimation response to the environment, ruled for distinct pathways. Then, in the well-watered soil conditions, the combination of VPD_{air} and PPFD peaks at midday together with higher MDP in ‘A1’ than in ‘19’ could be considered not as disadvantage or advantage, respectively, but as the resulted of a distinct acclimation responses (adaptative trait) adopted by each clone. In the projection to the water deficit conditions (not studied in this work), high stomatal sensibility associated to poor deep rooting, as found in ‘A1’, could be one disadvantage. In an agricultural context, where the ‘tolerance concept’ is related to plant survival, growth and mainly to production capacity, the maintenance of the plant water status and stomatal aperture under water deficit conditions would be fundamental.

The combination of the stomatal control of CO_2 access to carboxylation sites and the deep rooting, including the carbon assimilation for biomass partitioning, is characterizing a drought-tolerant coffee plants (DaMatta et al., 2003; Pinheiro et al., 2005; Silva et al., 2013). Then, although the plants showed an acclimation capacity as a function of the daily variation in environment conditions, the higher g_s at midday and deep rooting of ‘19’, even in well-watered soil conditions, are traits that could enable a higher water deficit tolerance when compared to ‘A1’.

CONCLUSION

We study can show that the MDP phenomenon can not be adoted as a parameter to define the tolerance or sensibility of the plant to the high VPD_{air} and PPFD, since the plants use different strategies to face the environment conditions, which can result in an increase or decrease of MDP, do not meaning lower or higher plant adaptability. Than, the maintenance of photosynthesis intensity around midday did not contribute to higher net carbon gain over the day and hence higher plant growth, discarding we first hypothesis. The MDP was influenced by stomatal and non-stomatal effects for both clones, being the MDP in ‘A1’ more influenced by stomatal effects, whilst in ‘19’ by non-stomatal effects. The deep rooting (RDM 0.75-1.0m) impacted on stomatal responses due the positive correlation with gs and Ɛ, enabling a better water supply to the plant tissues.

REFERENCES

Adams III, W. W., Cohu, C. M., Muller, O., Demmig-Adams, B. (2013) Foliar phloem infrastructure in support of photosynthesis. *Frontiers in Plant Science*, 4(194): 1-7.

Baltzer, J. L., Davies, S. J., Bunyavejchewin, S., Noor, N. S. M. (2008) The role of desiccation tolerance in determining tree species distributions along the Malay–Thai Peninsula. *Functional Ecology*, 22 (2): 221-231.

Bao, Y., Aggarwal, P., Robbins, N.E., Sturrock, C.J., Thompson, M.C., Tan, H.Q., Tham, C., Duan, L., Rodriguez, P.L., Vernoux, T. Money, S. J., Bennett, M.

J., Dinneny, J. R. (2014) Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proceedings of the National Academy of Sciences*, 111: 9319–9324.

Barros, R. S., Mota, J. W. S., DaMatta, F. M., Maestri, M. (1997) Decline of vegetative growth in *Coffea arabica* L. in relation to leaf temperature, water potential and stomatal conductance. *Field Crops Research*, 54: 65–72.

Bartlett, M. K., Scoffoni, C., Sack, L. (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecology Letters*, 15 (5): 393-405.

Batista, K. D., Araújo, W. L., Antunes, W. C., Cavatte, P. C., Moraes, G. A. B. K., Martins, S. C. V., DaMatta, F. M. (2012) Photosynthetic limitations in coffee plants are chiefly governed by diffusive factors. *Trees*, 26:459–468.

Blackman, C. J., Brodribb, T. J., Jordan, G. J. (2010) Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytologist*, 188 (4): 1113-1123.

Blum, A. (2016) Stress, strain, signaling, and adaptation—not just a matter of definition. *Journal of Experimental Botany,* 67: 563–566.

Brodribb, T. J., Holloway-Phillips, M. M., Bramley, H. (2015) Improving water transport for carbon gain in crops. *In:* Sadras, V. O., Calderini, D. F. (eds.) *Crop physiology*. San Diego: Academic Press, p. 251-281.

Buckley, T. N. (2005) The control of stomata by water balance. *New phytologist*, 168 (2), 275-292.

Chaves, A. R., Ten-Caten, A., Pinheiro, H. A., Ribeiro, A., DaMatta, F. M. (2008) Seasonal changes in photoprotective mechanisms of leaves from shaded and unshaded field-grown coffee (*Coffea arabica* L.) trees. *Trees*, 22 (3): 351-361.

Chaves, M. M., Harley, P. C., Tenhunen, J. D., Lange, O. L. (1987) Gas exchange studies in two portuguese grapevine cultivars. *Physiologia Plantarum*, 70 (4): 639-647.

Correia, M. J., Chaves, M. M. C., Pereira, J. S. (1990) Afternoon depression in photosynthesis in grapevine leaves- Evidences for a high light stress effect. *Journal of Experimental Botany*, 41: 417–426.

DaMatta, F. M. (2003) Drought as a multidimensional stress affecting photosynthesis in tropical tree crops. *Advances in Plant Physiology,* 5: 227-265.

DaMatta, F. M., Rahn, E., Läderach, P., Ghini, R., Ramalho, J. C. (2019) Why could the coffee crop endure climate change and global warming to a greater extent than previously estimated? *Climatic Change*, 152: 167–178.

De Dato, G., Pellizzaro, G., Cesaraccio, C., Sirca, C., De Angelis, P., Duce, P., Spano, D., Scarascia Mugnozza, G. (2008) Effects of warmer and drier climate conditions on plant composition and biomass production in a Mediterranean shrubland community. *iForest - Biogeosciences and Forestry*, 1:39–48.

Demmig-Adams, B., Cohu, C. M., Muller, O., Adams, W. W. (2012) Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. *Photosynthesis Research*, 113 (1): 75-88.

Demmig-Adams, B., Stewart, J. J., Adams III, W. W. (2017) Environmental regulation of intrinsic photosynthetic capacity: an integrated view. *Current Opinion in Plant Biology*, 37: 34-41.

Den Herder, G., Van Isterdael, G., Beeckman, T., De Smet, I. (2010) The roots of a new green revolution. *Trends Plant Science*, 15: 600–607.

dos Santos, C. M., Verissimo, V., de Lins Wanderley Filho, H. C., Ferreira, V. M., da Silva Cavalcante, P. G., Rolim, E. V., Endres, L. (2013) Seasonal variations of photosynthesis, gas exchange, quantum efficiency of photosystem II and biochemical responses of *Jatropha curcas* L. grown in semi-humid and semi-arid areas subject to water stress. *Industrial Crops and Products*, 41: 203-213.

Dwivedi, S. K., Basu, S., Kumar, S., Kumari, S., Kumar, A., Jha, S., Mishra, J. S., Bhatt, B. P., Kumar, G. (2019) Enhanced antioxidant enzyme activities in developing anther contributes to heat stress alleviation and sustains grain yield in wheat. *Functional Plant Biology,* 46(12): 1090-1102.

El-Sharkawy, M. A., Cock, M. J. H., Hernandez, A. D. P. (1985) Stomatal response to air humidity and its relation to stomatal density in a wide range of warm climate species. *Photosynthesis Research,* 7 (2): 137-149.

Flexas, J., Medrano, H. (2002) Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Annals of Botany*, 89 (2):183–189.

Franck, N., Vaast, P. (2009) Limitation of coffee leaf photosynthesis by stomatal conductance and light availability under different shade levels. *Trees*, 23:761–769.

Ghini, R., Torre-Neto, A., Dentzien, A. F. M., Guerreiro-Filho, O., Lost, R., Patrício, F. R. A., Prado, J. S. M., Thomaziello, R. A., Bettiol, W., DaMatta, F. M. (2015) Coffee growth, pest and yield responses to free-air CO_2 enrichment. *Climatic Change*, 132: 307–320.

Grassi, G., Magnani, F. (2005) Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell & Environment*, *28* (7): 834-849.

Guo, W. D., Guo, Y. P., Liu, J. R., Mattson, N. (2009) Midday depression of photosynthesis is related with carboxylation efficiency decrease and D1 degradation in bayberry (*Myrica rubra*) plants. *Scientia Horticulturae*, 123 (2): 188-196.

Gururani, M. A., Venkatesh, J., Tran, L. S. P. (2015) Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Molecular Plant*, 8 (9): 1304–1320.

Kalaji, H. M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I. A., Cetner, M. D., Lukasik, I., Goltsev, V., Ladle, R. J. (2016) Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum,* 38 (4): 1-11.

Kalaji, H. M., Schansker, G., Ladle, R. J., Goltsev, V., Bosa, K., Allakhverdiev, S. I., Brestic, M., Bussotti, F., Calatayud, A., Dabrowski, P., Elsheery, N. I., Ferroni, L., Guidi, L., Hogewoning, S. W., Jajoo, A., Misra, A.,Nebauer, S. G., Pancaldi, S., Penella, C., Poli, D., Pollastrini, M., Romanowska-Duda, Z. B., Rutkowska, B., Serôdio, J., Suresh, K., Szulc, W., Tambussi, E., Yanniccari, M., Zivcak, M. (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynthesis Research*, 122 (2): 121-158.

Klein, T. (2014) The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. *Functional Ecology*, 28 (6): 1313-1320.

Kramer, P.J.; Boyer, J.S. (1995) *Water Relations of Plants and Soils*. San Diego: Academic Press, 481p.

Kuppers, B. R., Wheeler, A. M., Kuppers, B. I. L., Krischbaum, M. U. F., Farquhar, G. D. (1986) Carbon fixation in eucalyptus in the field. Analysis of diurnal variations in photosynthetic capacity. *Oecologia,* 70: 273–282.

Lavorel, J., Etienne, A. L. (1977) In vivo chlorophyll fluorescence. *Primary Processes of Photosynthesis*, 2: 203-268.

Lawlor, D. W., Cornic, G. (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell & Environmental*, 25 (2):275–294.

Lemoine, R., La Camera, S., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, J. L., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrila, J., Durand, M. (2013) Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in Plant Science*, 4(272): 1-21.

Li, Y. T., Xu, W. W., Ren, B. Z., Zhao, B., Zhang, J., Liu, P., Zhang, Z. S. (2020) High temperature reduces photosynthesis in maize leaves by damaging chloroplast ultrastructure and photosystem II. *Journal of Agronomy and Crop Science*, 206 (5): 548-564.

Liu, H., Gleason, S. M., Hao, G., Hua, L., He, P., Goldstein, G., Ye, Q. (2019) Hydraulic traits are coordinated with maximum plant height at the global scale. *Science Advances*, 5 (2): eaav1332.

Ma, Y., Wang, T., Xie, Y., Lv, Q., Qiu, L. (2021) Alleviatory effect of rare earth micro-fertilizer on photosystem II (PSII) photoinhibition in Pseudostellaria heterophylla leaves at photosynthetic midday depression. *Journal of Rare Earths*, 40 (7): 1156-1164.

Machado Filho, J. A., Rodrigues, W. P., Baroni, D. F., Pireda, S., Campbell, G., de Souza, G. A. R., Verdin Filho, A. C., Arantes, S. D., Arantes, L. O., Cunha, M., Gambetta, G., A., Rakocevic, M., Ramalho, J. C., Campostrini, E. (2021) Linking root and stem hydraulic traits to leaf physiological parameters in Coffea canephora clones with contrasting drought tolerance. *Journal of Plant Physiology*, 258-259(153355): 1-13.

Martins, S. C., Sanglard, M. L., Morais, L. E., Menezes-Silva, P. E., Mauri, R., Avila, R. T., Vital, C. E., Cardoso, A. A., DaMatta, F. M. (2019) How do coffee trees deal with severe natural droughts? An analysis of hydraulic, diffusive and biochemical components at the leaf level. *Trees*, 33 (6): 1679-1693.

Matsuo N, Ozawa K, Mochizuki T. (2009). Genotypic differences in root hydraulic conductance of rice (*Oryza sativa* L.) in response to water regimes. *Plant and Soil*, 316: 25–34.

McAdam, S. A. M., Brodribb, T. J. (2014) Separating Active and Passive Influences on Stomatal Control of Transpiration. *Plant Physiology*, 164 (4): 1578–1586.

McAdam, S. A., Brodribb, T. J. (2015) The evolution of mechanisms driving the stomatal response to vapor pressure deficit. *Plant Physiology*, 167: 833–843

Meinzer, F. C., Smith, D. D., Woodruff, D. R., Marias, D. E., McCulloh, K. A., Howard, A. R., Magedman, A. L. (2017) Stomatal kinetics and photosynthetic gas exchange along a continuum of isohydric to anisohydric regulation of plant water status. *Plant, Cell & Environment*, 40 (8): 1618-1628.

Merilo, E., Yarmolinsky, D., Jalakas, P., Parik, H., Tulva, I., Rasulov, B., Kilk, K., Kollist, H. (2018) Stomatal VPD response: there is more to the story than ABA. *Plant Physiology*, 176 (1): 851-864.

Miao, Y., Cai, Y., Wu, H., Wang, D. (2021) Diurnal and seasonal variations in the photosynthetic characteristics and the gas exchange simulations of two rice cultivars grown at ambient and elevated CO_2. *Frontiers in Plant Science*, *12*: 559.

Mishar, M. K., Slater, A. (2012) Recent advances in the genetic transformation of Coffee. *Biotechnology Research International*, 2012 (580857): 1-17.

Murata, N., Allakhverdiev, S. I., Nishiyama, Y. (2012) The mechanism of photoinhibition in vivo: re-evaluation of the roles of catalase, α-tocopherol, non-photochemical quenching, and electron transport. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1817 (8): 1127-1133.

Nishiyama, Y., Allakhverdiev, S. I., Murata, N. (2006) Anewparadigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1757 (7): 742-749.

Nishiyama, Y., Allakhverdiev, S.I., Murata, N. (2011) Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II. *Physiologia Plantarum*, 142 (1): 35–46.

Nishiyama, Y., Murata, N. (2014) Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. *Applied Microbiology and Biotechnology,* 98 (21): 8777–8796.

Pallardy, S. G. (2008) *Physiology of woody plants*. 3rd edn. San Diego: Elsevier, Academic Press, 453p.

Patrick, J. W., Colyvas, K. (2014) Crop yield components–photoassimilate supply- or utilisation limited-organ development? *Functional Plant Biology*, 41 (9): 893-913.

Polutchko, S. K., Stewart, J. J., Demmig-Adams, B., Adams, W. W. (2018) Evaluating the link between photosynthetic capacity and leaf vascular organization with principal component analysis. *Photosynthetica*, 56 (1): 392-403.

Pons, T. L., Welschen, R. A. (2003) Midday depression of net photosynthesis in the tropical rainforest tree Eperua grandiflora: contributions of stomatal and internal conductances, respiration and Rubisco functioning. *Tree Physiology*, 23 (14): 937-947.

Powles, S. B. (1984) Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology*, 35 (1): 15-44.

Prasad, P. V. V., Vu, J. C. V., Boote, K. J., Allen, L. H. (2009) Enhancement in leaf photosynthesis and upregulation of Rubisco in the C4 sorghum plant at elevated growth carbon dioxide and temperature occur at early stages of leaf ontogeny. *Functional Plant Biology,* 36 (9): 761-769.

Rakocevic, M., Braga, K. S. M., Batista, E. R., Maia, A. H. N., Scholz, M. B. S., Filizola, H. F. (2020) The vegetative growth assists to reproductive responses of Arabic coffee trees in a long-term FACE experiment. *Plant Growth Regulation*, 91: 305-316.

Ramalho, J. C., Pais, I. P., Leitão, A. E., Guerra, M., Reboredo, F. H., Máguas, C. M., Carvalho, M. L., Scotti-Campos, P., Ribeiro-Barros, A. I., Lidon, F. L. C., DaMatta, F. M. (2018) Can elevated air [CO_2] conditions mitigate the predicted warming impact on the quality of coffee bean? *Frontiers in Plant Science*, *9*: 287.

Raschke, K., Resemann, A. (1986) The midday depression of CO_2 assimilation in leaves of *Arbutus unedo* L.: diurnal changes in photosynthetic capacity related to changes in temperature and humidity. *Planta,* 168: 546–558.

Ripoll, J., Bertin, N., Bidel, L. P., Urban, L. (2016) A user's view of the parameters derived from the induction curves of maximal chlorophyll a fluorescence: perspectives for analyzing stress. *Frontiers in Plant Science*, *7*: 1679.

Roche, D. (2015) Stomatal conductance is essential for higher yield potential of C3 crops. *Critical Reviews in Plant Sciences*, 34 (4), 429–453.

Rodrigues, W. P., Martins, M. Q., Fortunato, A. S., Rodrigues, A. P., Semedo, J. N., Simões-Costa, M. C., Pais, I. P., Leitão, A. E., Colwell, F., Goulao, L., Máguas, C., Maia, R., Partelli, F. L., Campostrini, E., Scotti-Campos, P., Ribeiro-Barros, A. I., Lidon, F. C., DaMatta, F. M., Ramalho, J. C. (2016) Long-term elevated air [CO_2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical *Coffea arabica* and *C. canephora* species. *Global Change Biology*, 22: 415-431.

Roessler, P. G., Monson, K. R. (1985) Midday depression in net photosynthesis and stomatal conductance in *Yucca glauca*. Relative contributions of leaf temperature and leaf-to-air water vapour concentration difference. *Oecologia,* 67: 380–387.

Ronquim, J. C., Prado, C. H. B. A., Novaes, P., Fahl, J. I., Ronquim, C. C. (2006) Carbon gain in *Coffea arabica* during clear and cloudy days in the wet season. *Experimental Agriculture*, 42: 147–164.

Sack, I., Pasquet-Kok, J. (2010) Leaf pressure-volume curve parameters: <https://sites.lifesci.ucla.edu/eeb-sacklab/protocols/>. Accessed in: Mar. 2020.

Schulze, E. D., Hall, A. E. (1982) Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. *In*: Lange, O. L., Nobel, P. S., Osmond, C. B., Ziegler, H. (eds.), *Physiological plant ecology II*. 12B. ed. Berlin: Springer, Heidelberg, p. 181-230.

Silva, E. A., DaMatta, F. M., Ducatti, C., Regazzi, A. J., Barros, R. S. (2004) Seasonal changes in vegetative growth and photosynthesis of Arabica coffee trees. *Field Crops Research*, 89 (2-3): 349-357.

Strasser, R. J., Srivastava, A., Tsimilli-Michael, M. (2004) Analysis of the chlorophyll a fluorescence transient. *In*: Papageorgiou, G. Govindjee (eds.) *Chlorophyll fluorescence: a signature of photosynthesis*. Dordrecht: Springer, p. 321– 362.

Takahashi, S., Murata, N. (2008) How do environmental stresses accelerate photoinhibition? *Trends Plant Science*, 13: 178–182.

Theroux-Rancourt, G., Ethier, G., Pepin, S. (2014) Threshold response of mesophyll CO_2 conductance to leaf hydraulics in highly transpiring hybrid poplar clones exposed to soil drying. *Journal of Experimental Botany*, 65: 741–753.

Tognetti, R., Raschi, A., Jones, M. B. (2000) Seasonal patterns of tissue water relations in three Mediterranean shrubs co-occurring at a natural CO_2 spring. *Plant Cell and Environment*, 11: 1341–1351.

Trueba, S., Pan, R., Scoffoni, C., John, G. P., Davis, S. D., Sack, L. (2019) Thresholds for leaf damage due to dehydration: declines of hydraulic function, stomatal conductance and cellular integrity precede those for photochemistry. *New Phytologist*, 223 (1): 134-149.

Tsimilli-Michael, M., Strasser, R. J. (2013) The energy flux theory 35 years later: formulations and applications. *Photosynthesis Research*, 117 (1): 289-320.

Vadez, V. (2014) Root hydraulics: the forgotten side of roots in drought adaptation. *Field Crops Research*, *165*: 15-24.

Venancio, L. P., Filgueiras, R., Mantovani, E. C., do Amaral, C. H., da Cunha, F. F., dos Santos Silva, F. C., Althoff, D., dos Santos, R. A., Cavatte, P. C. (2020) Impact of drought associated with high temperatures on *Coffea canephora* plantations: a case study in Espírito Santo State, Brazil. *Scientific Reports*, 10 (1): 1-21.

Venturin, A. Z., Guimarães, C. M., de Sousa, E. F., Machado Filho, J. A., Rodrigues, W. P., Serrazine, Í. A., Bressan-Smith, R., Marciano, C. R., Campostrini, E. (2020) Using a crop water stress index based on a sap flow method to estimate water status in conilon coffee plants. *Agricultural Water Management*, 241: 106343.

Weraduwage, S. M., Chen, J., Anozie, F. C., Morales, A., Weise, S. E., Sharkey, T. D. (2015) The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. *Frontiers in Plant Science*, *6*: 167.

Werner, C., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., Schmulling, T. (2003) Cytokinin-deficient transgenic arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *The Plant Cell*, 15: 2532–2550.

Xiong, D., Nadal, M. (2020) Linking water relations and hydraulics with photosynthesis. *The Plant Journal*, 101 (4): 800-815.

Xu, D.Q., Shen Y. (1996) Midday depression of photosynthesis. *In*: Pessarakli, M. (Ed.) *Handbook of photosynthesis*. 2. ed. New York: Marcel Dekker Inc., p.451-459.

Yokoyama, G., Yasutake, D., Tanizaki, T., Kitano, M. (2019) Leaf wetting mitigates midday depression of photosynthesis in tomato plants. *Photosynthetica*, 57 (3): 740-747.

Zhou, Y., Lam, H.M., Zhang, J. (2007) Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. Jornal of *Experimental Botany*, 58: 1207–1217.

Zhu, S. D., Chen, Y. J., Ye, Q., He, P. C., Liu, H., Li, R. H., Cao, K. F. (2018) Leaf turgor loss point is correlated with drought tolerance and leaf carbon economics traits. *Tree Physiology*, 38 (5): 658-663.

4. CAPÍTULO 2

MORPHOLOGICAL, PHYSIOLOGICAL AND ANATOMICAL RESPONSES IN CONILON COFFEA TREE UNDER REPETED WATER DEFICIT CONDITIONS: AN ANALYSIS FROM ROOT TO LEAF

ABSTRACT

The understanding of the morphological, anatomical, and physiological changes involved in the acclimation response to water deficit is of crucial importance to the selection process of tolerant/resilient clones, with high productive potential for regions where recurrent episodes of drought are frequent. The experiment was carried using two clones of *Coffea canephora* Pierre ex Froehner with contrasting tolerance to water deficit [clones ‘3V’ (deep root growth) and ‘A1’ (less deep root growth)]. All the plants were watered daily during the first 60 days after transplanting, and after, seven plants of each clone were exposed to soil water deficit (WD), whilst seven plants were maintained well-watered soil (WW, control). The WD was applied until the soil water potential achieve value ≤ -300 kPa in both clones and the two observed soil depths (10 and 50 cm), after which the plants had been rewatered until a complete recovery of net photosynthetic rate (A_{net}). Posteriorly, a new WD event was applied, totalizing two WD events. The following analyzes were performed: leaf gas exchanges, non-modulated and modulated chlorophyll *a* fluorescence, leaf hydraulic, leaf, branch and root morphology. The water deficit caused negative impact to plant growth in both clones (‘3V’ and ‘A1’). The ‘3V’ showed less decrease in the plant growth parameters under water deficit than ‘A1’. The root growth in deeper soil layer was advantageous only under the mild water deficit, in the beginning of the water withdrawn. The quicker A_{net} and g_s recoveries after rewatering were associated to higher deep root system and plant growth, evidence that ‘3V’ was more tolerant to water deficit than ‘A1’. The combined hydraulic, anatomic, photosynthetic, and photochemical adjustments were shown as more determinant in acclimation process to repeted several water deficits, than the deep root system.

Key words: Acclimation capacity, *Coffea canephora*, repeted water deficit, rewatering, root depth.

RESUMO

O entendimento das alterações morfológicas, anatômicas e fisiológicas envolvidas na resposta da aclimatação ao déficit hídrico é de fundamental importância para o processo de seleção de clones tolerantes/resilientes, com alto potencial produtivo para regiões onde episódios recorrentes de seca são frequentes. O experimento foi realizado usando dois clones de *Coffea canephora* Pierre ex Froehner com tolerância contrastante ao déficit hídrico [clones '3V' (crescimento radicular profundo) e 'A1' (crescimento radicular menos profundo)]. Todas as plantas foram regadas diariamente durante os primeiros 60 dias após o transplante e, após, sete plantas de cada clone foram expostas ao déficit hídrico do solo (WD), enquanto sete plantas foram mantidas em solo bem irrigado (WW, controle). O WD foi aplicado até que o potencial hídrico do solo atingisse valor ≤ -300 kPa em ambos os clones e nas duas profundidades de solo observadas (10 e 50 cm), após o que as plantas foram reidratadas até a recuperação completa da taxa fotossintética líquida (A_{net}). Posteriormente, foi aplicado um novo evento WD, totalizando dois eventos WD. Foram realizadas as seguintes análises: trocas gasosas foliares, fluorescência de clorofila a não modulada e modulada, hidráulica foliar, morfologia foliar, ramal e radicular. O déficit hídrico causou impacto negativo no crescimento das plantas em ambos os clones (‘3V’ e ‘A1’). O '3V' apresentou menor queda nos parâmetros de crescimento das plantas sob déficit hídrico do que o 'A1'. O crescimento radicular na camada mais profunda do solo foi vantajoso apenas sob déficit hídrico moderado, no início da retirada de água. As recuperações mais rápidas de Anet e gs após a reidratação foram associadas a maior sistema radicular profundo e crescimento das plantas, evidência de que ‘3V’ foi mais tolerante ao déficit hídrico do que ‘A1’. Os ajustes hidráulicos, anatômicos, fotossintéticos e fotoquímicos combinados mostraram-se mais determinantes no processo de aclimatação para repetir vários déficits hídricos, do que o sistema radicular profundo.

Palavras-chave: Capacidade de aclimatação, *Coffea canephora*, déficit hídrico repetido, reidratação, profundidade radicular.

INTRODUCTION

Brazil is the second largest producer of *Coffea canephora* in the world (Machado Filho et al., 2020), with the Espírito Santo State, Southeast Brazil, responsible for *ca.* 15% of the world's yield (Venancio et al., 2020). This region is characterized to have a dry climate (Machado Filho et al., 2020), demanding irrigation to allow adequate crop yields. However, the limitation of water availability for irrigation have increased due to larger areas of agricultural soil, and due to ongoing global climate change, which has increased the frequency and severity of drought events (DaMatta et al., 2010; 2018). Therefore, the selection of drought tolerant coffee cultivars, which can produce acceptable yields under conditions of water scarcity, is of crucial importance (Silva et al., 2013).

In general, plant drought resistance is associated to drought escape *via* a short plant and/or leaf life cycle or developmental plasticity (Manavalan et al., 2009), drought tolerance *via* antioxidant capacity, osmotic adjustment, and dehydration endurance (Yue et al., 2006; Luo, 2010), deep root (Hammer et al., 2009; Steele et al., 2013), or drought avoidance *via* reduced water loss and enhanced water uptake (Luo, 2010; Tardieu, 2013), being common to have a mix of several mechanisms. The avoidance of dehydration is associated to a variety of adaptive traits to maximize water uptake and minimize water loss, which can be brought about by a combination of morphological, physiological, and anatomical adaptations (Ennajeh et al., 2010).

The major effects often observed during the early phases of a drought are growth impairments and photosynthetic decline, due to reduced cell turgor and stomatal closure, respectively (Martins et al., 2019). Decreases in g_s and hydraulic conductance under drought conditions prevent excessive water loss and limit the leaf damages caused by low leaf water potentials, but parallelly decrease the carbon dioxide uptake and hence biomass accumulation and yield (Silva et al., 2013).

Photosynthetic capacity and water balance in plants are determined by leaf anatomy (Terashima et al., 2011). The thickness of palisade parenchyma cells is an important feature that governs the ability to withstand water stress (Ennajeh et al., 2010). Drought tolerant cultivars often present a thicker sponge parenchyma with large intercellular space, to facilitate CO_2 diffusion (Hajnajari et al., 2019).

Mild drought stress can cause a decrease in photosynthetic capacity by stomatal limitation, that are regulated for example, by expression of the antioxidant genes precursors of ABA synthesis involved in the stomata closure to minimize water loss (Fernandes et al., 2021). However, in several levels of the stress, non-stomatal limitations, including inhibition or damage of biochemical metabolism and photochemical reactions (Beyel and Bruggemann, 2005; Xu et al., 2008; Guóth et al., 2009) and proteomic changes can occur (Marques et al., 2022).

Measurements of chlorophyll *a* fluorescence is a useful and accurate technique to evaluate the photochemical machinery functioning (PSII activity), and to obtain insights regarding the efficiencies of energy transfer and heat dissipation (Ripoll et al., 2016), contributing to characterize the acclimation mechanisms to stress (Misra et al., 2012; Kalaji et al., 2014; 2016). Based in the fast fluorescence kinetics of chlorophyll *a* fluorescence, the JIP-test allows to measure several photochemical related parameters (Strasserf et al., 1995; Yin et al., 2010).

Additionally, chlorophyll fluorescence quenching analysis helps to evaluate photosystem II (PSII) photoinhibition (van Kooten and Snel, 1990; Bolhàr-Nordenkampf and Öquist, 1993). Quenching analysis permits the separation of the contributions of photochemical and non-photochemical processes in the quenching of variable fluorescence, inducing a temporary closure of all PSII reaction centers by a high saturating light pulse (Schreiber et al., 1995; Baker, 2008). Analyzing the PSII efficiency under drought can provide a rapid indication of variations in drought tolerance ability of plants. However, the relations between photosynthetic gas exchange and chlorophyll *a* fluorescence with hydraulic parameters under drought stress conditions has not been well characterized (Reddy et al., 2019).

In the last decade, the knowledge of the mechanisms associated to drought tolerance has grown, in which the majority was considering drought as a single event that occurs only once in the plant life (Silva et al., 2017). However, this is well different from natural conditions, in which repeated drought and recovery cycles are more common than a single prolonged drought event (Galle et al., 2011). Thus, some care must be taken to extrapolate information associated with drought responses obtained from a single drought event (Silva et al., 2017).

Non-irrigated and irrigated crops are subjected to cycles of drought stress and rewatering in both temperate and arid climates (Perrone et al., 2012). Interesting example of non-irrigated and irrigated cycles on the root is the partial rootzone drying (PRD) (Tesfaye et al., 2013). The process of the plant repairing of the drought-induced damages and restarting the growth is complex, because it involves the rearrangement of many metabolic pathways (Chen et al., 2016). Therefore, plants can develop a kind of acclimation when previously exposed to a stress agent, which can potentiate their defense responses, to prepare them to a subsequent exposure to that stress agent (Bruce et al., 2007; Iwasaki and Paszkoski, 2014; Fleta-Soriano and Munné-Bosch, 2016; Menezes-Silva et al., 2017). Still, the velocity of photosynthetic decline during drought, the velocity of recovery from drought after rewatering and the potential acclimation to recurrent drought cycles was only explored in a few cases (Galle et al., 2011), by comparison with single drought and recovery studies.

The response of the plants to water deficit is directly influenced by root system traits, that affect the amount of water and nutrient absorption, and are important for maintaining crop yield under water stress conditions (Narayanan et al., 2014). Several studies in various crops (sorghum, pulses, rice, maize, and wheat) have reported the importance of the deep root system for uptake of water from deeper soil layers under water deficit conditions (Eissenstat et al., 1992; Krishnamurthy et al., 1999; Kashiwagi et al., 2006; Reynolds et al., 2007; Hammer et al., 2009; Manschadi et al., 2010; Wasson et al., 2012; Steele et al., 2013). However, plants with a large root system are partitioning more photosynthetic products to roots, which implies a reduced partition to reproductive growth (Ma et al., 2013). Furthermore, clones with deep root growth could be more advantageous under the mild soil water deficit, during wich the drought does not achieve deeper soil profile.

Better understanding of morphological, anatomical, and physiological traits, from root to the leaf, involved in the mechanisms of drought responses, may provide insights and facilitate the selection of promising *C. canephora* clones for future coffee cropping in drought-prone regions. In this context, some questions were addressed as: (1) Does exist the difference in the photosynthetic and photochemical declines during water deficit and recovery time among contrasting deep root growth coffee clones? (2) Do the coffee clones show an acclimation response to previous water deficit when subjected to two consecutives water deficit events? (3) What are the morphological, physiological, and anatomical integrated acclimation responses in the contrasting deep root growth coffee clones when subjected to two consecutives water deficit event? We hypothesized that the higher deep root growth clone would have a vantage under the mild water deficit, being able to allow delay or avoid cell dehydration, maintaining higher leaf photosynthetic activity during the repeated drought event. Also, it was hypothized that under the several water deficits, the phenotypical plasticity (acclimation mechanisms) is determinant for the *C. canephora* plant establishment and growth.

MATERIAL AND METHODS

Local of experiment, specie characterization and useful information

The experiment was carried out in the greenhouse at the State University of Northern Fluminense, Campos dos Goytacazes (21º44’47” S and 41º18’24” W at 10 m altitude), using two clones of *C. canephora* Pierre ex Froehner with contrasting root growth coffee clones (clones ‘3V’ and ‘A1’, previously characterized as deeper root growth and lower deep root growth, respectively). On November 2020, five-month-old seedlings (14 of each clone, summing 28 seedlings) produced from cuttings were transplanted to PVC tubes (1.0 x 0.2 m of height and diameter, respectively). Tubes were filled with substrate composed of red-yellow latosol and sand in the 4:1 proportion, respectively. Soil chemicals analysis was performed to determine the soil fertility and ensure the adequate fertilization according to crop requirements (Prezotti et al., 2007).

All plants were watered daily to maintain the soil under the suitable water availability during the first 60 days after transplanting. After this period, seven plants of each clone were exposed to soil water deficit (WD), whilst remaining seven plants were maintained well-watered soil (WW, control). The WD was applied in the following: the water withdrawn until the soil water potential (Ψ_{soil}) achieve value ≅ -300 kPa (-0.3MPa) in both clones and both soil depths (10 and 50 cm) (Figure 1). After this moment, the plants were rewatering until a complete recovery of net photosynthetic rate at 1500 µmol m^{-2} s^{-1} ($A_{net1500}$). This process from the water withdrawn until the maximum $A_{net1500}$ recovery was named as a WD event. Thirty days after rewatering (sufficient period to permit a complete growth of new leaves developed after WD), a new WD event was applied (Figure 1). It mean that plants were subjects to two WD events, named as first WD event (WD-1) and second WD event (WD-2). The period after the first water withdrawn and at the end of the experiment was called DSWD (days of soil water deficit). After the end of the WD-2 and recovery, the plant root and shoot analyses were performed.

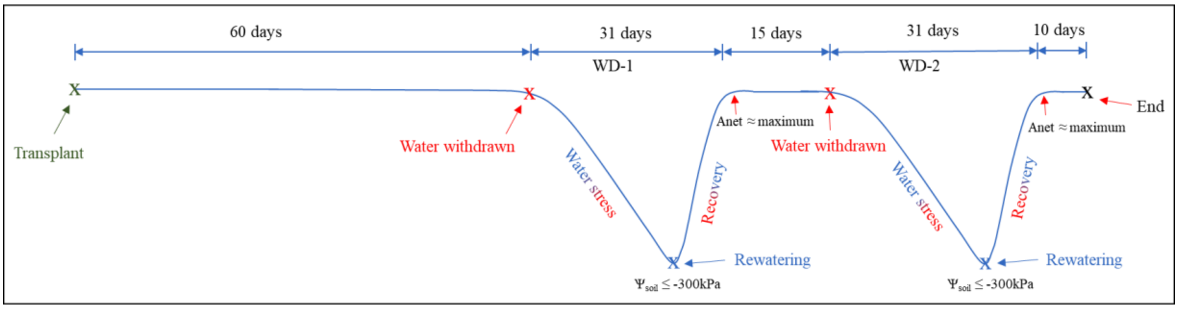

Figure 1. Scheme design water stress events applied in *Coffea canephora* plants. A_{net1500}= net photosynthetic rate at 1500 µmol m^{-2} s^{-1}. Rewatering was applied at 23 and 21 days after water cut in the WD-1 and WD-2, respectively, when the Ψ_{soil} achieved value ≤ -300 kPa.

The micrometeorological variables as air temperature (T_{air}, °C), relative humidity (RH, %), air vapor pressure deficit (VPD_{air}, kPa) and photosynthetic photons fluxes density (PPFD, µmol m^{-2} s^{-1}) were recorded each 60 minutes, using a Weather Station Watchdog 2000 (Spectrum Technologies, Plainfield, IL, USA). The soil water potential was recorded each 30 minutes in uninterrupted days since transplanting (November 12) until the end (April 20) of the experiment, using a TEROS 21 water potential sensor and stored to a data logger (ZL6 PRO, Meter Group, USA). One sensor at 10 cm of deep from soil surface was installed in each control (3VWW and A1WW), whilst in 3VWD and A1WD were installed two sensors for treatment, the first one at 10 cm and the second at 50 cm of deepth from soil surface.

Leaf water potential

Leaf water potential (Ψ_{leaf}) was measured in the 23 and 71 DSWD, benchmarks for the two WD events to start rewatering the soil, based in the Ψ_{soil} (≅ -300 kPa, -0.3MPa). This was made to attest that the plants were in a several WD. Measurements were made at predawn in the third pair of leaves from apex of the 3rd plagiotropic branch formed from the top of the plant. It was measured immediately after the leaf excision, using a pressure chamber (model 3000, Soil Moisture Corp., Santa Barbara, CA, USA), according to Schölander et al. (1965).

Leaf gas exchange measurements

$A_{net1500}$, stomatal conductance (g_s) and transpiration rate (E) were measured using a portable open-system IRGA (Li-Cor 6400xt, LI-COR, Lincoln, USA), with an external CO_2 supply of 400 µL L^{-1}, and *ca.* 1500 µmol m^{-2} s^{-1} of irradiance. The measurements were performed at midday (between 12-1 p. m.) each two days in the WD-1 and each three days in the WD-2, on the fully expanded leaves, which correspond to the third or fourth leaf pair from the apex of the 2nd order plagiotropic (lateral) branches, from the upper part of the plant.

From leaf gas exchange measured, it was possible to calculate the relative variation (%) for each parameter (´X´) between WD and WW conditions in the two clones. The following equation was applied.

$$'X'\ relative\ variation = \left(1 - \frac{´X´WD}{´X´WW}\right) x\ 100 \qquad \text{Eq. 1}$$

The results were shown as absolute values for a control treatment and as relative variation values for plants subjected to WD.

Chlorophyll* a *fluorescence evaluation

Non-modulated fluorescence

Chlorophyll (Chl) *a* fluorescence was measured in the same leaves used for the gas exchange evaluations, on the same period and days, using a Pocket PEA fluorometer (Hansatech, King‟s Lynn, UK). The sampled leaves were dark-adapted for 30-40 minutes using leaf clips (Hansatech, King‟s Lynn, UK) to turn the reaction centers into an "open" (oxidized Q_A) state (Bolhar-Nordenkampf et al., 1989).

From the rapid kinetics of fluorescence emission over time, some variables were obtained using JIP-test (Strasser and Tsimilli-Michael, 2001; Strasserf et al., 1995, 2004), such as those related to: (a) quantum yield [maximum quantum yield of primary photochemical reactions (ΦP_o), probability of electron transport beyond Q_A (Ψ_o), quantum efficiency of electron transfer from Q_A to electron transport chain beyond Q_A (ΦE_o)], (b) performance index of photosynthetic apparatus (PI_{ABS}) and (c) density of active PSII reaction centers (RC/CS).

From JIP-test variables, the relative variation (%) for each parameter (´X´) between WD and WW conditions in the two clones was calculated (Eq. 1). The results were shown as absolute values for control treatment of two clones and as relative variation values for plants subjected to WD.

Modulated fluorescence

With the portable photosynthesis system (LI6400XT, LI-COR, Lincoln, NE, USA) equipped with a fluorescence chamber (LI-6400-40), chlorophyll fluorescence was recorded at predawn and midday, in a frequency of two days during the WD-1, and in a frequency of three days during the WD-2. For the modulated fluorescence were used the same leaves as for leaf gas exchange measurments. Leaves were allowed for dark adaptation overnight, and at predawn, minimal (F_0) and maximal (F_m) fluorescence were recorded. A light intensity as high as possible to induce the fluorescence without inducing photosynthesis was applied to record F_0, followed by the application of saturating light pulse to record F_m. At midday, on the same leaves measured at predawn, but now adapted to light, an illumination by actinic light of 1500 µmol m^{-2} s^{-1} (10% blue, 90% red) was carried out in a sufficient time to enable a stabilization of gas exchange parameters and photochemical dissipation. It was followed by an application of saturating light pulse of 8000 µmol m^{-2} s^{-1} for 0.8 s, to record the light-adapted maximum fluorescence (F_m’). Immediately, the actinic light was turn off and far-red light was applied to determine F_0’. The following parameters were calculated:

- ΦPSII - proportion of absorbed light that is actually used in PSII photochemistry (Genty el al., 1992):

$$\Phi PSII = (F_m' - F_s)/F_m' \qquad \text{Eq.2}$$

- qP - relates PSII maximum efficiency to operating efficiency, which is non-linearly related to proportion of open PSII centres (Murchie and Lawson, 2013):

$$qP = (F_m' - F_s)/(F_m' - F_o') \qquad \text{Eq. 3}$$

- NPQ - photo-protective process that removes excess excitation energy within chlorophyll-containing complexes and prevents the likelihood of formation of damaging free radicals (Murchie and Lawson, 2013):

$$NPQ = (F_m - F_m')/F_m' \qquad \text{Eq. 4}$$

where F_s is the steady-state fluorescence yield.

- ETR - linear electron transport rate was calculated using the photochemical efficiency at electron transport of PSII (ΦPSII) (Krall and Edwards, 1992):

$$ETR = \Phi PSII * PPFD * \alpha_{leaf} * f \qquad \text{Eq. 5}$$

where α is the fractional absorbance of light by the leaf, assumed as 0.85 (LI-COR Biosciences manual, 2012) based on Baker (2008) and *f* is the partitioning of absorbed quanta between photosystems I and II, that was assumed to be 0.5 (LI-COR Biosciences, 2012).

From the obtained non-modulated fluorescence parameters, the relative variation (%) for each parameter (´X´) between WD and WW conditions in the two clones was calculated (Eq. 1). The results were shown as absolute values for control treatment of two clones and as relative variation values for plants subjected to WD.

Leaf spectral indices

The leaf spectral reflectance was measured on the same leaves used for the gas exchange evaluation, considering the same periods and days, using CI710/720 Miniature Leaf Spectrometer (CID - Bioscience, Camas, WA, USA). Measurements were conducted from 400 nm to 1000 nm wavelength as per the inbuilt software of the system and. We used various indices calculated through standard refereed techniques included in per the in-built software of the CI-710/720 system, as anthocyanin reflectance index (ARI), carotenoid reflectance index (CRI), structure intensive pigment index (SIPI) and plant senescence reflectance index (PSRI).

From the obtained values for spectral reflectance indices, the relative variation (%) for each parameter (´X´) between WD and WW conditions in the two clones was calculated (eq. 1). The results were showed as absolute values for control clones and as relative variation values for clones subjected to WD.

Pressure-volume (P-V) curve

P–V curves were determined after end of WD-2, measuring four plants per day, demanding seven days for whole set measurments. Previously, ten hours before the analyses, the plagiotropic branches containing between five to eight leaf pairs were carefully cut and submerged in water until full rehydration. The branches were maintained covered with dark plastic bag in the laboratory, during at least ten hours, sufficient time to ensure the full stomatal close and the full rehydration. Sampled leaves of the same age as those used in the leaf gas exchange measurements, were excised from rehydrated branches under water and dehydrated slowly on the bench top.

P–V curves were determined using a Scholander pressure chamber (Scholander et al., 1965) and following the free-transpiration method described in previous studies (Talbot et al., 1975; Hinckley et al., 1980; Dreyer et al., 1990). The leaf fresh mass and water potential were measured at short intervals at the beginning and at longer intervals with pass of the time until reaching values close to –3 MPa. Individual leaf area was measured using a leaf area meter (Li-3100, Li-Cor, Lincoln, NE, USA). Leaf dry masses were determined after desiccation in a forced-air oven at 65 °C for 72 h (Silva and Queiroz, 2006).

The P-V curve parameters were obtained from Sack and Pasquet-Kok (2010) protocol. Briefly, the turgor loss point (Ψ_{TLP}) was estimated as the point of transition between curvilinear and linear portions of the graph, plotting the inverse of Ψ_{leaf} against relative leaf water content (RWC). Osmotic potential (Ψ_o) was estimated by extrapolating the straight-line section to 100% RWC. The bulk modulus of elasticity (ε) was estimated from the slope of the pressure potential between full turgor and TLP. The leaf capacitance (*C*) was determined from the slopes of the pressure-volume relationship between full turgor and TLP.

Leaf anatomy

The leaf imprints from the abaxial leaf surface (from the tagged leaves developed after the WD-1) were observed after the end of WD-2, using a light microscope (Axioplan, ZEISS, Aalen, Germany) coupled to an image capture system (Moticam Pro 282B, Hong Kong). Stomatal density was determined as previously described by Ramalho et al. (2013).

The leaf blade fragments were obtained from the tagged leaves (n = 7), where 2 fields of view were examined for each repetition, fixed in a solution of 2.5% glutaraldehyde, 4% formaldehyde, and 0.05 M of sodium cacodylate buffer at pH 7.2. Thereafter, the material was post-fixed in 1% aqueous osmium tetroxide solution and 0.05 M sodium cacodylate buffer for 2 h and dehydrated in ascending series of acetone. Semi-thin cuts were obtained. The sections were stained with 1% Toluidine blue and 1% borax buffer for 1 min. Sections were mounted using Entellan® (Merck, Kenilworth, NJ, USA) and observed under bright field microscopy. Leaf tissue anatomical values (thickness of leaf, adaxial and abaxial epidermal, palisade parenchyma and spongy parenchyma) were calculated from cross sections of the middle third of the leaf blade.

Leaf, branch, and root xylem trait analyses

Cross sections thought thin cuts of leaf central vein, plagiotropic branch and root were obtained, with one, six and 11 fields of view examined for each of seven repetitions. These sections were subjected to the following procedures: clarification using sodium hypochlorite at 50% and 0.1% acidulated water, dehydration in an ascending ethanol series (Johansen, 1940), and staining with astra blue and hydro-alcoholic Safranin. Vessel density and vessel area were measured from the cross section. For this, was used a light microscopy to obtain the images (Axioplan, ZEISS, Aalen, Germany) coupled to an image capture system (Moticam Pro 282B, Hong Kong).

Plant growth traits

The height of plant (HP), diameter of trunk (DT), number of leaves (NL) and number of dropped leaves (NDL) were followed once a week, from the transplant until the final of the experiment. At the end of the experiment, the leaves, plagiotropic and orthotropic branches and roots were separated and dried in a forced-air oven (65 °C, 72 h, Silva and Queiroz, 2006) to obtain the final shoot [final plant height (FPH), final trunk diameter (FTD), final leaf number (FLN), final leaf area (FLA), final leaf dry mass (FLDM), final plagiotropic branch dry mass (FPDM), final orthotropic branch dry mass (FODM), final shoot dry mass (FSDM)] and root [final root dry mass (FRDM)] morphological traits.

Roots were previously separated in four parts according soil depth (0-0.25 m; 0.25-0.50 m, 0.50-0.75 m, and 0.75-1.0 m) to determine the root distribution over the soil profile.

The final total shoot dry mass (FTDM) was obtained by the sum of leaf, plagiotropic branches and orthotropic branch dry mass, while the FRDM was determined by sum of the four parts of the root. The final total dry mass (FTDM) was determined by sum of all parts of the plant. The FLA was measured using a leaf area meter (Li-3100, Li-Cor, Lincoln, NE, USA).

Experimental design and statistical analysis

The implemented experimental design considered a randomized block in a 2 x 2 factorial experimental design (two clones and two soil water conditions, WW and WD with seven repetitions (plants). Plants of the two clones (‘A1’ and ‘3V’) under the WW conditions were named A1WW and 3VWW, whilst under the WD conditions were named A1WD and 3VWD.

The absolute values of leaf gas exchange, non-modulated and modulated fluorescence, leaf spectral index and morphological parameters measured over the time were submitted to a two-way ANOVA, to test the effects of the water deficit over the DSWD. This analysis was performed independently for each clone. The relative variations in leaf gas exchange, non-modulated and modulated fluorescence, and leaf spectral indices were submitted to a two-way ANOVA, to compare the relative variation between the clones under water deficit over the DSWD. When significative, Tukey test ($p \leq 0.05$) was applied to compare the medium. The final morphological, P-V curve, leaf anatomy and leaf, branch, and root xylem parameters were submitted to a two-way ANOVA, to test the clone and water deficit effects. When significative, Tukey test ($p \leq 0.05$) was applied to compare the medium.

The MDP was normalized using minimum-maximum normalization, to eliminate the negative values presents in the data set. All data were evaluated for homogeneity of variance among treatments and, when appropriate, data transformation was adopted.

The growth leaf over time was represented by linear regression models. The effect of water deficit and clones on these models was compared using time as a covariable. The relation $A_{net1500}/g_s$ and $A_{net1500}$/ETR also were represented by linear regression models. All statistical analyzes were performed using a R software (R Core Team, 2020).

RESULTS

Climatic and soil variables, plant conditions and leaf potential

The PPFD (Figure S2A), T_{air} (Figure S2B), RH (Figure S2C), and VPD (Figure S2D) showed variation along of the DSWD, with lower and maximal values associated with cloudy and sunny days, respectively, but keeping in a general context a similar condition from beginning until the end of the experiment.

The Ψ_{soil} decreased in both clones after beginning of both WD events (WD-1 and WD-2), and recovered quickly after water supply (Figure 2). At the ‘3V’, the Ψ_{soil} at 50cm below of the soil superficies was lower than at 10 cm (Figure 2A), whilst in ‘A1’ one reverse behavior was found (Figure 3B). The control clones showed Ψ_{soil} values more negative than -120 kPa (-0.12 MPa).

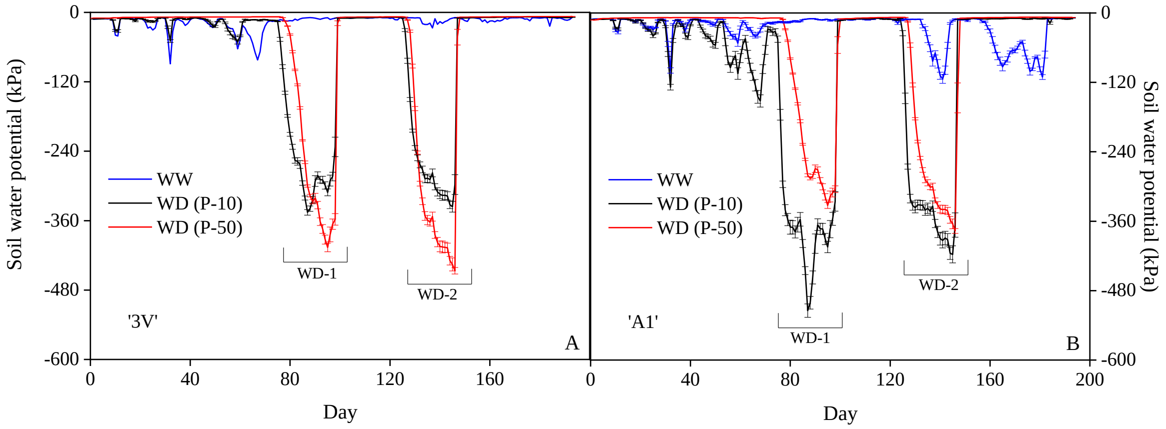

Figure 2. Soil water potential in (A) ‘3V’ (deeper root growth)) and (B) ‘A1’ (lower deep root growth) under well-water soil (WW) and soil water deficit (WD) conditions, registered every 30 minutes in continually since transplanting (November 12) until the end (April 20) of the experiment. P-10 (10 cm) and P-50 (50 cm) were equivalent to sensor position at soil profile.

The leaf water potential (Ψ_{leaf}) was significatively reduced in 3VWD and A1WD during both WD events, without difference between de clones (Table 1). Ψ_{leaf} decreased from -0.25 MPa under WW to -2.77 MPa under WD, equivalent to decreasing more intensive than 1100%.

Leaf gas exchange parameters

The absolute values of leaf gas exchange parameters ($A_{net1500}$, g_s, E and C_i) in WW plants kept a stability over the time, but with a certain fluctuation between 7 and 13 µmol m^{-2} s^{-1} over time of observation (Figure 3). $A_{net1500}$, g_s and E decreased in 3VWD and A1WD over the time of water withdrawn, during both WD-1 and WD-2 (Figures 4A, 4B and 4C).

The maximal decreased of $A_{net1500}$ was found at DSWD 19 (-96%), DSWD 23 (-96%) and DSWD 71 (-104%) in 3VWD and at DSWD 23 (-125%) and DSWD 71 (-106%) in A1WD (Figure 4A). $A_{net1500}$ relative reduction under water withdrawn was similar over the time for both clones, except at DSWD 17, 19, 23 and 25 during the WD-1, where A1WD showed higher relative reduction than 3VWD. Interestingly, no difference between 3VWD and A1WD was found in the WD-2.

The complete recovery of $A_{net1500}$ was achieved at 6th day after rewatering in 3VWD in both WD events, corresponding to DSWD 29 and 70, respectively. The complete recovery of $A_{net1500}$ inA1WD, was achieved at the 4th day after rewatering during the WD-1 (DSWD 27) and at the 9th day after rewatering during the WD-2 (DSWD 79). A quick g_s relative reduction were found until the DSWD 9 in 3VWD (-72%) and A1WD (-76%) during the WD-1 (Figure 4B), followed by a slow g_s relative reduction until DSWD 23, corresponding to -89% and -88% of g_s relative reduction in 3VWD and A1WD, respectively. Both clones showed a quick recovery of g_s after rewatering. The complete recovery was achieved at DSWD 29 in 3VWD and at DSWD 27 in A1WD, corresponding to periods after rewatering. In those periods, no difference was found between the clones subjected to water withdrawn versus the controls. At these DSWD no statistical significance in g_s relative variation was found in the two clones.

In the WD-2, 3VWD and A1WD showed 90% and 89% of g_s relative reduction at DSWD 61 (10 days of water withdrawn), respectively, maintaining similar values over the time of water withdrawn. The g_s recovery started immediately after rewatering for both clones, achieving similar values as control at DSWD 76 in 3VWD and at DSWD 79 in A1WD. At these DSWD no statistical significance in g_s relative variation was found in the two clones. Both clones showed E relative difference similar to g_s during all the experimental period (Figure 3C). The only difference was found during the recovery period of WD-1, at DSWD 27 in E relative difference and at DSWD 29 in g_s relative difference.

The C_i relative difference increased among DSWD 17 and 27 in A1WD, whilst no significance was found in 3VWD (Figure 3D). Hence, C_i relative difference was significantly higher at DSWD 19, 21 and 25 in A1WD than in 3VWD. The C_i recovery in A1WD was achieved at DSWD 27 during the WD-1. During the WD-2, both clones showed the C_i relative increase between DSWD 67 and 70, and a reduction immediately after rewatering, at DSWD 73, three days later. Differently than during the WD-1, during the WD-2 no significance was found in C_i relative difference between 3VWD and A1WD.

Table 1. Medium values ± s.e. and ANOVA *P*-values for leaf water potential (Ψ_{leaf}) at 23rd (WD-1) and 70th (WD-2) DSWD in conilon coffee clones (‘3V’ and ‘A1’) under WW (well-watered soil) and WD (soil water deficit) conditions. The medium values (n = 7) followed by different letters (a, b) express significant differences between WW and WD at $p \leq 0.05$ (marked in bold) using the Tukey test. *Percentage of leaf water potential decrease from WW to WD condition.

WD events	**Leaf water potential (MPa)**				**P_{value}<0.05**			
	3VWW	**A1WW**	**3VWD**	**A1WD**	**WD events**	**Clone**	**Water condition**	**Interation**
WD-1	-0.25 ± 0.026	-0.25 ± 0.016	-2.64 ± 0.16	-2.88 ± 0.12	0.9908	0.6203		0.1659
WD-2	-0.23 ± 0.024	-0.26 ± 0.022	-2.97 ± 0.02	-2.60 ± 0.40				
Medium	-0.247 ± 0.022 a (100%)		-2.77 ± 0.175 b (-1121%)				**<0.0001**	

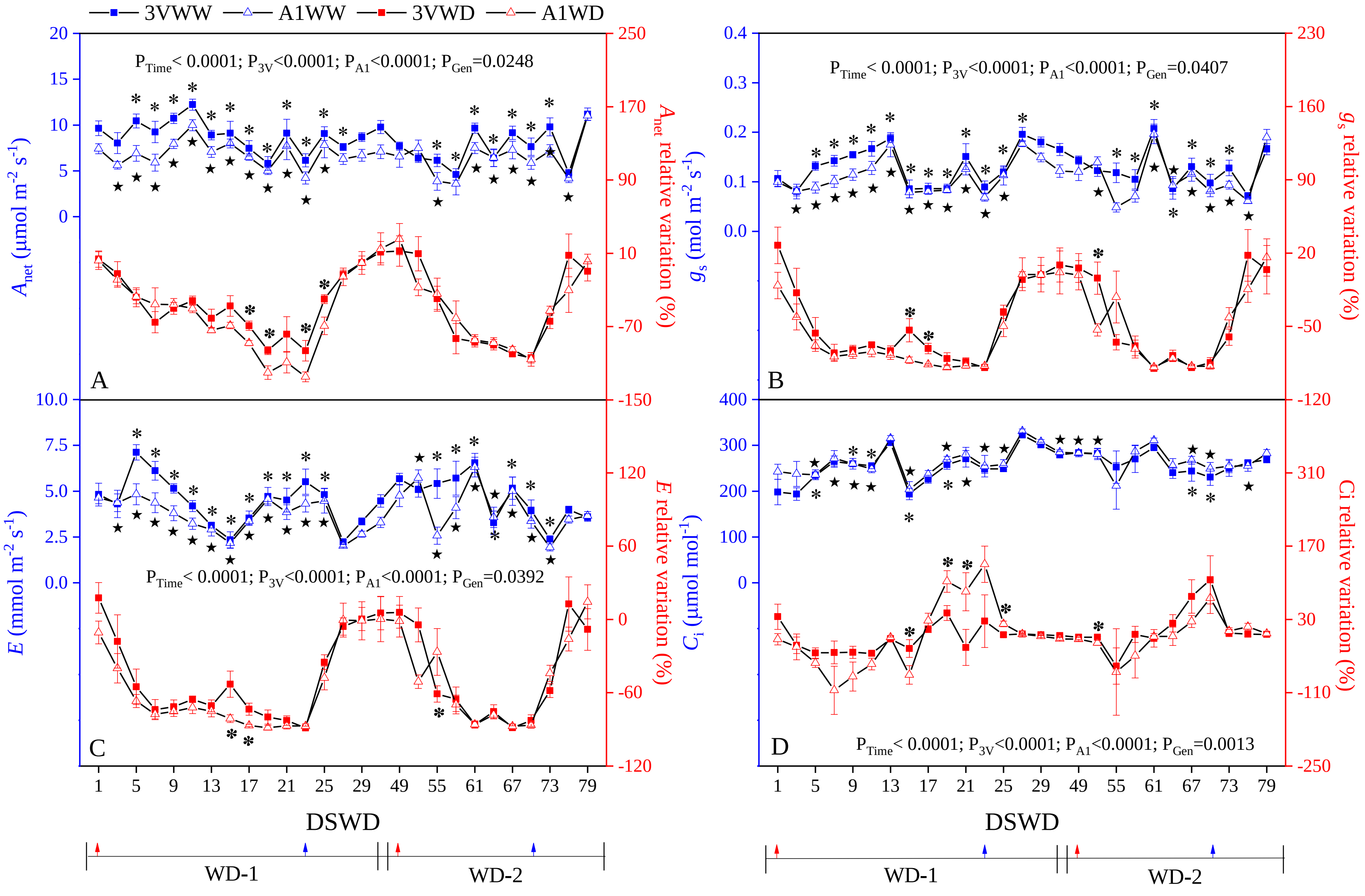
3VWW
A1WW
3VWD
A1WD
A
P_Time< 0.0001; P_3V<0.0001; P_A1<0.0001; P_Gen=0.0248
A_net (µmol m^-2 s^-1)
A_net relative variation (%)
20
15
10
5
0
250
170
90
10
-70
-150
B
P_Time< 0.0001; P_3V<0.0001; P_A1<0.0001; P_Gen=0.0407
g_s (mol m^-2 s^-1)
g_s relative variation (%)
0.4
0.3
0.2
0.1
0.0
230
160
90
20
-50
-120
C
P_Time< 0.0001; P_3V<0.0001; P_A1<0.0001; P_Gen=0.0392
E (mmol m^-2 s^-1)
E relative variation (%)
10.0
7.5
5.0
2.5
0.0
120
60
0
-60
-120
D
P_Time< 0.0001; P_3V<0.0001; P_A1<0.0001; P_Gen=0.0013
C_i (µmol mol^-1)
Ci relative variation (%)
400
300
200
100
0
310
170
30
-110
-250
1
5
9
13
17
21
25
29
49
55
61
67
73
79
DSWD
WD-1
WD-2

Figure 3. Medium values ± s.e. and ANOVA *P*-values for instantaneous leaf gas exchange parameters (left y-axis, blue) and relative difference between WW (well-watered soil) and WD (soil water deficit) (right y-axis, red) in conilon coffee clones (‘3V’ and ‘A1’). (A) Net leaf photosynthetic rate (A_{net} or A_{net1500}), (B) stomatal conductance (g_{s}), (C) leaf transpiration rate (E), and (D) intercellular CO_2 concentration (C_{i}). The medium values followed by “✻”, “*” and “⋆” express significant differences between clones under soil water deficit, between soil water conditions in ‘3V’ and soil water conditions in ‘A1’, respectively, at $p \leq 0.05$ using the Tukey test. P_{Time}= express significance level for the DSWD; P_{3V}= express significance level for interaction between 3VWW:3VWD over the DSWD; P_{A1}= express significance level for interaction between A1WW:A1WD over the DSWD; P_{Gen}= express significance level for interaction between 3VWD:A1WD over the DSWD. Red arrow: beginning of soil water deficit; blue arrow: rewatering.

In both WD events and in two clones a high influence of g_s on $A_{net1500}$ (Figures 4A and 4B) was observed. WD clearly reduced g_s in both clones, resulting in $A_{net1500}$ decrease. The '3V' showed higher influence of g_s on $A_{net1500}$ than 'A1', as observed by higher slope coefficient of the linear regression. During the WD-1, the slope coefficients were 45.018 µmol CO_2 mol H_2O^{-1} [intrinsic water use efficiency (iWUE)] in '3V' and 38.913 µmol CO_2 mol H_2O^{-1} in 'A1'. During the WD-2, the slope coefficients were 53.568 and 50.657 µmol CO_2 mol H_2O^{-1} in '3V' and 'A1', respectively. Thus, '3V' showed higher iWUE than 'A1' during WD-1 and WD-2. In the WD-2, both clones increased the iWUE, once the slope coefficient were higher than in the WD-1 in both clones.

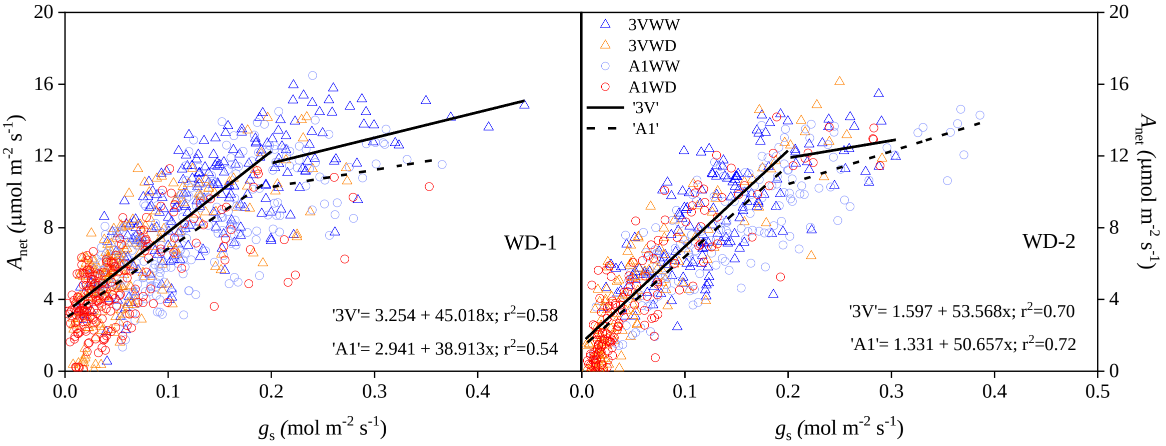

Figure 4. Linear regressions between stomatal conductance (g_s) and net photosynthetic rate (A_{net} or $A_{net1500}$) measured at midday in conilon coffee clones ('3V' and 'A1') under well water soil condition and subjected to two soil water deficit (WD) events, (A) WD-1 and (B) WD-2.

Leaf chlorophyll a fluorescence and spectral index

The absolute values of JIP-test indices (ΦPo, ΨEo, PI_{ABS} and RC/CS) in WW plants kept a stability over the experimental period, but with a certain fluctuation over time (Figure 5). ΦPo, ΨEo, PI_{ABS} and RC/CS decreased in 3VWD and A1WD over the time of water withdrawn in the WD-1, whilst in the WD-2 no difference was observed (Figures 5A, 5B, 5C, and 5D).

The continuous decrease in ΦPo absolute values was observed from DSWD 11 until DSWD 23 in both clones, during the WD-1 (Figure 5A). The maximum decrease in ΦPo, was achieved at 23 DSWD, equivalent to 11% of relative reduction in 3VWD and 28% in A1WD, without statistical difference between clones under WD. Interestingly, water deficit had no impact on ΦPo of two clones during the WD-2, and clone responses did not differ neither. The complete recovery of ΦPo in the WD-1 was achieved at the 2nd day after rewatering in 3VWD and at the 6th day in A1WD, corresponding to DSWD 23 and 29, respectively, whilst in the WD-2 no difference was observed (Figure 5A).

The ΦEo and PI_{abs} absolute values decreased over the time under WD in the two clones during the WD-1, achieving maximum ΦEo and PI_{abs} relative reduction at 23th DSWD (Figures 5B and 5C). On the other hand, no ΦEo and PI_{abs} relative reduction was found in the two clones during the WD-2. The complete recovery of ΦEo and PI_{abs} during the WD-1 was achieved at the 2nd day after rewatering in both clones, corresponding to DSWD 25, whilst in the WD-2 no temporal difference was observed (Figures 5B and 5C).

The continuous decrease in RC/CS absolute values was observed from the 7th DSWD to the 23th DSWD in both clones, during the WD-1 (Figure 5D). The maximum decrease in RC/CS, was achieved at DSWD 23, equivalent to 41% of relative reduction in 3VWD and 30% in A1WD, without statistical difference between clones under water deficit during the WD-1. During the WD-2, water deficit had no impact on RC/CS in both 3VWD and A1WD, and clone responses did not differ neither. The complete recovery of RC/CS was achieved at 4th day after rewatering for both clones during the WD-1, corresponding to DSWD 27, whilst during the WD-2 no difference was observed (Figure 5D).

The absolute values of modulated leaf chlorophyll *a* fluorescence parameters ($Φ_{PSII}$, qP, NPQ and ETR) in WW plants kept a stability over the time, but with a certain fluctuation over time (Figure 6). The $Φ_{PSII}$ absolute values decreased over the experiment under WD in both clones during the WD-1 and WD-2 (Figure 6A). The maximum decreased of $Φ_{PSII}$ was observed at DSWD 19 (-71%), DSWD 21 (-71%) and DSWD 67 (-83%) in 3VWD and at DSWD 21 (-84%) and DSWD 70 (-81%) in A1WD (Figure 6A). $Φ_{PSII}$ relative reduction under water withdrawn in the WD-1 was higher in A1WD at 11, 13, 15, 21, 23 and 25 DSWD when compared to 3VWD. Interestingly, no difference between 3VWD and A1WD was found in the WD-2. The complete recovery of $Φ_{PSII}$ in 3VWD was achieved at the 4th day after rewatering in the WD-1 and at the 6th day in the WD-2, corresponding to DSWD 27 and 76, respectively. The complete recovery of Φ_{PSII} in A1WD was achieved at the 6th day after rewatering during the two WD events, corresponding to 27 DSWD and 76 DSWD.

The qP (Figure 6B) showed a similar behavior than Φ_{PSII} (Figure 6A) in both clones during the two WD events. qP absolute values decreased over the time in the WD treatment in both clones during the WD-1 and WD-2 (Figure 6B). The maximum in qP decreasing was found at DSWD 19, 21 and 67 in 3VWD, and at DSWD 21 and 70 in A1WD. Both clones showed qP relative reduction under water withdrawn in the two WD events. However, qP relative reduction was higher in the A1WD than in the 3VWD from 7th to 13th DSWD and at DSWD 21, 23 and 25 during the WD-1, whilst no difference between the clones was observed during the WD-2.

The qP recovery started immediately after rewatering in both clones during the two WD events (Figure 6B). The complete recovery of qP in 3VWD was achieved at the 4th day after rewatering of the WD-1 and at the 6th of the WD-2, corresponding to 27 and 76 DSWD, respectively. The complete recovery in A1WD, was achieved at 8th (DSWD 29) and 6th (DSWD 76) day after rewatering in the WD-1 and WD-2, respectively.

The NPQ was statistically different between 3VWW and 3VWD only at the 1st and 31st DSWD of the WD-1 and at the 61st, 64th, 67th and 70th DSWD of the WD-2 (Figure 6C). The differences in NPQ between A1WW and A1WD were observed at the 15th, 17th, 23rd, and 29th DSWD of the WD-1 and at 64th, 67th and 70th and 73rd DSWD of the WD-2. During the WD-1, no effect of the water condition on NPQ was observe in both clones. Interestingly, during the WD-2, the NPQ was lower in 3VWD than in 3VWW from the 61st to 70th DSWD, as was lower in A1WD than in A1WW from the 64th to 73rd DSWD.

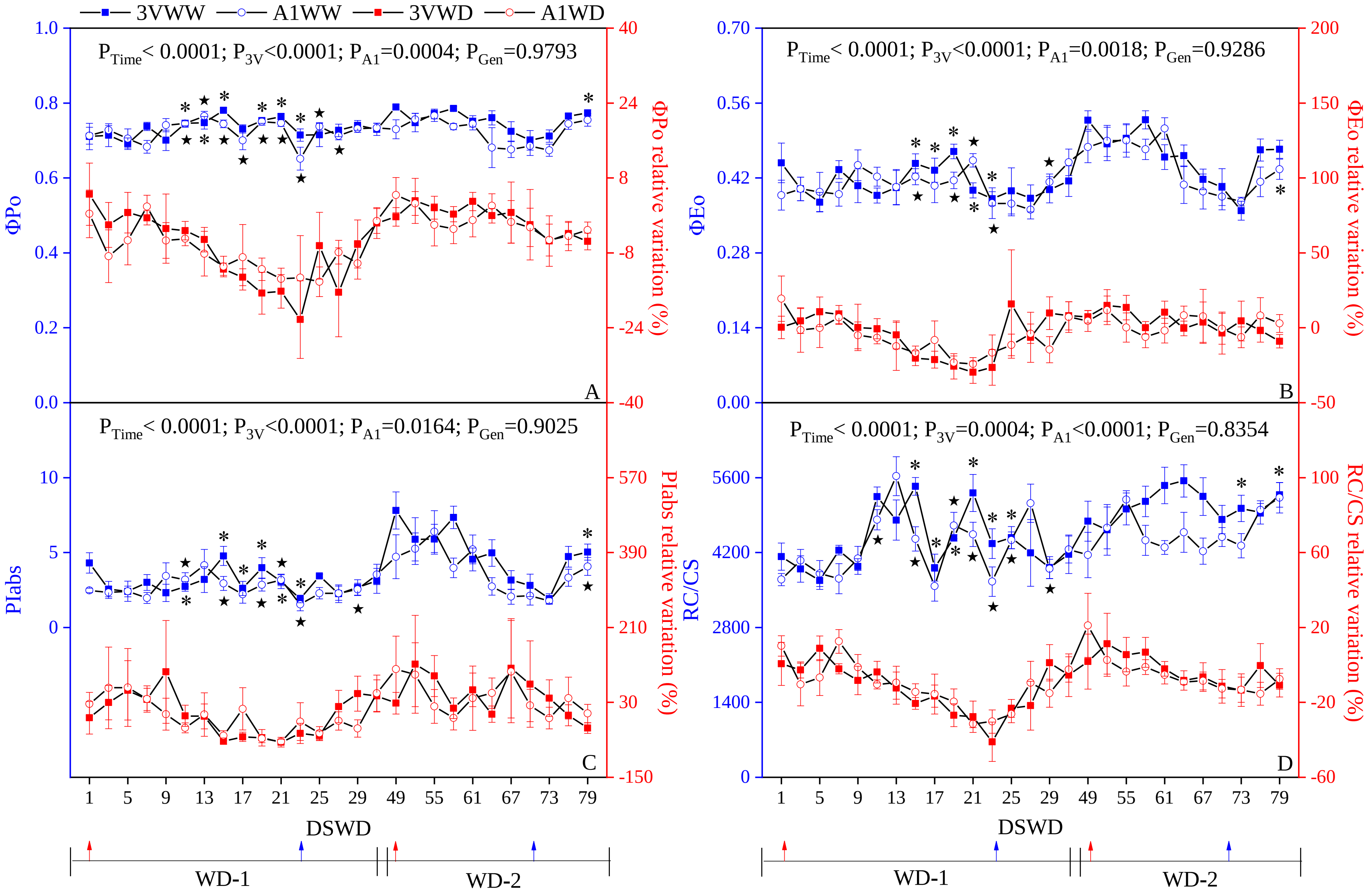
3VWW
A1WW
3VWD
A1WD
P_{Time}< 0.0001; P_{3V}<0.0001; P_{A1}=0.0004; P_{Gen}=0.9793
ΦPo
ΦPo relative variation (%)
A
P_{Time}< 0.0001; P_{3V}<0.0001; P_{A1}=0.0018; P_{Gen}=0.9286
ΦEo
ΦEo relative variation (%)
B
P_{Time}< 0.0001; P_{3V}<0.0001; P_{A1}=0.0164; P_{Gen}=0.9025
PIabs
PIabs relative variation (%)
C
P_{Time}< 0.0001; P_{3V}=0.0004; P_{A1}<0.0001; P_{Gen}=0.8354
RC/CS
RC/CS relative variation (%)
D
1.0
0.8
0.6
0.4
0.2
0.0
40
24
8
-8
-24
-40
0.70
0.56
0.42
0.28
0.14
0.00
200
150
100
50
0
-50
10
5
0
570
390
210
30
-150
5600
4200
2800
1400
0
100
60
20
-20
-60
1
5
9
13
17
21
25
29
49
55
61
67
73
79
DSWD
WD-1
WD-2

Figure 5. Medium values ± s.e. and ANOVA *P*-values for leaf chlorophyll *a* fluorescence parameters (left y-axis, blue) and relative difference between WW (well-watered soil) and WD (soil water deficit) (right y-axis, red) in conilon coffee clones (‘3V’ and ‘A1’). (A) Maximum quantum yield of primary photochemical reactions (ΦPo), (B) quantum efficiency of electron transfer from Q_{A-} to electron transport chain beyond (ΦEo), (C) photosynthetic performance index (PI_{ABS}), and (D) density of reaction centers capable of QA reduction (RC/CS). The medium values followed by “✻”, “*” and “⋆” express significant differences between clones under soil water deficit, between soil water condition in ‘3V’ and soil water condition in ‘A1’, respectively, at $p \leq 0.05$ using the Tukey test. P_{Time}= express significance level in the DSWD; P_{3V}= express significance level for interaction between 3VWW:3VWD over the DSWD; P_{A1}= express significance level for interaction between A1WW:A1WD over the DSWD; P_{Gen}= express significance level for interaction between 3VWD:A1WD over the DSWD. Red arrow: beginning of soil water deficit; Blue arrow: rewatering.

The ETR absolute values decreased over the time under WD in both clones during the WD-1 and WD-2 (Figure 6D). The maximum decrease of ETR was found at DSWD 19, 21 and 67 in 3VWD and at DSWD 21 and 70 in A1WD. Both clones showed relative reduction of ETR under water withdrawn during the two WD events. However, ETR relative reduction was higher in the A1WD than in the 3VWD from 7th to 13th DSWD and at DSWD 21 and 23 during the WD-1, whilst no difference between the clones was found during the WD-2.

The ETR recovery started immediately after rewatering in both clones during the two WD events (Figure 6D). The complete recovery of ETR in 3VWD was achieved at 4th day after rewatering during the WD-1 and at 6th after rewatering during the WD-2, corresponding to DSWD 27 and 76, respectively. The complete recovery in A1WD was achieved at 8th (DSWD 29) and 6th (DSWD 76) day after rewatering in the WD-1 and WD-2, respectively.

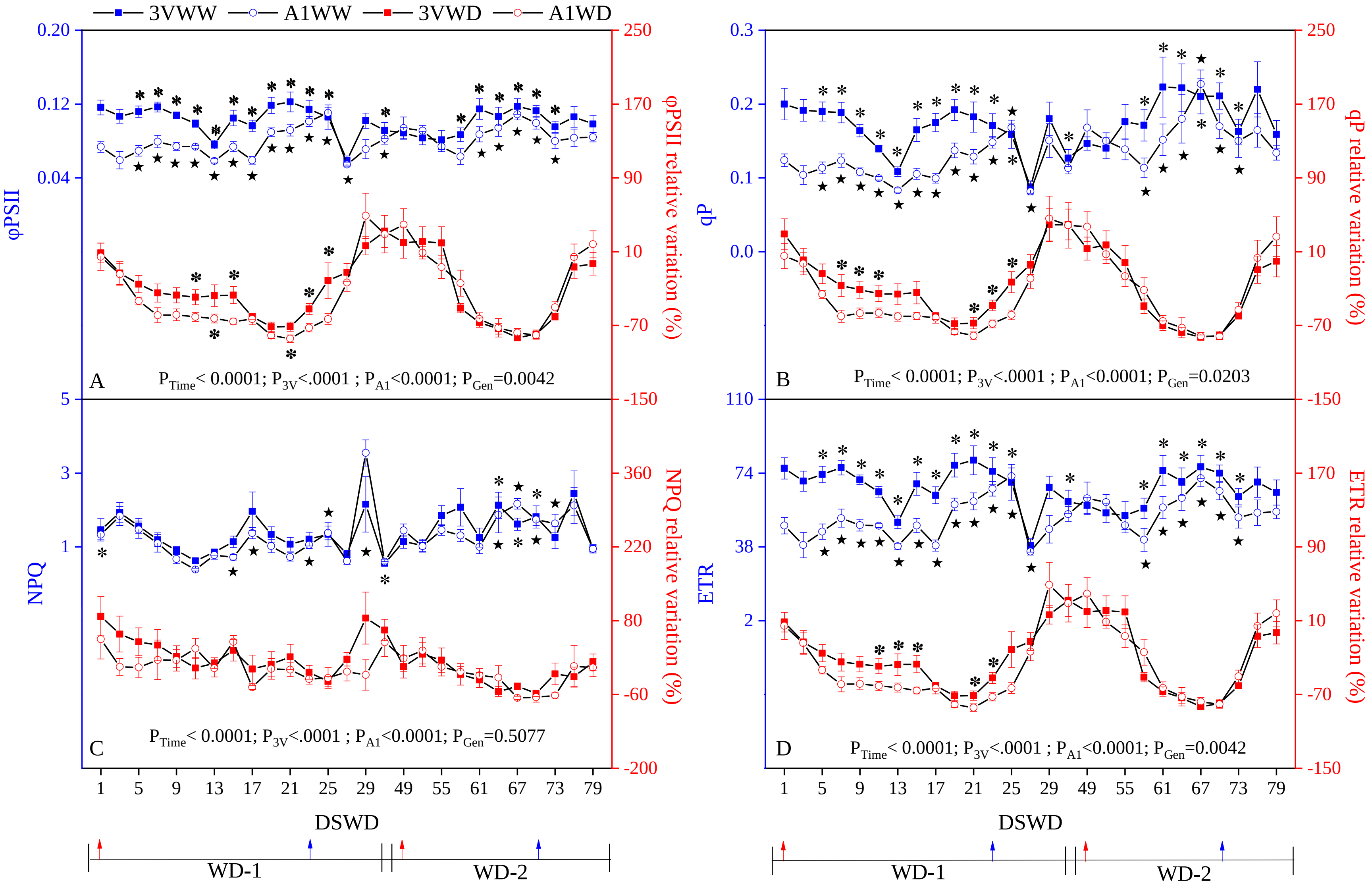
3VWW
A1WW
3VWD
A1WD
A
φPSII
φPSII relative variation (%)
0.20
0.12
0.04
250
170
90
10
-70
-150
PTime< 0.0001; P3V<.0001 ; PA1<0.0001; PGen=0.0042
B
qP
qP relative variation (%)
0.3
0.2
0.1
0.0
PTime< 0.0001; P3V<.0001 ; PA1<0.0001; PGen=0.0203
C
NPQ
NPQ relative variation (%)
5
3
1
360
220
80
-60
-200
PTime< 0.0001; P3V<.0001 ; PA1<0.0001; PGen=0.5077
D
ETR
ETR relative variation (%)
110
74
38
2
170
90
10
-70
-150
PTime< 0.0001; P3V<.0001 ; PA1<0.0001; PGen=0.0042
1
5
9
13
17
21
25
29
49
55
61
67
73
79
DSWD
WD-1
WD-2

Figure 6. Medium values ± s.e. and ANOVA *P*-values for leaf chlorophyll *a* fluorescence parameters (left y-axis, blue) and relative difrerence between WW (well-watered soil) and WD (soil water deficit) (right y-axis, red) in conilon coffee clones (‘3V’ and ‘A1’). (A) Effective quantum yield of PSII (Φ_{PSII}), (B) quenching photochemical (qP), (C) quenching non-photochemical (NPQ), and (D) electron transport rate (ETR). The medium values followed by “✻”, “*” and “⋆” express significant differences between clones under soil water deficit, between soil water condition in ‘3V’ and soil water condition in ‘A1’, respectively, at $p \leq 0.05$ using the Tukey test. P_{Time}= express significance level in the DSWD; P_{3V}= express significance level for interaction between 3VWW:3VWD over the DSWD; P_{A1}= express significance level for interaction between A1WW:A1WD over the DSWD; P_{Gen}= express significance level for interaction between 3VWD:A1WD over the DSWD. Red arrow: beginning of soil water deficit; Blue arrow: rewatering.

The ETR to $A_{net1500}$ linear regressions were similar in the two clones during the two WD events (Figures 7A and 7B). Decrease of ETR resulted in decrease of $A_{net1500}$. $A_{net1500}$ was more responsive to ETR during the WD-2 than during the WD-1, which was expressed through the higher slope coefficient of the linear regression during the WD-2 in both clones. ETR and $A_{net1500}$ were more affected by soil water deficit during the WD-1 (Figure 7A) than in during the WD-2 (Figure 7B) in both clones. High accumulation of the points was registered in the beginning of the linear regression during the WD-1 in both clones, whilst during the WD-2 the point distribution over the linear regression was more regular.

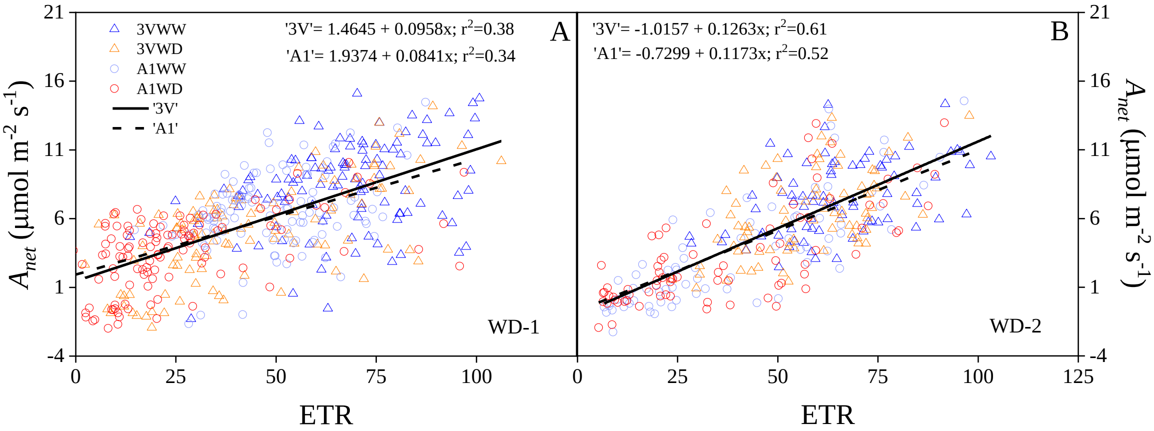

Figure 7. Linear regressions between electron transport rate (ETR) and net photosynthetic rate (A_{net} or $A_{net1500}$) in conilon coffee clones ('3V' and 'A1') under well-watered soil (WW) and in two soil water deficit events (WD-1 and WD-2).

The absolute values of leaf spectrometry indices (ARI, CRI, SIPI, and PSRI) in WW plants kept a stability over the time, but with a certain fluctuation over time of observation (Figure 8). No effect of water withdrawn was observed on ARI, CRI, SIPI and PSRI in both clones during the two WD events.

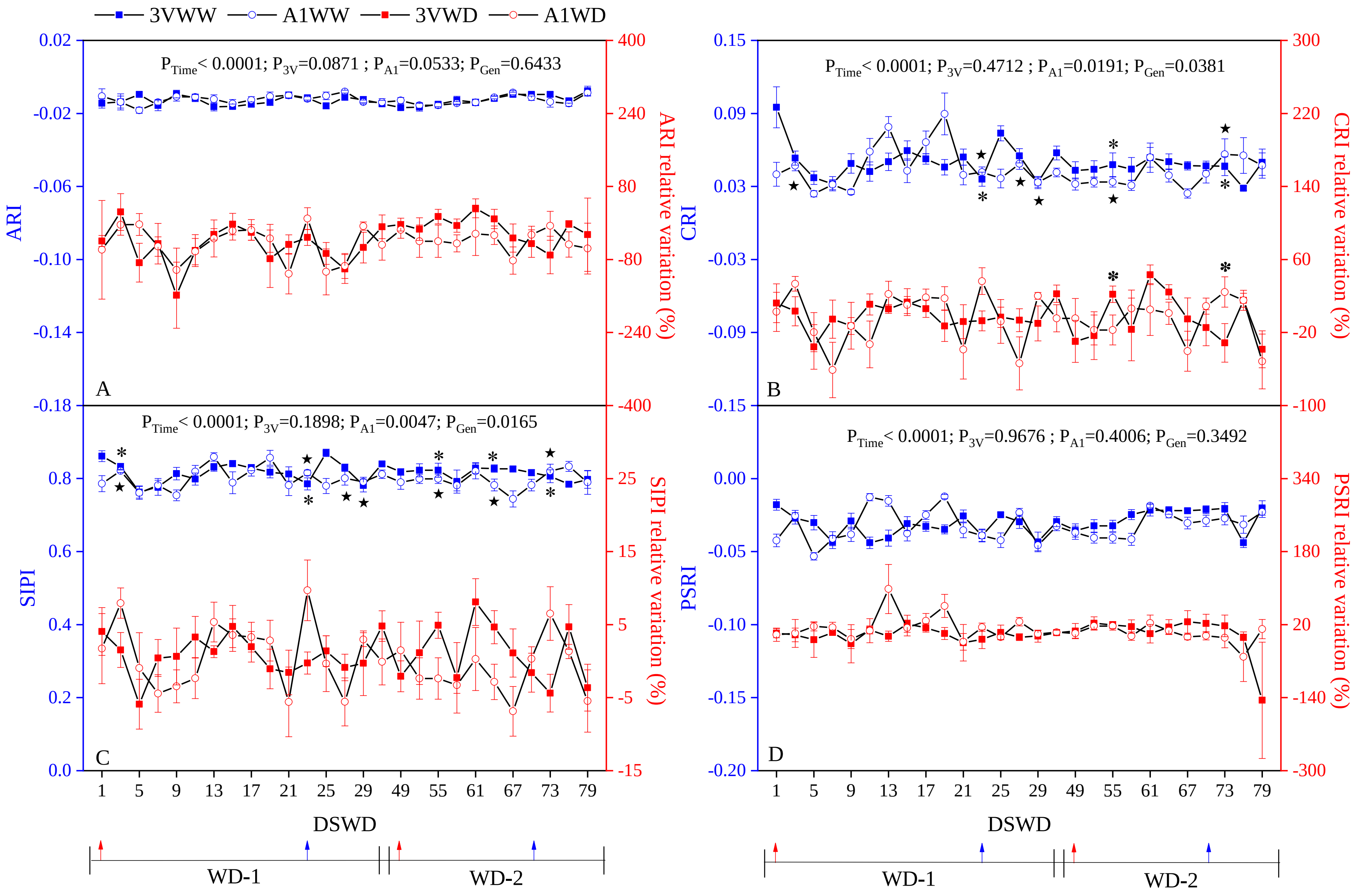
3VWW
A1WW
3VWD
A1WD
P_{Time}< 0.0001; P_{3V}=0.0871 ; P_{A1}=0.0533; P_{Gen}=0.6433
ARI
ARI relative variation (%)
A
P_{Time}< 0.0001; P_{3V}=0.4712 ; P_{A1}=0.0191; P_{Gen}=0.0381
CRI
CRI relative variation (%)
B
P_{Time}< 0.0001; P_{3V}=0.1898; P_{A1}=0.0047; P_{Gen}=0.0165
SIPI
SIPI relative variation (%)
C
P_{Time}< 0.0001; P_{3V}=0.9676 ; P_{A1}=0.4006; P_{Gen}=0.3492
PSRI
PSRI relative variation (%)
D
0.02
-0.02
-0.06
-0.10
-0.14
-0.18
400
240
80
-80
-240
-400
0.15
0.09
0.03
-0.03
-0.09
-0.15
300
220
140
60
-20
-100
0.0
0.2
0.4
0.6
0.8
-15
-5
5
15
25
-0.20
-0.15
-0.10
-0.05
0.00
-300
-140
20
180
340
1
5
9
13
17
21
25
29
49
55
61
67
73
79
DSWD
WD-1
WD-2

Figure 8. Medium values ± s.e. and ANOVA *P*-values for leaf spectral indices (left y-axis, blue) and relative difference between WW (well-watered soil) and WD (soil water deficit) (right y-axis, red) in conilon coffee clones (3V and A1). (A) anthocyanin reflectance index (ARI), (B) carotenoid reflectance index (CRI), (C) structure intensive pigment index (SIPI), and (D) plant senescence reflectance index (PSRI). The medium values followed by "✻", "*" and "⋆" express significant differences between clones under soil water deficit, between soil water condition in '3V' and soil water condition in 'A1', respectively, at $p \leq 0.05$ using the Tukey test. P_{Time}= express significance level in the DSWD; P_{3V}= express significance level for interaction between 3VWW:3VWD over the DSWD; P_{A1}= express significance level for interaction between A1WW:A1WD over the DSWD; P_{Gen}= express significance level for interaction between 3VWD:A1WD over the DSWD. Red arrow: beginning of soil water deficit; Blue arrow: rewatering.

Leaf hydraulic traits

No difference was found in relative water content at turgor loss point (RWC_{TLP}), modulus of cell elasticity (Ɛ), or absolute leaf capacitance at full turgor (*C*) between the 3VWD and A1WD (Table 2). RWC_{TLP} and Ɛ increased 5% and 40% in both clones under WD, respectively, whilst *C* decreased 30% (Table 2). Saturated water content (SWC), osmotic potential (Ψ_o) and water potential at turgor loss point (Ψ_{TLP}) were not affected by the WD in the two clones.

Leaf, branch, and root anatomical traits

The stomatal density was significatively higher (10%), and the leaf thickness was thinner (6%) in ‘3V’ than in ‘A1’, independently of the water conditions (Table 3). ‘A1’ showed a higher increase (5%) in the leaf thickness under WD than ‘3V’ (0.01%), despite no difference between 3VWD and A1WD was found. Both clones increased thickness of spongy parenchyma under WD, but the ‘A1’ kept a spongy parenchyma thicker than the ‘3V’, independently of the water condition. On the other hand, thickness of palisade parenchyma decreased under WD, manly in ‘3V’. This might explain the reason in ’3V’ had not increased the leaf thickness under WD.

The density of leaf xylem vessels was not affected by WD in the two clones, whilst medium area of leaf xylem vessel was reduced under WD, mainly in ‘3V’ (Table 3). Despite no difference in the medium area of leaf xylem vessel between the two clones under WD, the reduction in 3VWD (19%) was largely higher than in A1WD (2%) (Figure 9). The density of branch xylem vessels was similar in 3VWW and A1WW, whilst in 3VWD was lower than in A1WD.

No difference of branch xylem density vessels was found between A1WW and A1WD (Table 3). On the other hand, density of branch xylem vessels was reduced 14% in 3VWD when compared to 3VWW. Medium area of branch xylem vessel and density of root xylem vessels were similar in ‘3V’ and ‘A1’, independently of water condition. However, the medium area of root xylem vessel was higher in 3VWW than in A1WW (Figures 10A and 10B). The medium area of root xylem vessel decreased in 3VWD (58%) and in A1WD (7%), without differing descreases in two clones under WD conditions (Figure 10).

Table 2. Pressure volume curve variables: saturated water content (SWC), osmotic potential (Ψo), water potential at turgor loss point (Ψ_{TLP}), relative water content at turgor loss point (RWC_{TLP}), modulus of cell elasticity (ε), and leaf absolute capacitance at full turgor (*C*), measured in conilon coffee clones (‘3V’ and ‘A1’) under well-watered soil (WW), or soil water deficit (WD) conditions. These variables were obtained after the end of WD-2. The medium values ± SE (n = 5) were compared using the Tukey test at $p \leq 0.05$. $p_{value} \leq 0.05$ was highlighted in bold. * the percentage of increase or decrease of the pressure volume curve variables for each clone under WD in relation to WW.

Variables	**WW**		**WD**		**P_{value} <0.05**		
	‘3V’	**‘A1’**	**‘3V’**	**‘A1’**	**Clone**	**Water condition**	**Interation**
SWC	1.49 ± 0.14 (100%)	1.73 ± 0.07 (100%)	1.92 ± 0.25 (29%)*	1.66 ± 0.12 (-4%)*	0.407	0.292	0.134
Ψo (MPa)	-1.68 ± 0.07 (100%)	-1.71 ± 0.07 (100%)	-1.73 ± 0.05 (3%)	-1.58 ± 0.06 (-8%)	0.273	0.56	0.161
Ψ_{TLP} (MPa)	-1.96 ± 0.10 (100%)	-1.92 ± 0.07 (100%)	-1.92 ± 0.07 (2%)	-1.87 ± 0.14 (-3%)	0.664	0.669	0.956
RWC_{TLP} (MPa)	87.82 ± 1.26 (100%)	88.09 ± 3.06 (100%)	91.26 ± 0.22 (4%)	93.04 ± 0.50 (6%)	0.526	**0.003**	0.639
ε (MPa)	14.69 ± 1.60 (100%)	16.72 ± 2.13 (100%)	21.35 ± 0.84 (45%)	23.05 ± 2.03 (38%)	0.279	**0.001**	0.923
C (mol m^{-2} MPa^{-1})	0.524 ± 0.033 (100%)	0.628 ± 0.128 (100%)	0.422 ± 0.019 (-20%)	0.381 ± 0.032 (-39%)	0.6414	**0.004**	0.287

Table 3. Leaf, branch, and root anatomical variables, measured in conilon coffee clones (‘3V’ and ‘A1’) under well-watered soil (WW) and soil water deficit (WD) conditions. The medium values ± SE (n = 5) followed by different letters express significant differences between clones in each water condition (a, b) and between water condition for each clone (A, B) at $p \leq 0.05$ using the Tukey test. $p_{\text{value}} \leq 0.05$ was highlighted in bold. * represent the percentage of increase or decrease of the leaf, branch and root anatomical variables for each clone under WD in relation to WW.

Variables	**WW**		**WD**		***P* value (<0.05)**		
Leaf	**‘3V’**	**‘A1’**	**‘3V’**	**‘A1’**	**Clone**	**Water condition**	**Interation**
Stomatal density (mm^2)	268.51 ± 8.73 (100%) a	251.51 ± 6.93 (100%) b	278.03 ± 18.20 (3%) a	250.72 ± 7.53 (0%) b	**0.0370**	0.892	0.634
Thickness of leaf (µm^2)	221.71 ± 2.95 (100%) b	228.62 ± 3.56 (100%) a	221.74 ± 3.73 (0%) b	241.34 ± 2.60 (7%) a	**<0.0001**	**0.042**	0.059
Thickness of adaxial epidermal (µm^2)	21.07 ± 0.62 (100%)	20.87 ± 0.48 (100%)	20.39 ± 0.43 (-3%)	21.08 ± 0.62 (1%)	0.481	0.653	0.41
Thickness of abaxial epidermal (µm^2)	13.98 ± 0.40 (100%)	13.72 ± 0.52 (100%)	13.42 ± 0.31 (-4%)	13.90 ± 0.37 (1%)	0.602	0.493	0.372
Thickness of palisade parenchyma (µm^2)	54.01 ±1.58 (100%)	48.58 ± 0.85 (100%)	46.61 ± 1.38 (-14%)	46.19 ± 3.00 (-5%)	0.298	**0.002**	0.191
Thickness of spongy parenchyma (µm^2)	131.37 ± 3.38 (100%) b	147.30 ± 3.77 (100%) a	140.70 ± 3.45 (7%) b	158.65 ± 3.70 (8%) a	**<0.0001**	**0.007**	0.78
Density of xylem vessels (vessel µm^{-2})	1.7e^{-3} ± 7.0e^{-5} (100%)	1.9e^{-3} ± 6.0e^{-5} (100%)	1.9e^{-3} ± 1.1e^{-4} (12%)	1.8e^{-3} ± 8.0e^{-5} (-5%)	0.243	0.857	0.213
Medium area of xylem vessel (µm^2)	234.12 ± 9.50 (100%)	203.64 ± 11.31 (100%)	190.03 ± 11.03 (-19%)	200.00 ± 9.70 (-2%)	0.205	**0.037**	0.076
Branch							
Density of xylem vessels (vessel µm^{-2})	4.2e^{-4} ± 2.3e^{-5} (100%) aA	3.9e^{-4} ± 1.4e^{-5} (100%) aA	3.6e^{-4} ± 1.8e^{-5} (-14%) bB	4.4e^{-4} ± 2.4e^{-5} (13%) aA	**0.088**	0.879	**0.006**
Medium area of xylem vessels (µm^2)	782.05 ± 26.39 (100%)	845.30 ± 23.35 (100%)	791.01 ± 27.70 (1%)	767.35 ± 30.94 (-9%)	0.25	0.216	0.112
Root							
Density of xylem vessels (vessel µm^{-2})	2.0e^{-4} ±1.4e^{-4} (100%)	6.7e^{-5} ± 1.9e^{-6} (100%)	7.1e^{-5} ± 1.5e^{-6} (-64%)	6.8e^{-5} ± 1.6e^{-6} (1%)	0.11	0.735	0.358
Medium area of xylem vessels (µm^2)	2696.01 ± 573.68 (100%) aA	1197.26 ± 21.43 (100%) bA	1121.35 ± 21.44 (-58%) aB	1109.48 ± 18.25 (-7%) aB	0.364	**0.001**	**0.01**

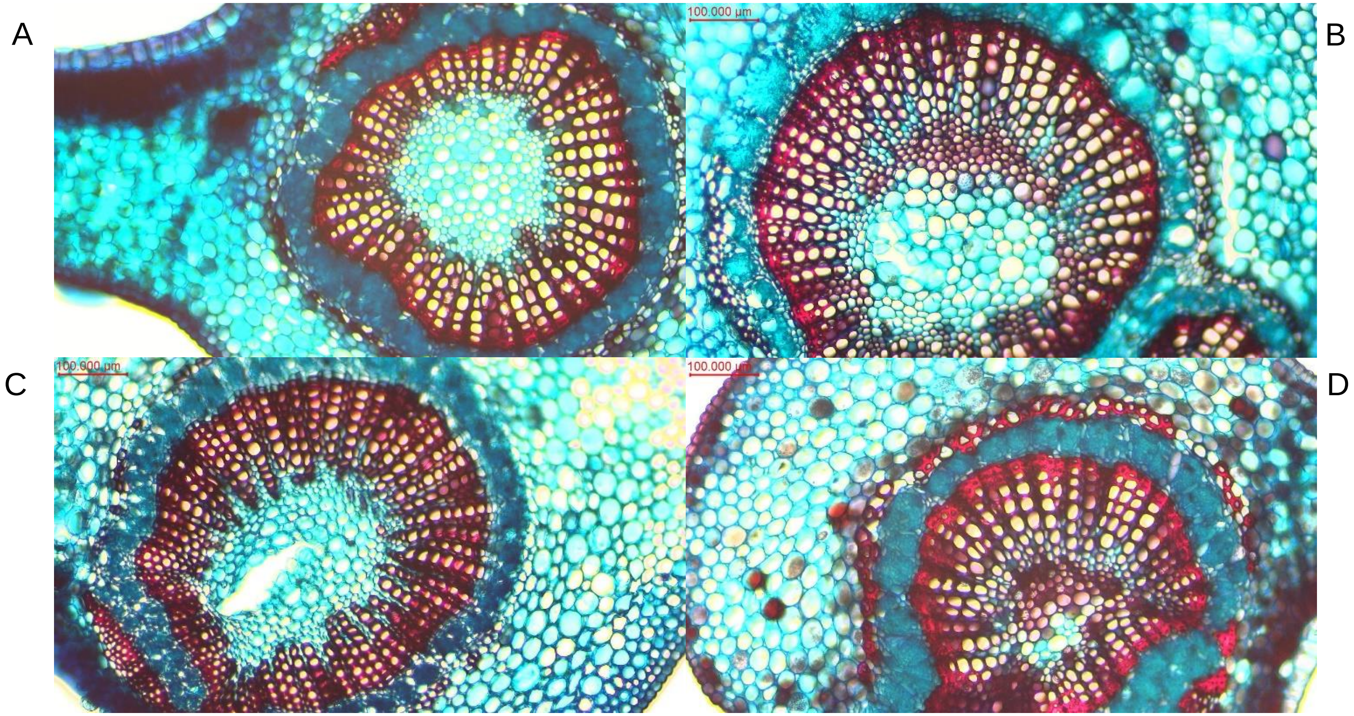

Figure 9. Representative medium area of leaf xylem vessel (µm^2) measured in conilon coffee clones (‘3V’ and ‘A1’) under well-watered soil (WW) and soil water deficit (WD) conditions. (A) A1WW, (B) 3VWW, (C) A1WD, and (D) 3VWD.

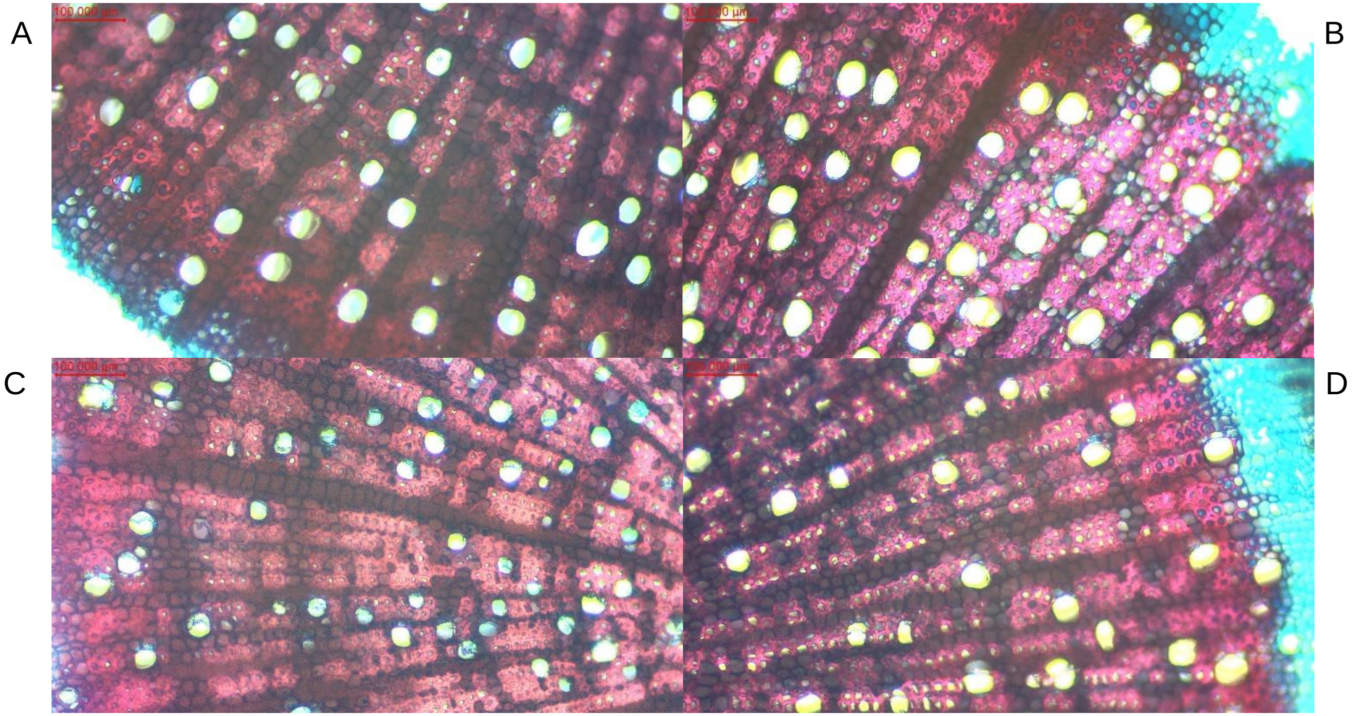

Figure 10. Representative medium area of root xylem vessel (µm^2) measured in conilon coffee clones ('3V' and 'A1') under well-watered soil (WW) and soil water deficit (WD) conditions. (A) A1WW, (B) 3VWW, (C) A1WD, and (D) 3VWD.

Root and shoot morphological traits

The final root and shoot morphological traits [final plant height (FPH), final trunk diameter (FTD), final leaf number (FLN), final leaf area (FLA), final leaf dry mass (FLDM), final plagiotropic branch dry mass (FPDM), final orthotropic branch dry mass (FODM), final shoot dry mass (FSDM), final root dry mass (FRDM), and final total dry mass (FTDM)] in both clones were affected by water withdrawn (Table 4).

The FLN, FPDM, FODM, and FRDM were higher in ’A1’ than in ‘3V’, independently of the water condition (Table 4). The FLDM and FSDM were lower in 3VWW than in A1WW, whilst no difference was found between 3VWD and A1WD (Table 4). On the other hand, FLDM decreased in both clones under WD, 36% in 3VWD and 51% in A1WD. The FSDM also decreased in 3VWD (41%) and in A1WD (52%). The FTDM kept the similar trend as FLDM and FSDM. The FTDM was lower in 3VWW when compared to A1WW, whilst no difference was found between 3VWD and A1WD. On the other hand, FTDM decreased in both clones under WD, with a reduction of 45% in 3VWD and 57% in A1WD.

The increase in the plant height (PH), trunk diameter (TD) and leaf number (LN) over the time was stagnated immediately after the beginning of the water withdrawn, for both clones in the two WD events (Figure 11). The restartions in growth of the PL, TD, and LN was reestablished 10 days after rewatering in both clones during the WD-1. During the WD-2, until the last measurement (12 days after rewatering) of the PL, TD, and LN both clones did not had restarted the growth. The drop leaves number (DLN) increased over the time, without difference between clones and water condition.

Table 4. Root and shoot morphological variables, final plant height (FPH), final trunk diameter (FTD), final leaf number (FLN), final leaf area (FLA), final leaf dry mass (FLDM), final plagiotropic branch dry mass (FPBDM), final ortotropic branch dry mass (FOBDM), final shoot dry mass (FSDM), final root dry mass (FRDM), and final total dry mass (FTDM), measured in conilon coffee clones (3V and A1) under well-watered soil (WW) and soil water deficit (WD) conditions. The medium values ± SE (n = 5) followed by different letters express significant differences between clones in each water condition (a, b) and between water condition for each clone (A, B) at $p \leq 0.05$ using the Tukey test. $p_{value} \leq 0.05$ was highlighted in bold. * percentage of increase or decrease of the root and shoot morphological variables for each clone under WD in relation to WW.

Variables	**WW**		**WD**		***P* value (<0.05)**		
	‘3V’	**‘A1’**	**‘3V’**	**‘A1’**	**Clone**	**Water condition**	**Interation**
Plant height (cm)	52.56 ± 1.27 (100%)	52.66 ± 4.40 (100%)	40.51 ± 3.44 (-23%)	49.11 ± 1.46 (-7%)	0.382	**0.007**	0.167
Trunk diameter (cm)	1.560 ± 0.048 (100%)	1.54 ± 0.029 (100%)	1.14 ± 0.048 (-27%)	1.16 ± 0.22 (-25%)	0.985	**<0.0001**	0.554
Leaf total number	136 ± 10.20 (100%)	198.14 ± 7.98 (100%)	79.86 ± 6.28 (-41%)	130.28 ± 6.81 (-34%)	**<0.0001**	**<0.0001**	0.471
Leaf area (cm^2)	62.73 ± 3.99 (100%)	73.42 ± 2.19 (100%)	37.50 ± 4.06 (-40%)	44.02 ± 2.07 (-40%)	0.11	**<0.0001**	0.508
Leaf dry mass (g)	51.53 ± 3.88 (100%) bA	72.57 ± 4.09 (100%) aA	32.94 ± 5.60 (-36%) aB	35.39 ± 1.90 (-51%) aB	**0.002**	**<0.0001**	**0.033**
Plagiotropic branch dry mass (g)	20.85 ± 2.20 (100%)	24.81 ± 1.51 (100%)	11.22 ± 1.94 (-46%)	11.50 ± 0.50 (-54%)	**0.013**	**<0.0001**	0.265
Ortotropic branch dry mass (g)	33.20 ± 1.67 (100%)	39.39 ± 1.77 (100%)	17.96 ± 2.48 (-46%)	18.25 ± 0.80 (-54%)	**<0.0001**	**<0.0001**	0.104
Shoot dry mass (g)	105.58 ± 6.68 (100%) bA	136.78 ± 6.60 (100%) aA	62.12 ± 9.76 (-41%) aB	65.14 ± 2.73 (-52%) aB	**0.0003**	**<0.0001**	**0.05**
Root dry mass (g)	58.94 ± 6.62 (100%)	71.78 ± 7.26 (100%)	28.96 ± 3.98 (-51%)	24.32 ± 0.93 (-66%)	**0.002**	**<0.0001**	0.114
Total dry mass (g)	164.53 ± 12.82 (100%) bA	208.57 ± 11.56 (100%) aA	91.08 ± 11.85 (-45%) aB	89.46 ± 3.49 (-57%) aB	**0.0005**	**<0.0001**	**0.039**

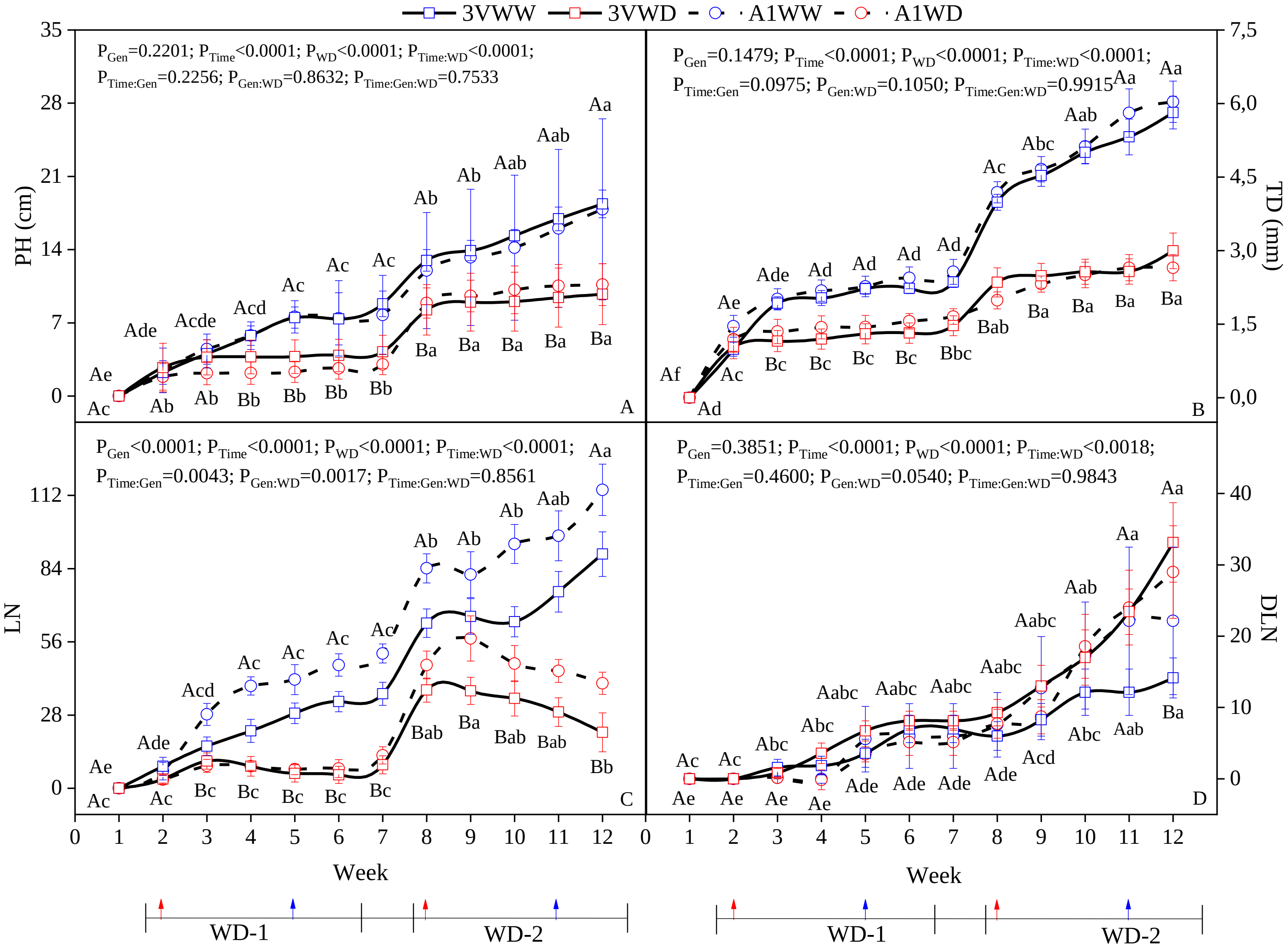
3VWW
3VWD
A1WW
A1WD
P_{Gen}=0.2201; P_{Time}<0.0001; P_{WD}<0.0001; $P_{Time:WD}$<0.0001;
$P_{Time:Gen}$=0.2256; $P_{Gen:WD}$=0.8632; $P_{Time:Gen:WD}$=0.7533
PH (cm)
35
28
21
14
7
0
Ae
Ade
Acde
Acd
Ac
Ac
Ac
Ab
Ab
Aab
Aab
Aa
Ac
Ab
Ab
Bb
Bb
Bb
Bb
Ba
Ba
Ba
Ba
Ba
A
P_{Gen}=0.1479; P_{Time}<0.0001; P_{WD}<0.0001; $P_{Time:WD}$<0.0001;
$P_{Time:Gen}$=0.0975; $P_{Gen:WD}$=0.1050; $P_{Time:Gen:WD}$=0.9915
TD (mm)
7,5
6,0
4,5
3,0
1,5
0,0
Af
Ae
Ade
Ad
Ad
Ad
Ad
Ac
Abc
Aab
Aa
Aa
Ad
Ac
Bc
Bc
Bc
Bc
Bbc
Bab
Ba
Ba
Ba
Ba
B
P_{Gen}<0.0001; P_{Time}<0.0001; P_{WD}<0.0001; $P_{Time:WD}$<0.0001;
$P_{Time:Gen}$=0.0043; $P_{Gen:WD}$=0.0017; $P_{Time:Gen:WD}$=0.8561
LN
112
84
56
28
0
Ae
Ade
Acd
Ac
Ac
Ac
Ac
Ab
Ab
Ab
Aab
Aa
Ac
Ac
Bc
Bc
Bc
Bc
Bc
Bab
Ba
Bab
Bab
Bb
C
P_{Gen}=0.3851; P_{Time}<0.0001; P_{WD}<0.0001; $P_{Time:WD}$<0.0018;
$P_{Time:Gen}$=0.4600; $P_{Gen:WD}$=0.0540; $P_{Time:Gen:WD}$=0.9843
DLN
40
30
20
10
0
Ac
Ac
Abc
Abc
Aabc
Aabc
Aabc
Aabc
Aabc
Aab
Aa
Aa
Ae
Ae
Ae
Ae
Ade
Ade
Ade
Ade
Acd
Abc
Aab
Ba
D
0 1 2 3 4 5 6 7 8 9 10 11 12
Week
0 1 2 3 4 5 6 7 8 9 10 11 12
Week
WD-1
WD-2
WD-1
WD-2

Figure 11. Medium values ± s.e. and ANOVA *P*-values for growth variables measured in conilon coffee clones (‘3V’ and ‘A1’) under well-watered soil (WW) and soil water deficit (WD) conditions. (A) Plant height (PH), (B) trunk diameter (TD), (C) leaf number (LN), and (D) droped leaves number (DLN). The medium values followed by different letters express significant difference between soil water condition in each DSWD (A, B), at $p \leq 0.05$ using the *t* test or between DSWD for each soil water condition (a, b, c, d, e) at $p \leq 0.05$ using the Tukey test. P_{Time}= express significance level in the DSWD; P_{3V}= express significance level for interaction between 3VWW:3VWD over the DSWD; P_{A1}= express significance level for interaction between A1WW:A1WD over the DSWD; P_{Gen}= express significance level for interaction between 3VWD:A1WD over the DSWD. Red arrow: beginning of soil water deficit; Blue arrow: rewatering.

The main leaf vein elongation (MLVE) was higher in ‘3V’ than in ‘A1’ during the WD-1, independently of the water condition (Figure 12A). The MLVE was continuous in control plants over the time, whilst in 3VWD and in A1WD decreased from 4th DSWD, without the growth recovery after rewatering, due the senescence and drop of leaves.

During the WD-2, the control plants kept a continuous MLVE until 25 days after beginning of the elongation (Figure 12B). After this moment, there was a stabilization of the MLVE, meaning that leaves achieved their maximum length. The MLVE decreased from the 4th DSWD in 3VWD and A1WD, restarting the growth at the 4th day after rewatering (DSWD 74).

The absolute root dry mass distribution was affected by the water withdrawn for both clones over the whole soil profile (Figure 13A). A1WW had higher absolute root dry mass than 3VWW at the 0-0.25 m layer, similar absolute root dry mass at the 0.25-0.50 m layer, and higher absolute root dry mass at the 0.5-0.75 m layer. No difference was found between 3VWW and A1WW at the 0.75-1.0 m layer, as well as between 3VWD and A1WD from 0-0.25 m to 0.25-0.5 m. Interestingly, 3VWD showed a higher absolute root mass at deep layers (0.5-0.75 m and 0.75-1.0 m) when compared to A1WD.

The relative root dry mass distribution was not affected by the water condition. (Figure 13B) but was statistically different between the clones over the soil profile. ‘A1’ showed higher relative root dry mass than ‘3V’at the 0-0.25 m soil layer, and lower ate the 0.5-0.75 m soil layer. No difference was observed between the clones at the 0.25-0.50 m and 0.75-1.0 m soil layers.

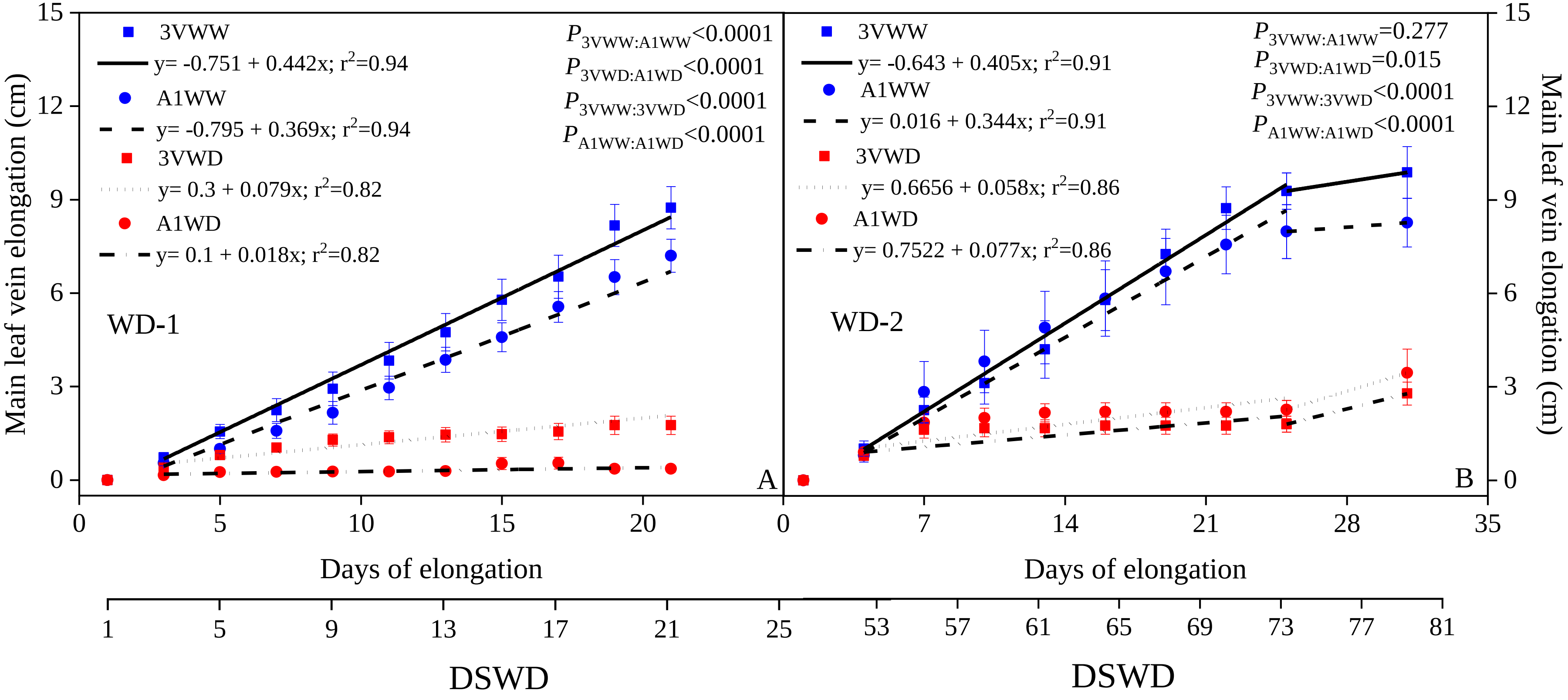

Figure 12. Linear regressions for main leaf vein elongation in conilon coffee clones ('3V' and 'A1') under well-watered soil (WW) and soil water deficit (WD) conditions in two WD events, WD-1 (A) and WD-2 (B). $P_{3VWW:A1WW}$= express significance level for interaction between clones under WW conditions; $P_{3VWD:A1WD}$= express significance level for interaction between clones under WD conditions; $P_{3VWW:3VWD}$= express significance level for interaction between water condition in '3V'; $P_{A1WW:A1WD}$= express significance level for interaction between water condition in 'A1'. Red arrow: beginning of soil water deficit; Blue arrow: rewatering.

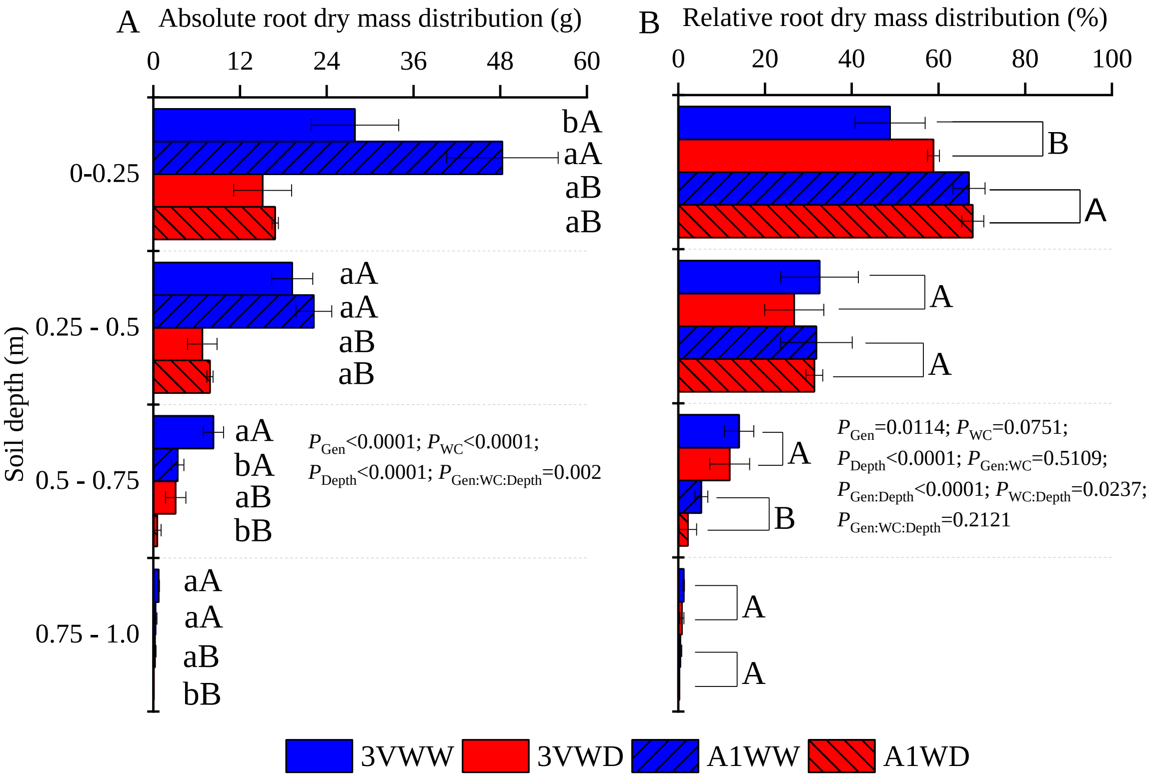

Figure 13. Medium values ± s.e. and ANOVA *P*-values for absolute (A) and relative (B) root dry mass distribution over the soil profile in conilon coffee clones (‘3V’ and ‘A1’) under well-watered soil (WW) and soil water deficit (WD) conditions. In the Figure 13A the medium values followed by different letters express significant differences between water condition at each clone and soil profile (A, B), or between clones at each water condition and soil profile (a, b) at $p \leq 0.05$ using the Tukey test. In the Figure 13B, the medium values followed by different letters express significant differences between the clones at each soil depth (A, B). P_{Gen}= express significance level for clone; P_{WC}= express significance level for water condition; P_{Depth}= express significance level for soil depth; $P_{Gen:WC}$= express significance level for interaction between clone and water condition; $P_{Gen:Depth}$= express significance level for interaction between clone and soil depth; $P_{WC:Depth}$= express significance level for interaction between water condition and soil depth; $P_{Gen:WC:Depth}$= express significance level for interaction between clone, water condition and soil depth.

DISCUSSION

Understanding morphological, physiological, and anatomical responses to water deficit, can provide the robust information related to drought tolerance and acclimation, as well as an important information for plant breeding programs.

Net CO_2 assimilation is fundamental for plant growth. The water deficit reduced leaf photosynthesis (Figure 3A), and reduced plant growth in both clones (Figure 11). Growth traits were affected initially due to reduced cell turgor and net CO_2 assimilation rate, because of stomatal closure in both WD events (Figure 3B), where the major effects were during the early phases of a drought event, as also is observed by Martins et al. (2019). After 15th and 55th DSWD the increase in C_i was observed in both clones (Figure 3D), being one of the consequence of photochemical damage, that was inevitable over the time under water withdrawn in both clones during the two subsequent WD events. However, in the WD-2, both clones showed a higher adjust of water consumption and hydraulic safety, through the hydraulic system acclimation, stomatal (Sperry, 2000; Choat et al., 2012; Schuldt et al., 2016) and anatomical regulations (Table 2, Figure 4B and Table 3).

An increase in the stomatal response to dehydration was observed in both clones, during the WD-2, resulting in a quick stomatal closing with the increase of water deficit. The plants increased stomatal control of water loss to avoid or delay too large a xylem pressure drop across, as a mechanism involved in protecting xylem from catastrophic hydraulic failure (Tyree and Sperry, 1988; Sperry et al., 1998; Whitehead, 1998; McDowell et al., 2008), that was linked to ε. A high ε can contributes to the less negative Ψ_{0TLP}, and this would enable a quickly stomatal close over turgor loss (Walter and Stadelmann, 1968; Read et al., 2006), maintaining a high RWC_{TLP} (Table 2).

Both clones also decreased the medium area of root and leaf xylem vessels and the density of branch xylem vessels (Table 3), as another mechanism of acclimation to avoid or delay the hydraulic failure, once xylem vessels with a large diameter are more susceptible to embolism than smaller vessels (Tyree and Sperry, 1989; Smith et al., 2013), which result in a water transport failure and dieback of trees under water deficit (Tyree and Sperry, 1988; Anderegg et al., 2012). That is, both clones increased the hydraulic safety, but decreased the efficiency to water transport, once the maximum hydraulic conductivity of a xylem vessel is proportional to the fourth power of the diameter of a xylem vessel (Tyree and Zimmermann, 2002).

Water deficit can change the leaf anatomical structures, such as leaf thickness and density, proportion of palisade tissue and spongy tissue, cell arrangement and ultrastructural characteristic (Ennajeh et al., 2010, Galmés et al., 2013; Zhang et al., 2015; Haffani et al., 2017), which was also observed in the present research (Table 3). A1WD increased the leaf thickness, with a notable trend to be higher than 3VWD, as well as the thickness of spongy parenchyma. Increase in the thickness of the spongy parenchyma could improve the diffusion of CO_2 through the inter-cellular spaces from the sub-stomatal cavity to the outer surface of the mesophyll cells (Ennajeh et al., 2010) and then improve the A_{net}. In *C. canephora* the A_{net} was similar in both clones during the two WD events, mainly in the days of higher water withdrawn (from 17th to 23rd DSWD and from 64th to 70th DSWD, Figure S3).

The water deficit causes a decrease in the carbon assimilation due the reduction of internal CO_2 concentration, because of either stomatal closure, or reduced internal CO_2 diffusion (Tang et al., 2002). The decrease of the parameters related to quantum yield of PSII (ΦPo and ΦEo), performance of photosynthetic apparatus (PI_{ABS}), and density of reaction centers that reduce Q_A (primary acceptor of PSII) (RC/CS) over the time of water withdrawn in both clones, during the WD-1, showed a gradual inactivation of the PSII, inducing photoinhibition. In such situation, reactive oxygen species can be produced, due the excessive reduction of plastoquinone Q_A, or due the charge recombination between acceptor and donor side of PSII (Aro et al., 1993).

The decline of ΦPo (determined in dark-adapted conditions) during the WD-1 was a good indicator of photoinhibition, however Φ_{PSII} is related to electron transport rate and on the nature of photoinhibition (Guidi et al., 2019). Thus, Φ_{PSII} reinforced the increase of photoinhibition for both clones over the time of water withdrawn in the two WD events, followed by decrease of the ETR (Figures 6A and 6D).

qP showed the similar trend as Φ_{PSII} and ETR in both clones during the two WD events, as expected (Figure 6B). However, there was a tendency of decrease of NPQ with water deficit increase, contrary than expected (Figure 6C). NPQ is related to the dissipation of excess light energy absorbed as heat (Guidi et al., 2019), once this process is related to xanthophyll cycle activity (Demmig-Adams et al., 2014).

Xanthophyll cycle protects plastids from over-excitation of the photosynthetic pigments and over-reduction of the electron transport chain that may led to the generation of reactive oxygen species (Guidi et al., 2019). In the xanthophyll cycle, ascorbate is a cofactor for violaxanthin de epoxidase (Muller-Moule et al., 2002; Chen et al., 2011), an enzyme that converts violaxanthin to zeaxanthin when exposed to light excess (Niu et al., 2013). Interestingly, ARI and CRI were unchanged for both clones in the two WD events, indicating the low protective activity of the carotenoids (Figures 8A and 8B). All ascorbate-deficient mutants reduce NPQ levels and show smaller xanthophyll cycle pool than the wild-type (Muller-Moule et al., 2004). Then, the unchanged of NPQ in both WD events indicated the low activity of xanthophyll cycle when the clones where subjects to water deficit, resulting in a damage of PSII reaction centers.

The ΦPo, ΦEo, PI_{ABS}, RC/CS, Φ_{PSII}, qP and ETR relative reduction increased over the time of water withdrawn for both clones, as consequence of photochemical damage. However, during the WD-2, both clones developed a hardening mechanism to previous water deficit (WD-1). ΦPo, ΦEo, PI_{ABS} and RC/CS were not impacted over the time of water withdrawn, as well as there was a lower increase in the Φ_{PSII}, qP and ETR relative reduction in the first nine DSWD (from 49th to 58th DSWD) during the WD-2 compared to WD-1. Plant water status and photosynthetic performance are directly/indirectly associated with the whole plant hydraulic conductivity through soil-root-shoot-leaf continuum (Reddy et al., 2018), showing that change in the photochemical response in the WD-2 was an acclimation response of the clones to the water conditions, influenced by hydraulic and anatomic adjustments. This was reinforced by $A_{net1500}$/ETR linear regression (Figure 7), which showed the high influence of photochemical phase on Calvin cycle functionality, due to NADPH and ATP suplly. During the WD-2, low values of $A_{net1500}$ were not always related to low ETR in plants subjected to water withdrawn, as observed during the WD-1. That is, the decrease of A_{net1500} in the WD-2 was more influenced by stomatal than non-stomatal factors.

Interestingly, 3VWD showed higher delay of Φ_{PSII}, qP and ETR relative reduction at the initial six DSWD (from 49th to 55th DSWD) during the WD-2 compared to A1WD (Figures 6A, 6B, and 6D). 3VWD also showed a high value of A_{net1500}, g_s and E (Figures 3A, 3B, and 3C) at the initial three DSWD (from 49th to 52nd DSWD) during the WD-2. These responses could be related to greater absolute distribution of the root system in the soil profile, intrinsic to '3V' (Figure 13A), ensuring a greater water absorption and hence a maintenance of photochemical apparatus vitality and suitable cell water status. The combination of stomatal control of CO_2 access to carboxylation sites and deep rooting, including carbon assimilation for biomass partitioning are traits that characterize drought-tolerant coffee plants (DaMatta et al., 2003; Pinheiro et al., 2005; Silva et al., 2013).

At the beginning of water withdrawn during the WD-2 (from 49th to 52nd DSWD), the deep root could uptake the water from deeper layers in 3VWD, maintaining the functionality of the plant. However, after this time, soil drying achieved deeper soil layers and consequently deeper parts of root system, resulting in g_s and E decreases (Figures 3B and 3C) and posteriorly in responses of photochemical efficiency parameters (Figure 6). That is, the deep root system in 3VWD was advantageous only in the beginning of the water deficit period, when deeper soil layers were still wet, going to agreement to our hypothesis.

A great root system may improve partially the competitive ability of plants to access water during the water deficit. Such plants demand elevated partition of photosynthetic products to the roots, by reducing partition to the reproductive (Ma et al., 2012) and to the shoot growth. This trait was clearly found in '3V', which showed a higher deep root growth than 'A1' under both soil water conditions (WW and WD), but lower growth of the shoot in the WW and same shoot growth in WD conditions, when compared to 'A1' (Table 4). On the other hand, 3VWD showed a lower decrease in the shoot growth than A1WD, when compared to respectively controls.

Both clones showed a quick and complete recovery of photosynthetic and photochemical efficiency parameters after rewatering, during the two WD events

(Figures 3, 5, and 6). The time to Φ_{PSII}, qP and ETR complete recovery was similar between the clones in the WD-2 (Figures 6A, 6B, and 6D). Interestingly, the time to $A_{net1500}$ and g_s complete recovery in A1WD was more than double from WD-1 to WD-2, whilst in 3VWD was similar (six days after rewatering) in the two WD events (Figures 5A and 5B). The recovery time of the water deficit is fundamental and can be considered as a major component of drought tolerance (Fang and Xiong, 2015). Despite of the difference in the recovery time, both clones showed a quick and complete recovery of photosynthetic and photochemical efficiency parameters after rewatering in the two WD events. This could be influenced by unchanged of the PSRI, since both clones supported the two WD events without leaf chlorophyll degradation (Figure 8D).

CONCLUSION

The repeated water deficit caused negative impact to plant growth in both clones, but the ‘3V’ showed lower decrease in the plant growth than ‘A1’. This can be related to the higher deep root system from ‘3V’, that resulted in a quicker $A_{net1500}$ and g_s recovery time after rewatering and hence a higher plant growth, evidencing its higher tolerance to water deficit. The root growth in deeper soil layers was more advantageous in the mild drought, which happend in the beginning of the water withdrawn, time wich there was disponible water in the deeper soil layers. The increase of the time under water withdrawn increased the dry in the deeper soil layer, reducing the water disponibility to the deep root. On the other hand, the deep root system allowed a quicker recovery of the plant after rehydration, which makes this trait crucial in the process of tolerant clones selection to water deficit.

Both clones showed the combined hydraulic (increase in ε and RWC_{TLP}), anatomic (decrease in thickness of palisade parenchyma, medium area of leaf and root xylem vessel and increase in thickness of spongy parenchyma), photosynthetic (increase in gs sensibility), and photochemical adjustments as acclimation responses to several water deficit repeted. This mechanisms were determinants as acclimation responses to several water deficit repeted for both clones.

REFERENCES

Anderegg, W. R., Berry, J. A., Field, C. B. (2012) Linking definitions, mechanisms, and modeling of drought-induced tree death. *Trends in Plant Science*,17 (12): 693-700.

Aro, E. M., Virgin, I., Andersson, B. (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1143: 113–134.

Avila, R. T., Cardoso, A. A., de Almeida, W. L., Costa, L. C., Machado, K. L., Barbosa, M. L., Souza, R. P. B., Oliveira, L. A., Batista, D. S., Martins, S. C.V., Ramalho, J. D.C., DaMatta, F. M. (2020) Coffee plants respond to drought and elevated [CO_2] through changes in stomatal function, plant hydraulic conductance, and aquaporin expression. *Environmental and Experimental Botany*, 177: 104148.

Avila, R. T., de Almeida, W. L., Costa, L. C., Machado, K. L., Barbosa, M. L., de Souza, R. P., Martino, P. B., Juárez, M. A. T., Marçal, D. M. S., Martins, S. C. V., Ramalho, J. C., DaMatta, F. M. (2020) Elevated air [CO_2] improves photosynthetic performance and alters biomass accumulation and partitioning in drought-stressed coffee plants. *Environmental and Experimental Botany*, 177: 104137.

Beyel, V., Bruggemann, W. (2005) Differential inhibition of photosynthesis during pre-flowering drought stress in sorghum bicolor genotypes with different senescence traits. *Physiologia Plantarum*, 124 (2): 249–259.

Bolhar-Nordenkampf, H. R., Long, S. P., Baker, N. R., Oquist, G., Schreiber, U., Lechner, E. G. (1989) Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. *Functional Ecology*, 3: 497-514.

Bolhar-Nordenkampf, H. R., Öquist, G. (1993) Chlorophyll fluorescence as a tool in photosynthesis research. In *Photosynthesis and production in a changing environment* (pp. 193-206). Springer, Dordrecht.

Bruce, T. J. A., Matthes, M. C., Napier, J. A., Pickett, J. A. (2007) Stressful 'memories' of plants: evidence and possible mechanisms. *Plant Science*, 173: 603–608.

Chen, D., Wang, S., Cao, B., Cao, D., Leng, G., Li, H., Yin, L., Shan, L., Deng, X. (2016) Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Frontiers in Plant Science*, 6: 1241.

Chen, X. Y., Li, W., Lu, Q. T., Wen, X. G., Li, H. W., Kuang, T. Y., Li, Z. S., Lu, C. M. (2011) The xanthophyll cycle and antioxidative defense system are enhanced in the wheat hybrid subjected to high light stress. *Journal Plant Physiology,* 167:1828–1836.

Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S. J., Field, T. S., Gleason, S. M., Hacke, U. G., Jacobsen, A. L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P. J., Nardini, A., Pittermann, J., Pratt, R. B., Sperry, J. S., Westoby, M., Wright, I. J., Zanne, A. E. (2012). Global convergence in the vulnerability of forests to drought. *Nature,* 491:752–755.

DaMatta, F. M., Avila, R. T., Cardoso, A. A., Martins, S. C., Ramalho, J. C. (2018) Physiological and agronomic performance of the coffee crop in the context of climate change and global warming: A review. *Journal of Agricultural and Food Chemistry*, 66 (21): 5264-5274.

DaMatta, F. M., Chaves, A. R. M., Pinheiro, H. A., Ducatti, C., Loureiro, M. E. (2003) Drought tolerance of two field-grown clones of *Coffea canephora*. *Plant Science*, 164 (1): 111−117.

DaMatta, F. M., Grandis, A., Arenque, B. C., Buckeridge, M. S. (2010) Impacts of climate changes on crop physiology and food quality. *Food Research International*, 43 (7): 1814-1823.

Demmig-Adams, B., Koh, S. C., Cohu, C., Muller, O., Stewart, J., Adams, W., III (2014) Non-photochemical fluorescence quenching in contrasting plant species and environments. *In*: Demmig-Adams, B., Garab, G., Adams, W.

III, Govindjee. (eds.) *Non-photochemical quenching and energy dissipation in plants, algae and cyanobacteria*. Dordrecht: Springer, p. 531-552.

Dubberstein, D., Lidon, F. C., Rodrigues, A. P., Semedo, J. N., Marques, I., Rodrigues, W. P., Gouveia, D., Armengaud, J., Semedo, M. C., Martins, S., Simões-Costa, M. C., Moura, I., Pais, I. P., Scotti-Campos, P., Partelli, F., Campostrini, E., DaMatta, F. M., Ramalho, J. C. (2020) Resilient and sensitive key points of the photosynthetic machinery of *Coffea spp*. to the single and superimposed exposure to severe drought and heat stresses. *Frontiers in Plant Science*, 11: 1049.

Eissenstat, D. M. (1992) Costs and benefits of constructing roots of small diameter. *Journal of Plant Nutrition*, 15 (6-7): 763-782.

Ennajeh, M., Vadel, A. M., Cochard, H., Khemira, H. (2010) Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. *The Journal of Horticultural Science and Biotechnology*, 85 (4), 289-294.

Fang, Y., Xiong, L. (2015) General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and. Molecular Life Science*, 72: 673–689.

Fernandes, I., Marques, I., Paulo, O. S., Batista, D., Partelli, F. L., Lidon, F. C., DaMatta, F. M., Ramalho, J. C., Ribeiro-Barros, A. I. (2021) Understanding the impact of drought in Coffea genotypes: transcriptomic analysis supports a common high resilience to moderate water deficit but a genotype dependent sensitivity to severe water deficit. *Agronomy*, 11 (11): 2255.

Fleta-Soriano, E., Munné-Bosch, S. (2016) Stress memory and the inevitable effects of drought: a physiological perspective. *Frontiers in Plant Science*, 7: 143.

Galle, A., Florez-Sarasa, I., Aououad, H. E., Flexas, J. (2011) The Mediterranean evergreen *Quercus ilex* and the semi-deciduous *Cistus albidus* differ in their leaf gas exchange regulation and acclimation to repeated drought and re-watering cycles. *Journal of Experimental Botany,* 62: 5207–5216.

Galmés, J., Ochogavia, J. M., Gago, J., Roldan, E. J., Cifre, J., Conesa, M. A. (2013). Leaf responses to drought stress in Mediterranean accessions of *Solanum lycopersicum*: anatomical adaptations in relation to gas exchange parameter. *Plant Cell Environmental*, 36: 920-935.

Genty, B., Goulas, Y., Dimon, B., Peltier, G., Briantais, J. M., Moya, I. (1992) Modulation of efficiency of primary conversion in leaves, mechanisms involved at PS2. *Research in Photosynthesis*, 4: 603-610.

Guidi, L., Lo Piccolo, E., Landi, M. (2019) Chlorophyll fluorescence, photoinhibition and abiotic stress: does it make any difference the fact to be a C3 or C4 species? *Frontiers in Plant Science*, 10: 174.

Guóth, A., Tari, I., Gallé, A., Csiszár, J., Pécsváradi, A., Cseuz, L., Erdei, L. (2009) Comparison of the drought stress responses of tolerant and sensitive wheat cultivars during grain filling: changes in flag leaf photosynthetic activity, ABA levels, and grain yield. *Journal of Plant Growth Regulation,* 28:167–176.

Haffani, S., Mezni, M., Nasri, M. B., Chaibi, W. (2017) Comparative leaf water relations and anatomical responses of three vetch species (*Vicia narbonensis* L., V. *sativa* L. and V. *villosa* Roth.) to cope with water stress. *Crop Pasture Science*, 68 (7): 691-702.

Hajnajari, H., Akbari, H., Abdossi, V. (2019) Genesis of ultra-specialized histology with stable traits in mesophyll of drought tolerant apple cultivars. *Scientia Horticulturae*, 249: 168-176.

Hammer, G. L., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., Zinselmeier, C., Paszkiewicz, S., Cooper, M. (2009) Can changes in canopy and/or root system architecture explain historical maize yield trends in the US corn belt? *Crop Science*, 49 (1): 299-312.

Iwasaki, M., Paszkowski, J. (2014). Epigenetic memory in plants. *The EMBO Journal*, 33: 1–12.

Kalaji, H. M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I. A., Cetner, M. D., Lukasik, I., Goltsev, V., Ladle, R. J. (2016) Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum*, 38 (4): 1-11.

Kalaji, H. M., Schansker, G., Ladle, R. J., Goltsev, V., Bosa, K., Allakhverdiev, S. I., Brestic, M., Bussotti, F., Calatayud, A., Dabrowski, P., Elsheery, N. I., Ferroni, L., Guidi, L., Hogewoning, S. W., Jajoo, A., Misra, A.,Nebauer, S. G., Pancaldi, S., Penella, C., Poli, D., Pollastrini, M., Romanowska-Duda, Z. B., Rutkowska, B., Serôdio, J., Suresh, K., Szulc, W., Tambussi, E., Yanniccari, M., Zivcak, M. (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynthesis Research*, 122 (2): 121-158.

Kashiwagi, J., Krishnamurthy, L., Crouch, J. H., Serraj, R. (2006) Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. *Field Crops Research*, *95* (2-3): 171-181.

Krishnamurthy, L., Johansen, C., Sethi, S. C. (1999) Investigation of factors determining genotypic differences in seed yield of non-irrigated and irrigated chickpeas using a physiological model of yield determination. *Journal of Agronomy and Crop Science*, 183 (1): 9-17.

Li, F. M., Yang, S. J., Li, C. X., Xu, B. C., Zhang, X. C. (2013) Effects of root pruning on non-hydraulic root-sourced signal, drought tolerance and water use efficiency of winter wheat. *Journal of Integrative Agriculture*, 12 (6): 989-998.

Luo, L. J. (2010) Breeding for water-saving and drought-resistance rice (WDR) in China. *Journal of Experimental Botany*, 61: 3509–3517.

Machado Filho, J. A., Rodrigues, W. P., Baroni, D. F., Pireda, S., Campbell, G., de Souza, G. A. R., Verdin Filho, A. C., Arantes, S. D., Arantes, L. O., Cunha, M., Gambetta, G., A., Rakocevic, M., Ramalho, J. C., Campostrini, E. (2021) Linking root and stem hydraulic traits to leaf physiological parameters in *Coffea canephora* clones with contrasting drought tolerance. *Journal of Plant Physiology*, 258-259:153355.

Manavalan, L. P., Guttikonda, S. K., Tran, L. S., Nguyen, H. T. (2009) Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiol*ogy, 50: 1260–1276.

Manschadi, A. M., Christopher, J. T., Hammer, G. L., Devoil, P. (2010) Experimental and modelling studies of drought-adaptive root architectural traits in wheat (*Triticum aestivum* L.). *Plant Biosystems*, 144 (2): 458-462.

Marques, I., Gouveia, D., Gaillard, J. C., Martins, S., Semedo, M. C., Lidon, F. C., DaMatta, F. M., Ribeiro-Barros, A. I., Armengaud, J., Ramalho, J. C. (2022) Next-generation proteomics reveals a greater antioxidative response to drought in *Coffea arabica* than in *Coffea canephora*. *Agronomy*, 12 (1): 148.

Martins, S. C., Sanglard, M. L., Morais, L. E., Menezes-Silva, P. E., Mauri, R., Avila, R. T., Vital, C. E., Cardoso, A. A., DaMatta, F. M. (2019) How do coffee trees deal with severe natural droughts? An analysis of hydraulic, diffusive and biochemical components at the leaf level. *Trees*, 33 (6): 1679-1693.

McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D. G., Yepez, E. A. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist,* 178:719–739.

Menezes-Silva, P. E., Sanglard, L. M., Ávila, R. T., Morais, L. E., Martins, S. C., Nobres, P., Patreze, C. M., Ferreira, M. A., Araújo, W. L., Fernie, A. R., DaMatta, F. M. (2017) Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in coffee. *Journal of Experimental Botany*, 68 (15): 4309-4322.

Misra, A. N., Misra, M., Singh, R. (2012) Chlorophyll fluorescence in plant biology. *In:* Misra, A. N. (ed.) *Biophysics*. Croatia: Intech, p.171–192.

Muller-Moule, P., Conklin, .P. L., Niyogi, K. K. (2002) Ascorbate deficiency can limit violaxanthin de-epoxidase activity in vivo. *Plant Physiology,* 128: 970–977.

Muller-Moule, P., Golan, T., Niyogi, K. K. (2004) Ascorbate-deficient mutants of Arabidopsis grow in high light despite chronic photooxidative stress. *Plant Physiology*, 134: 1163–1172.

Narayanan, S., Mohan, A., Gill, K. S., Prasad, P. V. (2014) Variability of root traits in spring wheat germplasm. *PLoS One*, 9 (6): e100317.

Niinemets, U. (2010) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management,* 260: 1623–1639.

Niu, Y., Wang, Y., Li, P., Zhang, F., Liu, H., Zheng, G. (2013) Drought stress induces oxidative stress and the antioxidant defense system in ascorbate-deficient *vtc1* mutants of *Arabidopsis thaliana*. *Acta Physiologiae Plantarum*, 35 (4): 1189-1200.

Perrone, I., Pagliarani, C., Lovisolo, C., Chitarra, W., Roman, F., Schubert, A. (2012) Recovery from water stress affects grape leaf petiole transcriptome. *Planta*, 235: 1383–1396.

Pinheiro, H. A., DaMatta, F. M., Chaves, A. R., Loureiro, M. E., Ducatti, C. (2005) Drought tolerance is associated with rooting depth and stomatal control of water use in clones of *Coffea canephora*. *Annals of Botany*, 96 (1): 101-108.

Ramalho, J. C., Rodrigues, A. P., Lidon, F. C., Marques, L. M., Leitão, A. E., Fortunato, A. S., Pais, I. P., Silva, M. J., Scotti-Campos, P., Lopes, A., Reborredo, F. H., Ribeiro-Barros, A. I. (2018) Stress cross-response of the antioxidative system promoted by superimposed drought and cold conditions in *Coffea* spp. *PLoS one*, 13 (6): e0198694.

Ramalho, J. C., Rodrigues, A. P., Semedo, J. N., Pais, I. P., Martins, L. D., Simões-Costa, M. C., Leitão, A. E., Fortunato, A. S., Batista-Santos, P., Palos, I. M., Tomaz, M. A., Scotti-Campos, P., Lidon, F. C., DaMatta, F. M. (2013) Sustained photosynthetic performance of *Coffea* spp. under long-term enhanced [CO_2]. *PLoS One*, 8 (12): e82712.

Read, J., Sanson, G. D., de Garine-Wichatitsky, M., Jaffre, T. (2006). Sclerophylly in two contrasting tropical environments: low nutrients vs. low rainfall. *American Journal of Botany,* 93 (11): 1601–1614.

Reddy, K. S., Sekhar, K. M., Sreeharsha, R. V., Reddy, A. R. (2019) Hydraulic dynamics and photosynthetic performance facilitate rapid screening of field grown mulberry (*Morus* spp.) genotypes for drought tolerance. *Environmental and Experimental Botany*, 157: 320-330.

Reynolds, M., Dreccer, F., Trethowan, R. (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. *Journal of Experimental Botany*, 58 (2): 177-186.

Ripoll, J., Bertin, N., Bidel, L. P., Urban, L. (2016) A user’s view of the parameters derived from the induction curves of maximal chlorophyll a fluorescence: perspectives for analyzing stress. *Frontiers in Plant Science*, 7: 1679.

Sack, l., Pasquet-Kok, J. (2010) Leaf pressure-volume curve parameters: <https://sites.lifesci.ucla.edu/eeb-sacklab/protocols/>. Accessed in: Mar. 2020.

Scholander, P. F., Hammel, H. T., Bradstreet, E. D., Hemmingsen, E. A. (1965) SAP pressure in vascular plants. *Science*, 148: 339–346.

Schreiber, U., Hormann, H., Neubauer, C., Klughammer, C. (1995) Assessment of photosystem II photochemical quantum yield by chlorophyll fluorescence quenching analysis. *Functional Plant Biology*, 22 (2): 209-220.

Schuldt, B., Knutzen, F., Delzon, S., Jansen, S., Müller-Haubold, H., Burlett, R., Clough, Y., Leuschner, C. (2016) How adaptable is the hydraulic system of European beech in the face of climate change-related precipitation reduction? *New Phytologist,* 210: 443–458.

Semedo, J. N., Rodrigues, A. P., Lidon, F. C., Pais, I. P., Marques, I., Gouveia, D., Armengaud, J., Silva, M. J., Martins, S., Semedo, M. C., Dubberstein, D., Partelli, F. L., Reboredo, F. H., Scotti-Campos, P., Ribeiro-Barros, R. I., DaMatta, F. M., Ramalho, J. C. (2021) Intrinsic non-stomatal resilience to drought of the photosynthetic apparatus in *Coffea* spp. is strengthened by elevated air [CO_2]. *Tree Physiology*, 41 (5): 708-727.

Silva, P. E. M., Cavatte, P. C., Morais, L. E., Medina, E. F., DaMatta, F. M. (2013) The functional divergence of biomass partitioning, carbon gain and water use in *Coffea canephora* in response to the water supply: Implications for breeding aimed at improving drought tolerance. *Environmental and Experiental Botany*, 87: 49−57.

Smith, M. S., Fridley, J. D., Yin, J., Bauerle, T. L. (2013) Contrasting xylem vessel constraints on hydraulic conductivity between native and non-native woody understory species. *Frontiers in Plant Science*, 4: 1–12.

Sperry, J. S. (2000) Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology*, 104:13–23.

Sperry, J. S., Adler, F. R., Campbell, G. S., Comstock, J. P. (1998) Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant, Cell and Environment,* 21: 347–359.

Steele, K. A., Price, A. H., Witcombe, J. R., Shrestha, R., Singh, B. N., Gibbons, J. M., Virk, D. S. (2013) QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. *Theoretical and Applied Genetics*, 126 (1): 101-108.

Strasser, R. J., Srivastava, A., Tsimilli-Michael, M. (2004) Analysis of the chlorophyll a fluorescence transient. *In*: Papageorgiou, G. Govindjee (eds.) *Chlorophyll fluorescence: a signature of photosynthesis*. Dordrecht: Springer, p. 321– 362.

Strasser, R.J., Tsimilli-Michael, M. (2001) Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-test. *Chimie Nouvelle*, 75: 3321–3326.

Strasserf, R. J., Srivastava, A. (1995) Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochemistry and Photobiology*, 61 (1): 32-42.

Tardieu, F. (2013) Plant response to environmental conditions: assessing potential production, water demand, and negative effects of water deficit. *Frontiers in Physiology*, 4 (17): 1-11.

Terashima, I., Hanba, Y. T., Tholen, D., Niinemets, Ü. (2011) Leaf functional anatomy in relation to photosynthesis. *Plant Physiology*, 155 (1): 108-116.

Tesfaye, S. G., Ismail, M. R., Kausar, H., Marziah, M., Ramlan, M. F. (2013) Plant water relations, crop yield and quality in coffee ('*Coffea arabica*' L.) as influenced by partial root zone drying and deficit irrigation. *Australian Journal of Crop Science*, 7 (9): 1361-1368.

Tyree, M. T., Sperry, J. S. (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. *Plant Physiology*, 88 (3): 574-580.

Tyree, M. T., Sperry, J. S. (1989) Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Biology*, 40: 19–36.

Tyree, M. T., Zimmermann, M. H. (2002) *Xylem structure and the ascent of sap*. 2. ed. Berlin: Springer, 284p.

Van Kooten, O., Snel, J. F. (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research*, 25 (3): 147-150.

Venancio, L. P., Filgueiras, R., Mantovani, E. C., do Amaral, C. H., da Cunha, F. F., dos Santos Silva, F. C., Althoff, D., dos Santos, R. A., Cavatte, P. C. (2020) Impact of drought associated with high temperatures on *Coffea canephora* plantations: a case study in Espírito Santo State, Brazil. *Scientific Reports*, 10 (1): 1-21.

Walter, H., Stadelmann, E. J. (1968) The physiological prerequisites for the transition of autotrophic plants from water to terrestrial life. *Bioscience*, 18: 694–701.

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S. C., Prasad, S. S., Rebetzke, G. J., Kirkegaard, J. A., Christopher, J., Watt, M. (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany*, 63 (9): 3485-3498.

Whitehead, D. (1998) Regulation of stomatal conductance and transpiration in forest canopies. *Tree Physiology*, 18: 633–644.

Xu, Z. Z., Zhou, G. S., Wang, Y. L., Han, G. X., Li, Y. J. (2008) Changes in chlorophyll fluorescence in maize plants with imposed rapid dehydration at different leaf ages. *Journal of Plant Growth Regulation*, 27: 83–92.

Yin, Z., Meng, F., Song, H., He, X., Xu, X., Yu, D. (2010) Mapping quantitative trait loci associated with chlorophyll a fluorescence parameters in soybean (*Glycine max* (L.) Merr.). *Planta*, 231 (4): 875-885.

Yue, B., Xue, W., Xiong, L., Yu, X., Luo, L., Cui, K., Jin, D., Xing, Y., Zhang, Q. (2006) Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. *Genetics,* 172 (2): 1213–1228.

Zhang, F.J., Zhang, K.K., Du, C.Z., Li, J., Xing, Y.X., Yang, L.T., Li, Y.R. (2015) Effect of drought stress on anatomical structure and chloroplast ultrastructure in leaves of sugarcane. *Sugar Tech*, 17: 41-48.

5. CONCLUDING REMARKS

The high DPV_{air} and PPFD, conditions that currently occur in the main producing regions of the crop, significantly reduce the CO_2 assimilation by the plants and, therefore, reduce the maximum performance of the crop. However, maintaining photosynthesis at higher values at times of high DPV_{air} and PPFD was not an indicative of greater plant growth, due to higher costs and/or greater inefficiency of the phochemical damage repair mechanisms. It is understood that the clones presented different ways to deal with the conditions of the environment, but that they provided the same final growth. It was also possible to understand that the development of a deeper root system is advantageous even in conditions of great water availability, but that leaf aspects are more determined in a context of high DPV_{air} and PPFD under great soil water availability. Under conditions of severe water deficit, the clones showed several mechanisms of acclimation to water deficit, from deep growth of the root system to hydraulic adjustments (increase in $\mathcal{E}$ and RWCTLP), anatomical (decrease in thickness of palisade parenchyma, medium area of leaf and root xylem vessel and increase in thickness of spongy parenchyma), photosynthetic (increase in g_s sensitivity) and photochemical combined. However, appears that the deep root system loses its efficiency in conditions of severe water deficit, since under these conditions the deeper layers of the soil dry out, and therefore the water deficit affects these roots. On the other hand, the root system in depth allowed a quicker recovery of the plant after rehydration, which makes this trait crucial in the process of selecting clones tolerant to water deficit.

REFERENCES

Abrams, M. D., Kubiske, M. E. (1990) Photosynthesis and water relations during drought in Acer rubrum L. genotypes from contrasting sites in central Pennsylvania. *Functional Ecology*, 4:727-733.

Adams III, W. W., Cohu, C. M., Muller, O., Demmig-Adams, B. (2013) Foliar phloem infrastructure in support of photosynthesis. *Frontiers in Plant Science*, 4: 194.

Ainsworth, E. A., Rogers, A. (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant Cell and Environment*, 30: 258-270.

Allen, D. J., Ort, D. R. (2001) Impacts of chilling temperatures on photosynthesis in warm-climate plants.*Trends in Plant Science*, 6 (1): 36-42.

Anderegg, W. R., Berry, J. A., Field, C. B. (2012) Linking definitions, mechanisms, and modeling of drought-induced tree death. *Trends in Plant Science*,17 (12): 693-700.

Anderegg, W.R.L., Berry, J.A., Smith, D.D., Sperry, J.S., Anderegg, L.D.L., Field, C.B. (2012) The roles of hydraulic and carbon stress in a widespread climate-induced forest die-of. *Proceedings of the National Academy of Sciences*, 109: 233–237.

Andrade Júnior, S. D., Alexandre, R. S., Schmildt, E. R., Partelli, F. L., Ferrão, M. A. G., Mauri, A. L. (2013) Comparison between grafting and cutting as vegetative propagation methods for conilon coffee plants. *Acta Scientiarum. Agronomy*, 35 (4): 461-469.

Anjum, S. A., Xie, X. Y., Wang, L. C., Saleem, M. F., Man, C., Lei, W. (2011) Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, 6 (9): 2026-2032.

Aro, E. M., Virgin, I., Andersson, B. (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1143: 113–134.

Attia, Z., Domec, J. C., Oren, R., Way, D. A., Moshelion, M. (2015) Growth and physiological responses of isohydric and anisohydric poplars to drought. *Journal of Experimental Botany*, 66 (14): 4373-4381.

Avila, R. T., Cardoso, A. A., de Almeida, W. L., Costa, L. C., Machado, K. L., Barbosa, M. L., Souza, R. P. B., Oliveira, L. A., Batista, D. S., Martins, S. C.V., Ramalho, J. D.C., DaMatta, F. M. (2020) Coffee plants respond to drought and elevated [CO_2] through changes in stomatal function, plant hydraulic conductance, and aquaporin expression. *Environmental and Experimental Botany*, *177*: 104148.

Azcon-Bieto, J., Berry, J., Bunce, J., Dijkstra, P., Farrar, J., Gifford, R. M., Gonzalez-Meler, M. A., Koch, G., Lambers, H., Siedow, J., Wullschleger, S. (1999) Does elevated atmospheric CO_2 concentration inhibit mitochondrial respiration in green plants? *Planty Cell and Environment*, 22: 649-657.

Baitelle, D. C., Baroni, D. F., Vieira, K. M., Freitas, S. J., Meneghelli, C. M., Berilli, S. S., Haddade, I. R., Verdin Filho, A. C. (2018) Pit dimensions in the early development of Conilon coffee propagated by seeds and cuttings. *Journal of Experimental Agriculture International* 20 (1): 1-8.

Baker, D. N., Myhre, D. L. (1969) Effects of leaf shape and boundary layer thickness on photosynthesis in cotton (*Gossypium hirsutum*). *Physiologia Plantarum*, 22 (5): 1043-1049.

Baker, N. R. (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annual Review of Plant Biology*, 59: 89-113.

Baltzer, J. L., Davies, S. J., Bunyavejchewin, S., Noor, N. S. M. (2008) The role of desiccation tolerance in determining tree species distributions along the Malay–Thai Peninsula. *Functional Ecology*, *22* (2): 221-231.

Bao, Y., Aggarwal, P., Robbins, N.E., Sturrock, C.J., Thompson, M.C., Tan, H.Q., Tham, C., Duan, L., Rodriguez, P.L., Vernoux, T. Money, S. J., Bennett, M. J., Dinneny, J. R. (2014) Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proceedings of the National Academy of Sciences*, 111: 9319–9324.

Barros, R. S., Mota, J. W. S., DaMatta, F. M., Maestri, M. (1997) Decline of vegetative growth in *Coffea arabica* L. in relation to leaf temperature, water potential and stomatal conductance. *Field Crops Research*, 54: 65–72.

Bartlett, M. K., Scoffoni, C., Sack, L. (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecology Letters*, 15 (5): 393-405.

Batista, K. D., Araújo, W. L., Antunes, W. C., Cavatte, P. C., Moraes, G. A. B. K., Martins, S. C. V., DaMatta, F. M. (2012) Photosynthetic limitations in coffee plants are chiefly governed by diffusive factors. *Trees*, 26:459–468.

Bauer, H., Ache, P., Lautner, S., Fromm, J., Hartung, W., Al-Rasheid, K. A., Sonnewald, S. Sonnewald, U., Kneitz, S., Lachmann, N., Mendel, R. R., Bitter, F., Hetherington, A. M., Hedrich, R. (2013) The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. *Current Biology*, 23 (1): 53-57.

Bauerle, T. L., Centinari, M., Bauerle, W. L. (2011) Shifts in xylem vessel diameter and embolisms in grafted apple trees of differing rootstock growth potential in response to drought. *Planta*, 234 (5): 1045-1054.

Berthaud, J. (1980) L'incompatibilité chez *Coffea canephora* methode de test et déterminisme génétique. *Café Cacao Thé,* 24 (4): 267-274.

Beyel, V., Bruggemann, W. (2005) Differential inhibition of photosynthesis during pre-flowering drought stress in Sorghum bicolor genotypes with different senescence traits. *Physiologia Plantarum*, 124 (2): 249–259.

Bita, C., Gerats, T. (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science*, 4: 273.

Blackman, C. J., Brodribb, T. J., Jordan, G. J. (2010) Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytologist*, 188 (4): 1113-1123.

Blackman, P. G., Davies, W. J. (1985) Root to shoot communication in maize plants of the effects of soil drying. *Journal of Experimental Botany*, 36 (1): 39-48.

Blum, A. (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research*, 56 (11): 1159-1168.

Blum, A. (2016) Stress, strain, signaling, and adaptation—not just a matter of definition. *Journal of Experimental Botany* 67: 563–566.

Bolhar-Nordenkampf, H. R., Long, S. P., Baker, N. R., Oquist, G., Schreiber, U., Lechner, E. G. (1989) Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. *Functional Ecology*, 3: 497-514.

Bolhàr-Nordenkampf, H. R., Öquist, G. (1993) Chlorophyll fluorescence as a tool in photosynthesis research. *In*: Hall, D. O., Scurlock, J. M. O., Bolhàr-Nordenkampf, H. R., Leegood, R. C., Long, S. P. (eds.) *Photosynthesis and production in a changing environment*. Dordrecht: Springer, p. 193-206.

Boughalleb, F., Abdellaoui, R., Hadded, Z., Neffati, M. (2015) Anatomical adaptations of the desert species *Stipa lagascae* against drought stress. *Biologia*, 70 (8): 1042-1052.

Bowman, W. D., Roberts, S. W. (1985) Seasonal changes in tissue elasticity in chaparral shrubs. *Physiologia Plantarum*, 65 (3), 233-236.

Boyer, J. S. (1982) Plant productivity and environment. *Science,* 218: 443–448.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248–254.

Brodribb, T. J. Holbrook, N. M. Zwieniecki, M. A. Palma, B. (2005) Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts on photosynthetic maxima. *New Phytologist*, 165 (3), 839-846.

Brodribb, T. J., Feild, T. S., Jordan, G. J. (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology*, 144 (4), 1890-1898.

Brodribb, T. J., Holbrook, N. M. (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology*, 132 (4): 2166-2173.

Brodribb, T. J., Holbrook, N. M. (2005) Leaf physiology does not predict leaf habit; examples from tropical dry forest. *Trees*, 19 (3): 290-295.

Brodribb, T. J., Holloway-Phillips, M. M., Bramley, H. (2015) Improving water transport for carbon gain in crops. *In:* Sadras, V. O., Calderini, D. F. (eds.) *Crop physiology*. San Diego: Academic Press, p. 251-281.

Brown, A. L.; Cavagnaro, T. R.; Gleadow, R., Miller, R. E. (2016) Interactive effects of temperature and drought on cassava growth and toxicity: implications for food security? *Global Change Biology*, 22 (10): 3461-3473.

Bruce, T. J. A., Matthes, M. C., Napier, J. A., Pickett, J. A. (2007) Stressful 'memories' of plants: evidence and possible mechanisms. *Plant Science*, 173: 603–608.

Buckley, T. N. (2005) The control of stomata by water balance. *New Phytologist*, 168 (2), 275-292.

Buckley, T. N. (2019) How do stomata respond to water status? *New Phytologist,* 224 (1): 21-36.

Bunn, C., Läderach, P., Rivera, O. O., Kirschke, D. (2015) A bitter cup: climate change profile of global production of Arabica and Robusta coffee. *Climatic Change*, 129: 89-101.

Campbell, G. S., Norman, J. M. (1998) *An introduction to environmental biophysics*. 2. ed. New York: Springer, 286p.

Cao, X., Du, W., Shang, C., Shen, Q., Liu, L., & Cheng, J. (2018). Comparative transcriptome reveals circadian and hormonal control of adventitious rooting in mulberry hardwood cuttings. *Acta Physiologiae Plantarum*, 40(11): 1-16.

Carvalho, A. C., Carvalho, D. F., Filgueiras, G. C., Araújo, A. C. S., Carvalho, A. V. (2018) Panorama e importância econômica do café no mercado internacional de commodities agrícolas: uma análise espectral. *Revista Agroecossistemas*, 9 (2): 223-249.

Carvalho, M., Jesus, A. M. S., Carvalho, S. P. D., Gomes, C. N., Soares, Â. M. (2008) Comportamento em condições de campo de cafeeiros (*Coffea arabica* L.) propagados vegertativas e por semeadora. *Coffee Science*, 3 (2): 108-114.

Castro, P., Puertolas, J., Dodd, I. C. (2019) Stem girdling uncouples soybean stomatal conductance from leaf water potential by enhancing leaf xylem ABA concentration. *Environmental and Experimental Botany*, 159: 149-156.

Cavender-Bares, J., Cortes, P., Rambal, S., Joffre, R., Miles, B., Rocheteau, A. (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. *New Phytologist*, 168 (3): 597-612.

Charrier, A., Berthaud, J. (1985) Botanical classification of coffee. *In*: Clifford, M. N., Willson, K. C. (eds.) *Coffee.* Boston: Springer, p. 13-47.

Chaves, A. R., Ten-Caten, A., Pinheiro, H. A., Ribeiro, A., DaMatta, F. M. (2008) Seasonal changes in photoprotective mechanisms of leaves from shaded and unshaded field-grown coffee (*Coffea arabica* L.) trees. *Trees*, 22 (3): 351-361.

Chaves, M. M., Harley, P. C., Tenhunen, J. D., Lange, O. L. (1987) Gas exchange studies in two portuguese grapevine cultivars. *Physiologia Plantarum*, 70 (4), 639-647.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P., Osório, M. L., Carvalho, I., Faria, T., Pinheiro, C. (2002) How plants cope with water stress in the field? Photosynthesis and growth. *Annals of Botany*, 89 (7): 907-916.

Chen, D., Wang, S., Cao, B., Cao, D., Leng, G., Li, H., Yin, L., Shan, L., Deng, X. (2016) Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Frontiers in Plant Science*, 6: 1241.

Chen, X.Y., Li, W., Lu, Q.T., Wen, X.G., Li, H.W., Kuang, T.Y., Li, Z.S., Lu, C.M. (2011) The xanthophyll cycle and antioxidative defense system are enhanced in the wheat hybrid subjected to high light stress. *Journal Plant Physiology,* 167:1828–1836.

Cheserek, J. J., Gichimu, B. M. (2012) Drought and heat tolerance in coffee: a review. *International Research Journal of Agricultural Science and Soil Science*, 2 (12): 498-501.

Cheung, Y. N. S., Tyree, M. T., Dainty, J. (1975) Water relations parameters on single leaves obtained in a pressure bomb and some ecological interpretations. *Canadian Journal of Botany*, 53 (13): 1342-1346.

Chevallier, F., De Noblet, N., Friend, A. D., Friedlingstein, P., Grünwald,T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J. M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J. F., Sanz, M. J., Schulze, E. D., Vesala, T., Valentini, R. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature,* 437: 529–533.

Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E. (2012). Global convergence in the vulnerability of forests to drought. *Nature,* 491:752–755.

Companhia nacional de abastecimento (2017). Acompanhamento da safra brasileira de café – Safra 2017: 4 (1). Brasília.

Companhia nacional de abastecimento (2018). Acompanhamento da safra brasileira de café – Safra 2018: 5 (3). Brasília.

Conagin, C. H. T. M., Mendes, A. J. T. (1961) Pesquisas citológicas e genéticas em três espécies de *Coffea*: auto-incompatibilidade em *Coffea canephora*. *Bragantia*, 20:787-804.

Cornic, G., Briantais, J. M. (1991) Partitioning of photosynthetic electron flow between CO_2 and O_2 reduction in a C3 leaf (*Phaseolus vulgaris* L.) at different CO_2 concentrations and during drought stress. *Planta*, 183 (2), 178-184.

Correia, M. J., Chaves, M. M. C., Pereira, J. S. (1990) Afternoon depression in photosynthesis in grapevine leaves - Evidences for a high light stress effect. *Journal of Experimental Botany*, 41: 417–426.

Costa, G. F., Marenco, R. A. (2007) Fotossíntese, condutância estomática e potencial hídrico foliar em árvores jovens de andiroba (*Carapa guianensis*). *Acta Amazonica,* 37 (2): 229 – 234.

Coste, R., Cambrony, H. (1992) *Coffee: The Plant and the Product*. 2. ed. London: MacMillan Press Ltd, 328p.

Croker, J. L., Witte, W. T., Auge, R. M. (1998) Stomatal sensitivity of six temperate, deciduous tree species to non-hydraulic root-to-shoot signalling of partial soil drying. *Journal of Experimental Botany*, 49 (321): 761-774.

DaMatta, F. M. (2003) Drought as a multidimensional stress affecting photosynthesis in tropical tree crops. *Advances in Plant Physiology,* 5: 227-265.

DaMatta, F. M. (2004) Exploring drought tolerance in coffee: a physiological approach with some insights for plant breeding. *Brazilian Journal of Plant Physiology*, 16 (1): 1-6.

DaMatta, F. M., Avila, R. T., Cardoso, A. A., Martins, S. C., Ramalho, J. C. (2018) Physiological and agronomic performance of the coffee crop in the context of climate change and global warming: A review. *Journal of Agricultural and Food Chemistry*, 66 (21): 5264-5274.

DaMatta, F. M., Chaves, A. R. M., Pinheiro, H. A., Ducatti, C., Loureiro, M. E. (2003) Drought tolerance of two field-grown clones of *Coffea canephora*. *Plant Science*, 164 (1): 111−117.

DaMatta, F. M., Grandis, A., Arenque, B. C., Buckeridge, M. S. (2010) Impacts of climate changes on crop physiology and food quality. *Food Research International*, 43 (7), 1814-1823.

DaMatta, F. M., Rahn, E., Läderach, P., Ghini, R., Ramalho, J. C. (2019) Why could the coffee crop endure climate change and global warming to a greater extent than previously estimated? *Climatic Change*, 152: 167–178.

DaMatta, F. M., Ramalho, J. D. C. (2006) Impacts of drought and temperature stress on coffee physiology and production: A review. *Brazilian Journal of Plant Physiology*, 18 (1): 55-81.

Darwin, F. (1898) Observations on stomata. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 190: 531–621.

Davies, W. J., Tardieu, F., Trejo, C. L. (1994) How do chemical signals work in plants that grow in drying soil? *Plant Physiology*, 104 (2): 309.

Davis, A. P., Gole, T. W., Baena, S., Moat, J. (2012) The impact of climate change on indigenous Arabica coffee (*Coffea arabica*): predicting future trends and identifying priorities. *PloS One*, 7 (11): e47981.

Davis, A. P., Rakotonasolo, F. (2021) Six new species of coffee (*Coffea*) from northern Madagascar. *Kew Bulletin*, 76 (3): 497-511.

Davis, A. P., Tosh, J., Ruch, N., Fay, M. F. (2011) Growing coffee: *Psilanthus* (Rubiaceae) subsumed on the basis of molecular and morphological data;

implications for the size, morphology, distribution and evolutionary history of *Coffea*. *Botanical Journal of the Linnean Society*, 167: 357-377.

De Dato, G., Pellizzaro, G., Cesaraccio, C., Sirca, C., De Angelis, P., Duce, P., Spano, D., Scarascia Mugnozza, G. (2008) Effects of warmer and drier climate conditions on plant composition and biomass production in a Mediterranean shrubland community. *iForest - Biogeosciences and Forestry*, 1:39–48.

Demmig-Adams, B., Cohu, C. M., Muller, O., Adams, W. W. (2012) Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. *Photosynthesis Research*, 113 (1): 75-88.

Demmig-Adams, B., Koh, S. C., Cohu, C., Muller, O., Stewart, J., Adams, W., III (2014) Non-photochemical fluorescence quenching in contrasting plant species and environments. *In*: Demmig-Adams, B., Garab, G., Adams, W. III, Govindjee. (eds.) *Non-photochemical quenching and energy dissipation in plants, algae and cyanobacteria*. Dordrecht: Springer, p. 531-552.

Demmig-Adams, B., Stewart, J. J., Adams III, W. W. (2017) Environmental regulation of intrinsic photosynthetic capacity: an integrated view. *Current Opinion in Plant Biology*, 37: 34-41.

Den Herder, G., Van Isterdael, G., Beeckman, T., De Smet, I. (2010) The roots of a new green revolution. *Trends Plant Science*, 15: 600–607.

Derks, A., Schaven, K., Bruce, D. (2015) Diverse mechanisms for photoprotection in photosynthesis. Dynamic regulation of photosystem II excitation in response to rapid environmental change. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1847 (4-5): 468-485.

Devi, M. J., Reddy, V. R. R. (2018) Transpiration response of cotton to vapor pressure deficit and its relationship with stomatal traits. *Frontiers in Plant Science*, 9: 1572.

Dewar, R. C. (1995) Interpretation of an empirical model for stomatal conductance in terms of guard cell function. *Plant, Cell & Environment*, 18 (4): 365-372.

Dias, A. S., Barreiro, M. G., Campos, P. S., Ramalho, J. C., Lidon, F. C. (2010) Wheat cellular membrane thermotolerance under heat stress. *Journal of Agronomy and Crop Science,* 196:100-108.

Diaz, A., Lacuesta, M., Muñoz-Rueda, A. (1996) Comparative effects of phosphinothricin on nitrate and ammonium assimilation and on anaplerotic

CO_2 fixation in N-deprived barley plants. *Journal of Plant Physiology*, 149: 9-13.

dos Santos, C. M., Verissimo, V., de Lins Wanderley Filho, H. C., Ferreira, V. M., da Silva Cavalcante, P. G., Rolim, E. V., Endres, L. (2013) Seasonal variations of photosynthesis, gas exchange, quantum efficiency of photosystem II and biochemical responses of *Jatropha curcas* L. grown in semi-humid and semi-arid areas subject to water stress. *Industrial Crops and Products*, 41: 203-213.

Dubberstein, D., Lidon, F. C., Rodrigues, A. P., Semedo, J. N., Marques, I., Rodrigues, W. P., Gouveia, D., Armengaud, J., Semedo, M. C., Martins, S., Simões-Costa, M. C., Moura, I., Pais, I. P., Scotti-Campos, P., Partelli, F., Campostrini, E., DaMatta, F. M., Ramalho, J. C. (2020) Resilient and sensitive key points of the photosynthetic machinery of *Coffea* spp. to the single and superimposed exposure to severe drought and heat stresses. *Frontiers in Plant Science*, 11: 1049.

Dwivedi, S. K., Basu, S., Kumar, S., Kumari, S., Kumar, A., Jha, S., Mishra, J. S., Bhatt, B. P., Kumar, G. (2019) Enhanced antioxidant enzyme activities in developing anther contributes to heat stress alleviation and sustains grain yield in wheat. *Functional Plant Biology,* 46 (12): 1090-1102.

Eilmann, B., Sterck, F., Wegner, L., de Vries, S. M., Von Arx, G., Mohren, G. M., de Ouden, J., Sass-Klaassen, U. (2014) Wood structural differences between northern and southern beech provenances growing at a moderate site. *Tree Physiology*, 34 (8): 882-893.

Eissenstat, D. M. (1992) Costs and benefits of constructing roots of small diameter. *Journal of Plant Nutrition*, *15* (6-7): 763-782.

El-Sharkawy, M. A., Cock, M. J. H., Hernandez, A. D. P. (1985) Stomatal response to air humidity and its relation to stomatal density in a wide range of warm climate species. *Photosynthesis Research,* 7 (2): 137-149.

Emery, R. J. N., Salon, C. (2002) Water entry into detached root systems saturates with increasing externally applied pressure, a result inconsistent with models of simple passive diffusion. *Physiologia Plantarum*, 115: 406–416.

Ennajeh, M., Vadel, A. M., Cochard, H., Khemira, H. (2010) Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. *The Journal of Horticultural Science and Biotechnology*, 85 (4), 289-294.

Façanha, A. R., Meis, L. (1995) Inhibition of maize root H^+-ATPase by fluoride and fluoroaluminate complexes*. Plant Physiology,* 108:241–246.

Fang, Y., Xiong, L. (2015) General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Science*, 72: 673–689.

Farquhar, G. D. (1978) Feedforward responses of stomata to humidity. *Functional Plant Biology*, 5 (6): 787-800.

Farquhar, G. D., Sharkey, T. D. (1982) Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, 33 (1): 317-345.

Fernandes, I., Marques, I., Paulo, O. S., Batista, D., Partelli, F. L., Lidon, F. C., DaMatta, F. M., Ramalho, J. C., Ribeiro-Barros, A. I. (2021) Understanding the impact of drought in coffea genotypes: transcriptomic analysis supports a common high resilience to moderate water deficit but a genotype dependent sensitivity to severe water deficit. *Agronomy*, 11 (11): 2255.

Ferrão, M. A. G., Fonseca, A. F. A., Ferrão, R. G., Barbosa, W. M., Souza, E. M. R. (2009) Genetic divergence in conilon coffee revealed by RAPD markers. *Repositório Alice*, 9: 67-74

Ferrão, R. G., Fonseca, A. F. A., Ferrão, M. A. G., DeMuner, L. G. (2017) *Coffea canephora. In*: Ferrão, R. G.; Fonseca, A. F. A.; Ferrão, M. A. G.; DeMuner, L. G. *Café Conilon*. 2. ed. Vitória: Incaper, p.37-53.

Fleta-Soriano, E., Munné-Bosch, S. (2016) Stress memory and the inevitable effects of drought: a physiological perspective. *Frontiers in Plant Science*, 7: 143.

Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T. D. (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biology*, 6 (3): 269-279.

Flexas, J., Medrano, H. (2002) Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Annals of Botany,* 89 (2):183–189.

Force, L., Critchley, C., Rensen, J. S. V. (2003) New fluorescence parameters for monitoring photosynthesis in plants. The effect of illumination on the fluorescence parameters of the JIP-test. *Photosynthesis Research,* 78: 17–33.

Franck, N., Vaast, P. (2009) Limitation of coffee leaf photosynthesis by stomatal conductance and light availability under different shade levels. *Trees*, 23:761–769.

Freitas, M. S. M. (2006) Flavonóides e nutrientes minerais em folhas de maracujazeiro amarelo e deficiência de macronutrientes e boro em maracujazeiro doce. Tese (Doutorado em Produção Vegetal) – Campos dos Goytacazes – RJ, Universidade Estadual do Norte Fluminense – UENF, 116p.

Galle, A., Florez-Sarasa, I., Aououad, H. E., Flexas, J. (2011) The Mediterranean evergreen *Quercus ilex* and the semi-deciduous *Cistus albidus* differ in their leaf gas exchange regulation and acclimation to repeated drought and re-watering cycles. *Journal of Experimental Botany,* 62: 5207–5216.

Galmés, J., Ochogavia, J. M., Gago, J., Roldan, E. J., Cifre, J., Conesa, M. A. (2013). Leaf responses to drought stress in Mediterranean accessions of *Solanum lycopersicum*: anatomical adaptations in relation to gas exchange parameter. *Plant Cell Environmental*, 36: 920-935.

Gates, D. M. (1965) Energy, plants, and ecology. *Ecology*, 46 (1-2): 1-13.

Gates, D. M. (1968) Transpiration and leaf temperature. *Annual Review of Plant Physiology*, 19 (1): 211-238.

Genty, B., Goulas, Y., Dimon, B., Peltier, G., Briantais, J. M., Moya, I. (1992) Modulation of efficiency of primary conversion in leaves, mechanisms involved at PS2. *Research in Photosynthesis*, 4: 603-610.

Genty, B., Briantais, J. M., Baker, N. R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 990 (1): 87-92.

Gerard, V. A., Driscoll, T. (1996) A spectrophotometric assay for Rubisco activity: application to the kelp *Laminaria saccharina* and implications for radiometric assays. *Journal of Phycology*, 32:880-884.

Ghini, R., Torre-Neto, A., Dentzien, A. F. M., Guerreiro-Filho, O., Lost, R., Patrício, F. R. A., Prado, J. S. M., Thomaziello, R. A., Bettiol, W., DaMatta, F. M.

(2015) Coffee growth, pest and yield responses to free-air CO_2 enrichment. *Climatic Change*, 132: 307–320.

Goltsev, V. N., Kalaji, H. M., Paunov, M., Bąba, W., Horaczek, T., Mojski, J., Kociel, H., Allakhverdiev, S. I. (2016) Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. *Russian Journal of Plant Physiology*, 63 (6): 869-893.

Grassi, G., Magnani, F. (2005) Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell & Environment*, *28* (7): 834-849.

Gray, S. B., Dermody, O., Klein, S. P., Locke, A. M., Mcgrath, J. M., Paul, R. E., Rosenthal, D. M., Ruiz-Vera, U. M., Siebers, M. H., Strellner, R., Ainsworth, E. A., Bernacchi, C. J., Long, S. P., Ort, D. R., Leakey, A. D. B. (2016) Intensifying drought eliminates the expected benefits of elevated carbon dioxide for soybean. *Nature Plants*, 2 (9): 16132.

Groom, P. K., Lamont, B. B. (1999) Which common indices of sclerophylly best reflect differences in leaf structure? *Ecoscience*, 6 (3): 471-474.

Grubb, P. J. (1986) Sclerophylls, pachyphylls, and pycnophylls: The nature and significance of hard leaf surface. *Insects and the plant surface*, 137-150.

Guidi, L., Lo Piccolo, E., Landi, M. (2019) Chlorophyll fluorescence, photoinhibition and abiotic stress: does it make any difference the fact to be a C3 or C4 species? *Frontiers in Plant Science*, 10: 174.

Guo, W. D., Guo, Y. P., Liu, J. R., Mattson, N. (2009) Midday depression of photosynthesis is related with carboxylation efficiency decrease and D1 degradation in bayberry (*Myrica rubra*) plants. *Scientia Horticulturae*, 123 (2): 188-196.

Guo, Y. P., Zhou, H. F., Zhang, L. C. (2006) Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species. *Scientia Horticulturae*, 108 (3): 260-267.

Guóth, A., Tari, I., Gallé, A., Csiszár, J., Pécsváradi, A., Cseuz, L., Erdei, L. (2009) Comparison of the drought stress responses of tolerant and sensitive wheat cultivars during grain filling: changes in flag leaf photosynthetic activity, ABA levels, and grain yield. *Journal of Plant Growth Regulation,* 28:167–176.

Gururani, M. A., Venkatesh, J., Tran, L. S. P. (2015) Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Molecular Plant*, 8 (9): 1304–1320.

Hacke, U. G., Sperry, J. S., Wheeler, J. K., Castro, L. (2006) Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiology*, 26 (6): 689-701.

Hacke, U. G., Spicer, R., Schreiber, S. G., Plavcová, L. (2017) An ecophysiological and developmental perspective on variation in vessel diameter. *Plant, Cell & Environment*, 40 (6): 831-845.

Haffani, S., Mezni, M., Nasri, M. B., Chaibi, W. (2017) Comparative leaf water relations and anatomical responses of three vetch species (*Vicia narbonensis* L., V. *sativa* L. and V. *villosa* Roth.) to cope with water stress. *Crop Pasture Science*, 68 (7): 691-702.

Haggar, J., Schepp, K. (2012) Coffee and climate change impacts and options for adaption in Brazil, Guatemala, Tanzania and Vietnam. *Natural Resources Institute,* 4: 47

Hajnajari, H., Akbari, H., Abdossi, V. (2019) Genesis of ultra-specialized histology with stable traits in mesophyll of drought tolerant apple cultivars. *Scientia Horticulturae*, 249: 168-176.

Hammer, G. L., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., Zinselmeier, C., Paszkiewicz, S., Cooper, M. (2009) Can changes in canopy and/or root system architecture explain historical maize yield trends in the US corn belt? *Crop Science*, 49 (1): 299-312.

Hatfield, J. L., Prueger, J. H. (2015) Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes*, 10: 4-10.

Hirasawa, T., Iida, Y., Ishihara, K. (1989) Dominant factors in reduction of photosynthetic rate affected by air humidity and leaf water potential in rice plants. *Japanese Journal of Crop Science*, 58 (3): 383-389.

Horton, P., Ruban, A. (2005) Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. *Journal of Experimental Botany*, 56 (411): 365-373.

Horton, P., Ruban, A. V., Walters, R. G. (1996) Regulation of light harvesting in green plants. *Annual Review of Plant Biology*, 47 (1): 655-684.

Hu, M. J., Guo, Y. P., Shen, Y. G., Guo, D. P., Li, D. Y. (2009) Midday depression of photosynthesis and effects of mist spray in citrus. *Annals of Applied Biology*, 154 (1): 143-155.

Ikegami, K., Okamoto, M., Seo, M., Koshiba, T. (2009) Activation of abscisic acid biosynthesis in the leaves of *Arabidopsis thaliana* in response to water deficit. *Journal of Plant Research*, 122 (2): 235-243.

Ivoglo, M. G., Fazuoli, L. C., Oliveira, A. C. B., Gallo, P. B., Mistro, J. C., Silvarolla, M. B., Toma-Braghini, M. (2008) Divergência genética entre progênies de café robusta. *Bragantia*, 67: 823-831.

Iwasaki, M., Paszkowski, J. (2014). Epigenetic memory in plants. *The EMBO Journal*, 33: 1–12.

Jacobs, B. F. (1999) Estimation of rainfall variables from leaf characters in tropical Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 145 (1-3): 231-250.

Jee, G. (1995) Sixty-three years since Kautsky: Chlorophyll fluorescence. *Australian Journal of Plant Physiology*, 22: 131–160.

Jones, H. G. (1992) *Plants and Microclimate*, 2. ed. Cambridge: Cambridge University Press, p.428.

Jones, H. G. (1999) Use of infrared thermometry for estimation of stomatal conductance in irrigation scheduling. *Agricultural and Forest Meteorology,* 95: 139-149.

Kalaji, H. M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I. A., Cetner, M. D., Lukasik, I., Goltsev, V., Ladle, R. J. (2016) Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum*, 38 (4): 1-11.

Kalaji, H. M., Schansker, G., Brestic, M., Bussotti, F., Calatayud, A., Ferroni, L., Goltsev, V., Guidi, L., Jajoo, A., Li, P., Losciale, P., Mishra, V. K., Misra, A. N., Nebauer, S. G., Pancaldi, S., Penella, C., Pollastrini, M., Suresh, K., Tambussi, E., Yanniccari, M., Zivcak, M., Cetner, M. D., Samborska, I. A., Stirbet, A., Olsovska, K., Kunderlikova, K., Shelonzek, H., Rusinowski, S., Bąba, W. (2017) Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynthesis Research*, 132 (1): 13-66.

Kalaji, H. M., Schansker, G., Ladle, R. J., Goltsev, V., Bosa, K., Allakhverdiev, S. I., Brestic, M., Bussotti, F., Calatayud, A., Dabrowski, P., Elsheery, N. I., Ferroni, L., Guidi, L., Hogewoning, S. W., Jajoo, A., Misra, A.,Nebauer, S. G., Pancaldi, S., Penella, C., Poli, D., Pollastrini, M., Romanowska-Duda, Z. B., Rutkowska, B., Serôdio, J., Suresh, K., Szulc, W., Tambussi, E., Yanniccari, M., Zivcak, M. (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynthesis Research*, 122 (2): 121-158.

Kanechi, M., Uchida, N. U., Yasuda, T., Yamaguchi, T. (1996) Nonstomatal inhibition associated with inactivation of Rubisco in dehydrated coffee leaves under unshaded and shaded conditions. *Plant & Cell Physiology*, 37 (4): 455 – 460.

Kashiwagi, J., Krishnamurthy, L., Crouch, J. H., Serraj, R. (2006) Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. *Field Crops Research*, *95* (2-3): 171-181.

Kirschbaum, M. U. F. (2011) Does enhanced photosynthesis enhance growth? Lessons learned from CO_2 enrichment studies. *Plant Physiology*, 155:117-124.

Kitano, M., Eguchi, H. (1993) Dynamic analyses of water relations and leaf growth in cucumber plants under midday water deficit. *Biotronics*, *22*: 73-85.

Klein, T. (2014) The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. *Functional Ecology*, 28 (6): 1313-1320.

Kramer, P. J. (1969) *Plant and soil water relationships: A modern synthesis. Plant and soil water relationships: a modern synthesis.*1. ed. New York: McGraw-Hill Book Company, 482p.

Kramer, P.J.; Boyer, J.S. (1995) *Water Relations of Plants and Soils*. San Diego: Academic Press, 481p.

Krishnamurthy, L., Johansen, C., Sethi, S. C. (1999) Investigation of factors determining genotypic differences in seed yield of non-irrigated and irrigated chickpeas using a physiological model of yield determination. *Journal of Agronomy and Crop Science*, 183 (1): 9-17.

Kuhlgert, S., Austic, G., Zegarac, R., Osei-Bonsu, I., Hoh, D., Chilvers, M. I., Roth, M. G., Bi, K., TerAvest, D., Weebadde, P., Kramer, D. M. (2016) MultispeQ

Beta: a tool for large-scale plant phenotyping connected to the open PhotosynQ network. *Royal Society Open Science,* 3 (10): 160592.

Kuppers, B. R., Wheeler, A. M., Kuppers, B. I. L., Krischbaum, M. U. F., Farquhar, G. D. (1986) Carbon fixation in eucalyptus in the field. Analysis of diurnal variations in photosynthetic capacity. *Oecologia,* 70: 273–282.

Lamont, B. B., Groom, P. K., Cowling, R. M. (2002) High leaf mass per area of related species assemblages may reflect low rainfall and carbon isotope discrimination rather than low phosphorus and nitrogen concentrations. *Functional Ecology*, *16* (3): 403-412.

Larcher, W. (2000) *Ecofisiologia vegetal*. 1. ed. São Carlos: Rima, 531p.

Lavorel, J. Etienne, A. L. (1977) In vivo chlorophyll fluorescence. *Primary Processes of Photosynthesis*, 2: 203-268.

Lawlor, D. W. (2002) Limitation to photosynthesis in water stressed leaves: stomata versus metabolism and the role of ATP. *Annals of Botany*, 89 (7): 871–885.

Lawlor, D. W.; Cornic, G. (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell & Environmental,* 25 (2):275–294.

Leigh, A., Sevanto, S., Close, J. D., Nicotra, A. B. (2017) The influence of leaf size and shape on leaf thermal dynamics: does theory hold up under natural conditions? *Plant, Cell & Environment*, 40 (2): 237-248.

Lemoine, R., La Camera, S., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, J. L., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrila, J., Durand, M. (2013) Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in Plant Science*, *4*: 272.

Lenz, T. I., Wright, I. J., Westoby, M. (2006) Interrelations among pressure–volume curve traits across species and water availability gradients. *Physiologia Plantarum*, 127 (3): 423-433.

Li, F. M., Yang, S. J., Li, C. X., Xu, B. C., Zhang, X. C. (2013) Effects of root pruning on non-hydraulic root-sourced signal, drought tolerance and water use efficiency of winter wheat. *Journal of Integrative Agriculture*, 12 (6): 989-998.

Li, Q. M., Liu, B. B. (2010) Comparison of three methods for determination of root hydraulic conductivity of maize (*Zea mays* L.) root system. *Agricultural Sciences in China*, 9 (10): 1438-1447.

Li, Y. T., Xu, W. W., Ren, B. Z., Zhao, B., Zhang, J., Liu, P., Zhang, Z. S. (2020) High temperature reduces photosynthesis in maize leaves by damaging chloroplast ultrastructure and photosystem II. *Journal of Agronomy and Crop Science*, 206 (5): 548-564.

Lisar, S. Y., Motafakkerazad, R., Hossain, M. M., Rahman, I. M. (2012) Water stress in plants: causes, effects and responses. *In*: Rahman, I. Md. M., Hasegawa, H. (eds.) *Water Stress*. Croatia: Intech, 300p.

Liu, H., Gleason, S. M., Hao, G., Hua, L., He, P., Goldstein, G., Ye, Q. (2019) Hydraulic traits are coordinated with maximum plant height at the global scale. *Science Advances*, 5 (eaav1332): 1-14.

Liu, W.G., Shan L., Deng, X. P. (2001) Probe the method of measuring root system hydraulic conductivity using pressure chamber. *Acta Botanica Boreal-Occidental Sinica,* 21: 761-765.

Long, S. P., Humphries, S., Falkowski, P. G. (1994) Photoinhibition of photosynthesis in nature. *Annual Review of Plant Biology*, 45 (1): 633-662.

Long, S. P., Ainsworth, E. A., Rogers, A., Ort, D. R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology*, 55: 591-628.

López-Millán, A. F., Morales, F., Abadía, A., Abadía, J. (2000) Responses of sugar beet rots to iron deficiency. Changes in carbon assimilation and oxygen use. *Plant Physiology*, 124:885-897.

Loveless, A. R. (1961) A nutritional interpretation of sclerophylly based on differences in the chemical composition of sclerophyllous and mesophytic leaves. *Annals of Botany*, 25 (2): 168-184.

Luo, L. J. (2010) Breeding for water-saving and drought-resistance rice (WDR) in China. *Journal of Experimental Botany*, 61: 3509–3517.

Ma, S.C.; Li, F. M., Yang, S. J., Li, C. X., Xu, B. C., Zhang, X. C. (2013) Effects of root pruning on non-hydraulic root-sourced signal, drought tolerance and water use efficiency of winter wheat. *Journal of Integrative Agriculture*, 12 (6): 989-998.

Ma, Y., Wang, T., Xie, Y., Lv, Q., Qiu, L. (2021) Alleviatory effect of rare earth micro-fertilizer on photosystem II (PSII) photoinhibition in Pseudostellaria heterophylla leaves at photosynthetic midday depression. *Journal of Rare Earths*, 40 (7): 1156-1164.

Machado Filho, J. A. (2018) Condutividade hidraulica (raize folha) e capacidade fotossintetica de mudas de clones de *Coffea canephora* Pierre ex a. Froehner. Tese (Doutorado em Produção Vegetal) – Campos dos Goytacazes – RJ, Universidade Estadual do Norte Fluminense – UENF, 64f.

Machado Filho, J. A., Rodrigues, W. P., Baroni, D. F., Pireda, S., Campbell, G., de Souza, G. A. R., Verdin Filho, A. C., Arantes, S. D., Arantes, L. O., Cunha, M., Gambetta, G., A., Rakocevic, M., Ramalho, J. C., Campostrini, E. (2021) Linking root and stem hydraulic traits to leaf physiological parameters in Coffea canephora clones with contrasting drought tolerance. *Journal of Plant Physiology*, 258-259:153355.

Magrach, A., Ghazoul, J. (2015) Climate and pest-driven geographic shifts in global coffee production: implications for forest cover, biodiversity and carbon storage. *PLoS One*, 10 (7): e0133071.

Maier-Maercker, U. T. A. (1983) The role of peristomatal transpiration in the mechanism of stomatal movement. *Plant, Cell & Environment*, 6 (5): 369-380.

Manavalan, L. P., Guttikonda, S. K., Tran, L. S., Nguyen, H. T. (2009) Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiol*ogy, 50: 1260–1276.

Manschadi, A. M., Christopher, J. T., Hammer, G. L., Devoil, P. (2010) Experimental and modelling studies of drought-adaptive root architectural traits in wheat (*Triticum aestivum* L.). *Plant Biosystems*, *144* (2): 458-462.

Manzi, M., Lado, J., Rodrigo, M. J., Zacarías, L., Arbona, V., Gómez-Cadenas, A. (2015). Root ABA accumulation in long-term water-stressed plants is sustained by hormone transport from aerial organs. *Plant and Cell Physiology*, 56 (12): 2457-2466.

Markesteijn, L., Poorter, L., Bongers, F., Paz, H., Sack, L. (2011) Hydraulics and life history of tropical dry forest tree species: coordination of species' drought and shade tolerance. *New phytologist*, 191 (2): 480-495.

Marques, I., Gouveia, D., Gaillard, J. C., Martins, S., Semedo, M. C., Lidon, F. C., DaMatta, F. M., Ribeiro-Barros, A. I., Armengaud, J., Ramalho, J. C. (2022) Next-generation proteomics reveals a greater antioxidative response to drought in *Coffea arabica* than in *Coffea canephora*. *Agronomy*, 12 (1): 148.

Martins, L. D., Tomaz, M. A., Lidon, F. C., DaMatta, F. M., Ramalho, J. C. (2014a) Combined effects of elevated [CO_2] and high temperature on leaf mineral balance in *Coffea* spp. plants. *Climatic Change*, 126 (3-4): 365-379.

Martins, S. C., Galmés, J., Cavatte, P. C., Pereira, L. F., Ventrella, M. C., DaMatta, F. M. (2014b) Understanding the low photosynthetic rates of sun and shade coffee leaves: bridging the gap on the relative roles of hydraulic, diffusive and biochemical constraints to photosynthesis. *PLoS One*, 9 (4): e95571.

Martins, S. C., Sanglard, M. L., Morais, L. E., Menezes-Silva, P. E., Mauri, R., Avila, R. T., Vital, C. E., Cardoso, A. A., DaMatta, F. M. (2019) How do coffee trees deal with severe natural droughts? An analysis of hydraulic, diffusive and biochemical components at the leaf level. *Trees*, 33 (6): 1679-1693.

Matiello, J. B., Garcia, A. W. R., Almeida, S. R., Fernandes, D. R. (2010) *Cultura de café no Brasil: manual de recomendações*. 3. ed. Rio de Janeiro: Bom Pastor, 542p.

Matsuo, N., Ozawa, K., Mochizuki, T. (2009) Genotypic differences in root hydraulic conductance of rice (*Oryza sativa* L.) in response to water regimes. *Plant and Soil*, 316: 25–34.

Maxwell, K., Johnson, G. N. (2000) Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany*, 51 (345): 659-668.

McAdam, S. A. M., Brodribb, T. J. (2014) Separating active and passive influences on stomatal control of transpiration. *Plant Physiology*, 164 (4): 1578–1586.

McAdam, S. A., Brodribb, T. J. (2015) The evolution of mechanisms driving the stomatal response to vapor pressure deficit. *Plant Physiology*, 167: 833–843

McAdam, S. A., Brodribb, T. J., Ross, J. J. (2016a) Shoot-derived abscisic acid promotes root growth. *Plant, Cell & Environment*, 39 (3): 652-659.

McAdam, S. A., Sussmilch, F. C., Brodribb, T. J. (2016b) Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms. *Plant, Cell & Environment*, 39 (3): 485-491.

McCulloh, K. A., Johnson, D. M., Meinzer, F. C., Woodruff, D. R. (2014) The dynamic pipeline: hydraulic capacitance and xylem hydraulic safety in four tall conifer species. *Plant, Cell & Environment*, 37 (5), 1171-1183.

McDermitt, D. K. (1990) Sources of error in the estimation of stomatal conductance and transpiration from porometer data. *HortScience*, 25 (12): 1538-1548.

McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D. G., Yepez, E. A. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist,* 178:719–739.

Meidner, H. (1986). Cuticular conductance and the humidity response of stomata. *Journal of Experimental Botany*, 37 (4): 517-525.

Meinzer, F. C., McCulloh, K. A. (2013) Xylem recovery from drought-induced embolism: where is the hydraulic point of no return? *Tree Physiology*, 33 (4): 331-334.

Meinzer, F. C., Saliendra, N. Z., Crisosto, C. (1992) Carbon isotope discrimination and gas exchange in *Coffea arabica* during adjustment to different soil moisture regimes. *Functional Plant Biology*, 19 (2):171-184.

Meinzer, F. C., Smith, D. D., Woodruff, D. R., Marias, D. E., McCulloh, K. A., Howard, A. R., Magedman, A. L. (2017) Stomatal kinetics and photosynthetic gas exchange along a continuum of isohydric to anisohydric regulation of plant water status. *Plant, Cell & Environment*, 40 (8): 1618-1628.

Melis, A. (1999) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage in vivo? *Trends in Plant Science*, 4 (4): 130-135.

Mendonça, J. C., Freitas, R. M., Aguiar, D. A., Sousa, E. F., Muniz, R. A., Esteves, B. S. (2011) Mapeamento das áreas de cana-de-açúcar na região Norte fluminense – RJ por uso de técnicas de sensoriamento remoto. *Engenharia Agrícola,* 31 (3): 561-571.

Menezes-Silva, P. E., Sanglard, L. M., Ávila, R. T., Morais, L. E., Martins, S. C., Nobres, P., Patreze, C. M., Ferreira, M. A., Araújo, W. L., Fernie, A. R., DaMatta, F. M. (2017) Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in coffee. *Journal of Experimental Botany*, 68 (15): 4309-4322.

Merilo, E., Yarmolinsky, D., Jalakas, P., Parik, H., Tulva, I., Rasulov, B., Kilk, K., Kollist, H. (2018) Stomatal VPD response: there is more to the story than ABA. *Plant Physiology*, 176 (1): 851-864.

Miao, Y., Cai, Y., Wu, H., Wang, D. (2021) Diurnal and seasonal variations in the photosynthetic characteristics and the gas exchange simulations of two rice cultivars grown at ambient and elevated CO_2. *Frontiers in Plant Science*, *12*: 559.

Michaletz, S. T., Weiser, M. D., McDowell, N. G., Zhou, J., Kaspari, M., Helliker, B. R., Enquist, B. J. (2016) The energetic and carbon economic origins of leaf thermoregulation. *Nature Plants*, 2 (9): 16129.

Mishar, M. K., Slater, A. (2012) Recent advances in the genetic transformation of coffee. *Biotechnology Research International*, 2012: 17.

Ministério da Agricultura, Pecuária e Abastecimento – MAPA (2018) Café no Brasil. Disponível em: <http://www.agricultura.gov.br/assuntos/politica-agricola/cafe/cafeicultura-brasileira>. Accessed in: Feb. 2018.

Misra, A. N., Misra, M., Singh, R. (2012) Chlorophyll fluorescence in plant biology. *In:* Misra, A. N. (ed.) *Biophysics*. Croatia: Intech, p.171–192.

Mitchell, P. J., Veneklaas, E. J., Lambers, H., Burgess, S. S. (2008) Leaf water relations during summer water deficit: differential responses in turgor maintenance and variation in leaf structure among different plant communities in South-Western Australia. *Plant, Cell & Environment*, 31 (12): 1791-1802.

Muller, P., Li, X. P., Niyogi, K. K. (2001) Non-photochemical quenching. A response to excess light energy. *Plant Physiology*, 125 (4): 1558-1566.

Muller-Moule, P., Conklin, .P. L., Niyogi, K. K. (2002) Ascorbate deficiency can limit violaxanthin de-epoxidase activity in vivo. *Plant Physiology,* 128: 970–977.

Muller-Moule, P., Golan, T., Niyogi, K. K. (2004) Ascorbate-deficient mutants of Arabidopsis grow in high light despite chronic photooxidative stress. *Plant Physiology*, 134: 1163–1172.

Murata, N., Allakhverdiev, S. I., Nishiyama, Y. (2012) The mechanism of photoinhibition in vivo: re-evaluation of the roles of catalase, α-tocopherol, non-photochemical quenching, and electron transport. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1817 (8): 1127-1133.

Murchie, E. H., Lawson, T. (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany*, 64 (13): 3983-3998.

Musoli, P., Cubry, P., Aluka, P., Billot, C., Dufour, M., De Bellis, F., Pot, D., Bieysse, D., Charrier, A., Leroy, T. (2009) Genetic differentiation of wild and cultivated populations: diversity of *Coffea canephora* Pierre in Uganda. *Genome*, 52 (7): 634-646.

Narayanan, S., Mohan, A., Gill, K. S., Prasad, P. V. (2014) Variability of root traits in spring wheat germplasm. *PLoS One*, 9 (6): e100317.

Nardini, A., Õunapuu-Pikas, E., Savi, T. (2014) When smaller is better: leaf hydraulic conductance and drought vulnerability correlate to leaf size and venation density across four *Coffea arabica* genotypes. *Functional Plant Biology*, 41 (9): 972-982.

Nardini, A., Pedà, G., La Rocca, N. (2012) Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. *New Phytologist*, 196 (3): 788-798.

Niinemets, Ü. (2001) Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology*, 82 (2): 453-469.

Niinemets, U. (2010) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management,* 260: 1623–1639.

Nishiyama, Y., Allakhverdiev, S. I., Murata, N. (2006) Anewparadigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1757 (7): 742-749.

Nishiyama, Y., Allakhverdiev, S.I., and Murata, N. (2011) Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II. *Physiologia Plantarum*, 142 (1): 35–46.

Nishiyama, Y., and Murata, N. (2014) Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. *Applied Microbiology and Biotechnology* 98 (21): 8777–8796.

Niu, Y., Wang, Y., Li, P., Zhang, F., Liu, H., Zheng, G. (2013) Drought stress induces oxidative stress and the antioxidant defense system in ascorbate-deficient *vtc1* mutants of *Arabidopsis thaliana*. *Acta Physiologiae Plantarum*, 35 (4): 1189-1200.

Niyogi, K. K. (2000) Safety valves for photosynthesis. *Current Opinion in Plant Biology*, 3 (6): 455-460.

Nunes, M. A. (1988) Environmental effects of stomatal and mesophyll regulation of photosynthesis in coffee leaves. *Photosynthetica*, 22 (4): 547–553.

Ogburn, R. M., Edwards, E. J. (2010) The ecological water-use strategies of succulent plants. *In*: Kader, J. C., Delseny, M. (eds.) *Advances in botanical research*. London: Academic Press, p.179-225.

Pallardy, S. G. (2008) Physiology of woody plants, 3rd edn. Elsevier, Amsterdam.

Palliotti, A., Tombesi, S., Frioni, T., Silvestroni, O., Lanari, V., D'Onofrioc, C., Mataresec, F., Bellincontro, A., Poni, S. (2015) Physiological parameters and protective energy dissipation mechanisms expressed in the leaves of two *Vitis vinifera* L. genotypes under multiple summer stresses. *Journal of Plant Physiology*, 185: 84–92.

Parkash, V., Singh, S. (2020) A review on potential plant-based water stress indicators for vegetable crops. *Sustainability*, 12 (10): 3945.

Parkhurst, D. F., Loucks, O. L. (1972) Optimal leaf size in relation to environment. *The Journal of Ecology*, 505-537.

Partelli, F. L., Batista-Santos, P., Scotti-Campos, P., Pais, I. P., Quantin, V. L., Vieira, H. D., Ramalho, J. C. (2011) Characterization of the main lipid components of chloroplast membranes and cold induced changes in *Coffea* spp. *Environmental and Experimental Botany*, 74: 194-204.

Patakas, A., Stavrakas, D., Fisarakis, I. (2003) Relationship between CO_2 assimilation and leaf anatomical characteristics of two grapevine cultivars. *Agronomie*, 23 (4): 293-296.

Patrick, J. W., Colyvas, K. (2014) Crop yield components–photoassimilate supply-or utilisation limited-organ development? *Functional Plant Biology*, 41 (9): 893-913.

Peppe, D. J., Royer, D. L., Cariglino, B., Oliver, S. Y., Newman, S., Leight, E., Enikolopov, G., Fernandez-Burgos, M., Herrera, F., Adams, J. M., Correa, E. Currano, E. D., Erickson, J. M., Hinojosa, L. F., Hoganson, J. W., Iglesias, A., Jaramillo, C. A., Johnson, K. R., Jordan, G. J., Kraft, N. J. B., Lovelock, E. C., Lusk, C. H., Niinemets, U., Peñuelas, J., Rapson, G., Wing, S. L., Wrightm, I. J. (2011) Sensitivity of leaf size and shape to climate: global patterns and paleoclimatic applications. *New Phytologist*, 190 (3): 724-739.

Perrone, I., Pagliarani, C., Lovisolo, C., Chitarra, W., Roman, F., Schubert, A. (2012) Recovery from water stress affects grape leaf petiole transcriptome. *Planta*, 235: 1383–1396.

Pinheiro, H. A., DaMatta, F. M., Chaves, A. R., Fontes, E. P., Loureiro, M. E. (2004) Drought tolerance in relation to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought. *Plant Science*, 167 (6): 1307-1314.

Pinheiro, H. A., DaMatta, F. M., Chaves, A. R., Loureiro, M. E., Ducatti, C. (2005) Drought tolerance is associated with rooting depth and stomatal control of water use in clones of *Coffea canephora*. *Annals of Botany*, 96 (1): 101-108.

Polutchko, S. K., Stewart, J. J., Demmig-Adams, B., Adams, W. W. (2018) Evaluating the link between photosynthetic capacity and leaf vascular organization with principal component analysis. *Photosynthetica*, 56 (1): 392-403.

Pons, T. L., Welschen, R. A. (2003) Midday depression of net photosynthesis in the tropical rainforest tree *Eperua grandiflora*: contributions of stomatal and internal conductances, respiration and Rubisco functioning. *Tree Physiology*, 23 (14): 937-947.

Powles, S. B. (1984) Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology*, 35 (1): 15-44.

Prasad, P. V. V., Vu, J. C. V., Boote, K. J., Allen, L. H. (2009) Enhancement in leaf photosynthesis and upregulation of Rubisco in the C4 sorghum plant at elevated growth carbon dioxide and temperature occur at early stages of leaf ontogeny. *Functional Plant Biology,* 36 (9): 761-769.

Rakocevic, M., Braga, K. S. M., Batista, E. R., Maia, A. H. N., Scholz, M. B. S., Filizola, H. F. (2020) The vegetative growth assists to reproductive responses of Arabic coffee trees in a long-term FACE experiment. *Plant Growth Regulation*, 91: 305-316.

Ramalho, J. C., Pais, I. P., Leitão, A. E., Guerra, M., Reboredo, F. H., Máguas, C. M., Carvalho, M. L., Scotti-Campos, P., Ribeiro-Barros, A. I., Lidon, F. L. C., DaMatta, F. M. (2018) Can elevated air [CO_2] conditions mitigate the predicted warming impact on the quality of coffee bean? *Frontiers in Plant Science*, *9*: 287.

Ramalho, J. C., Rodrigues, A. P., Lidon, F. C., Marques, L. M., Leitão, A. E., Fortunato, A. S., Pais, I. P., Silva, M. J., Scotti-Campos, P., Lopes, A., Reborredo, F. H., Ribeiro-Barros, A. I. (2018) Stress cross-response of the antioxidative system promoted by superimposed drought and cold conditions in *Coffea* spp. *PLoS one*, 13 (6): e0198694.

Ramalho, J. C., Rodrigues, A. P., Semedo, J. N., Pais, I. P., Martins, L. D., Simões-Costa, M. C., Leitão, A. E., Fortunato, A. S., Batista-Santos, P., Palos, I. M., Tomaz, M. A., Scotti-Campos, P., Lidon, F. C., DaMatta, F. M. (2013) Sustained photosynthetic performance of *Coffea* spp. under long-term enhanced [CO_2]. *PLoS One*, 8 (12): e82712.

Ramalho, J., C., Campos, P. S., Quartin, V. L., Silva, M. J., Nunes, M. A. (1999) High irradiance impairments on photosynthetic electron transport, ribulose-1,5-bisphosphate carboxylase/oxygenase and N assimilation as a function of N availability in *Coffea arabica* L. plants. *Journal of Plant Physiology*, 154:319-326.

Raschke, K., Resemann, A. (1986) The midday depression of CO_2 assimilation in leaves of *Arbutus unedo* L.: diurnal changes in photosynthetic capacity related to changes in temperature and humidity. *Planta,* 168: 546–558.

Read, J., Sanson, G. D., de Garine-Wichatitsky, M., Jaffre, T. (2006). Sclerophylly in two contrasting tropical environments: low nutrients vs. low rainfall. *American Journal of Botany,* 93 (11): 1601–1614.

Reddy, K. S., Sekhar, K. M., Sreeharsha, R. V., Reddy, A. R. (2019) Hydraulic dynamics and photosynthetic performance facilitate rapid screening of field grown mulberry (*Morus* spp.) genotypes for drought tolerance. *Environmental and Experimental Botany*, 157: 320-330.

Ren, H., Gao, Z., Chen, L., Wei, K., Liu, J., Fan, Y., Zhang, J. (2007) Dynamic analysis of ABA accumulation in relation to the rate of ABA catabolism in maize tissues under water deficit. *Journal of Experimental Botany*, 58 (2): 211-219.

Rena, A. B., Barros, R. S., Maestri, M., Söndahl, M. R. (1994) Coffee. *In:* Schaffer, B.; Andersen, P.C. (eds.). *Handbook of environmental physiology of tropical* *fruit crops: sub-tropical and tropical crops*. 1. ed. Boca Raton: CRC Press, p. 101-122.

Reynolds, M., Dreccer, F., Trethowan, R. (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. *Journal of Experimental Botany*, 58 (2): 177-186.

Ribeiro, R. V., Machado, E. C., Santos, M. G., Oliveira, R. F. (2009) Photosynthesis and water relations of well-watered orange plants as affected by winter and summer conditions. *Photosynthetica,* 47: 215–222.

Ripley, B. S., Redfern, S. P., Dames, J. (2004) Quantification of the photosynthetic performance of phosphorus-deficient *Sorghum* by medium of chlorophyll-*a* fluorescence kinetics. *South African Journal of Science*, 100 (11): 615-618.

Ripoll, J., Bertin, N., Bidel, L. P., Urban, L. (2016) A user's view of the parameters derived from the induction curves of maximal chlorophyll a fluorescence: perspectives for analyzing stress. *Frontiers in Plant Science*, *7*: 1679.

Roche, D. (2015) Stomatal conductance is essential for higher yield potential of C3 crops. *Critical Reviews in Plant Sciences*, 34 (4), 429–453.

Rodrigues, W. P., Martins, M. Q., Fortunato, A. S., Rodrigues, A. P., Semedo, J. N., Simões-Costa, M. C., Pais, I. P., Leitão, A. E., Colwell, F., Goulao, L., Máguas, C., Maia, R., Partelli, F. L., Campostrini, E., Scotti-Campos, P., Ribeiro-Barros, A. I., Lidon, F. C., DaMatta, F. M., Ramalho, J. C. (2016) Long-term elevated air [CO_2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical *Coffea arabica* and *C. canephora* species. *Global Change Biology*, 22: 415-431.

Roessler, P. G., Monson, K. R. (1985) Midday depression in net photosynthesis and stomatal conductance in *Yucca glauca*. Relative contributions of leaf temperature and leaf-to-air water vapour concentration difference. *Oecologia,* 67: 380–387.

Ronquim, J. C., Prado, C. H. B. A., Novaes, P., Fahl, J. I., Ronquim, C. C. (2006) Carbon gain in *Coffea arabica* during clear and cloudy days in the wet season. *Experimental Agriculture*, 42: 147–164.

Ruban, A. V., Johnson, M. P., Duffy, C. D. (2012) The photoprotective molecular switch in the photosystem II antenna. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1817 (1): 167-181.

Ryan, P. R., Delhaize, E., Watt, M., Richardson, A. E. (2016) Plant roots: understanding structure and function in an ocean of complexity. *Annals of Botany*, 118 (4): 555–559.

Sack, I., Pasquet-Kok, J. (2010) Leaf pressure-volume curve parameters: <https://sites.lifesci.ucla.edu/eeb-sacklab/protocols/>. Accessed in: Mar. 2020.

Sack, L, Scoffoni, C. (2013) Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytologist,* 198: 983–1000.

Sack, L. (2004) Responses of temperate woody seedlings to shade and drought: do trade-offs limit potential niche differentiation? *Oikos*, 107 (1): 110-127.

Sack, L., Cowan, P. D., Jaikumar, N., Holbrook, N. M. (2003) The ‘hydrology’of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment*, 26 (8): 1343-1356.

Sack, L., Holbrook, N. M. (2006) Leaf hydraulics. *Annual Review of Plant Biology*, 57: 361-381.

Sack, L.; Frole, K. (2006) Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology*, 87 (2): 483-491.

Sack, L.; Scoffoni, C. (2012) Measurement of leaf hydraulic conductance and stomatal conductance and their responses to irradiance and dehydration using the Evaporative Flux Method (EFM). *Journal of Visualized Experiments*, 70 (e4179): 1-7.

Scafaro, A. P., Von Caemmerer, S., Evans, J. R., Atwell, B. J. (2011) Temperature response of mesophyll conductance in cultivated and wild *Oryza* species with contrasting mesophyll cell wall thickness. *Plant, Cell & Environment*, 34 (11): 1999-2008.

Scholander, P. F., Hammel, H. T., Bradstreet, E. D., Hemmingsen, E. A. (1965) SAP pressure in vascular plants. *Science*, 148: 339–346.

Schreiber, U., Hormann, H., Neubauer, C., Klughammer, C. (1995) Assessment of photosystem II photochemical quantum yield by chlorophyll fluorescence quenching analysis. *Functional Plant Biology*, 22 (2): 209-220.

Schuldt, B., Knutzen, F., Delzon, S., Jansen, S., Müller-Haubold, H., Burlett, R., Clough, Y., Leuschner, C. (2016) How adaptable is the hydraulic system of

European beech in the face of climate change-related precipitation reduction? *New Phytologist,* 210: 443–458.

Schulze, E. D., Hall, A. E. (1982) Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. *In*: Lange, O. L., Nobel, P. S., Osmond, C. B., Ziegler, H. (eds.) *Physiological plant ecology II*. 12B. ed. Berlin: Springer, Heidelberg, p. 181-230.

Scoffoni, C. Albuquerque, C. Brodersen, C. Townes, S. V. John, G. P. Bartlett, M. K. Buckley, T. N. McElrone, A. J. Sack, L. (2017) Outside-xylem vulnerability, not xylem embolism, controls leaf hydraulic decline during dehydration. *Plant Physiology*, 173: 1197–1210.

Scoffoni, C., Rawls, M., McKown, A., Cochard, H., Sack, L. (2011) Decline of leaf hydraulic conductance with dehydration: relationship to leaf size and venation architecture. *Plant Physiology,* 156: 832–843.

Scoffoni, C., Vuong, C., Diep, S., Cochard, H., Sack, L. (2014) Leaf shrinkage with dehydration: coordination with hydraulic vulnerability and drought tolerance. *Plant Physiology*, 164 (4): 1772-1788.

Semedo, J. N., Rodrigues, A. P., Lidon, F. C., Pais, I. P., Marques, I., Gouveia, D., Armengaud, J., Silva, M. J., Martins, S., Semedo, M. C., Dubberstein, D., Partelli, F. L., Reboredo, F. H., Scotti-Campos, P., Ribeiro-Barros, R. I., DaMatta, F. M., Ramalho, J. C. (2021) Intrinsic non-stomatal resilience to drought of the photosynthetic apparatus in *Coffea* spp. is strengthened by elevated air [CO_2]. *Tree Physiology*, 41 (5): 708-727.

Silva, A. M., Lima, E. P., Coelho, M. R., Coelho, G. S. (2003) Produtividade, rendimento de grãos e comportamento hídrico foliar em função da época de irrigação do parcelamento e do método de adubação do cafeeiro Catuaí. *Engenharia Agrícola*, 23 (3): 434 – 440.

Silva, E. A., DaMatta, F. M., Ducatti, C., Regazzi, A. J., Barros, R. S. (2004) Seasonal changes in vegetative growth and photosynthesis of Arabica coffee trees. *Field Crops Research*, 89 (2-3): 349-357.

Silva, P. E. M., Cavatte, P. C., Morais, L. E., Medina, E. F., DaMatta, F. M. (2013) The functional divergence of biomass partitioning, carbon gain and water use in *Coffea canephora* in response to the water supply: Implications for breeding aimed at improving drought tolerance. *Environmental and Experiental Botany*, 87: 49−57.

Simonin, K. A., Limm, E. B., Dawson, T. E. (2012) Hydraulic conductance of leaves correlates with leaf lifespan: implications for lifetime carbon gain. *New Phytologist,* 193: 939–947.

Sinclair, T. R., Zwienieckib, M. A., Holbrook, N. M. (2007) Low leaf hydraulic conductance associated with drought tolerance in soybean, *Physiologia Plantarum*, 132: 446–451.

Smith, M. S., Fridley, J. D., Yin, J., Bauerle, T. L. (2013) Contrasting xylem vessel constraints on hydraulic conductivity between native and non-native woody understory species. *Frontiers in Plant Science*, 4: 1–12.

Souza, C. R., Maroco, J., Santos, T. P., Rodrigues, M. L., Lopes, C., Pereira, J. S., Chaves, M. M. (2005) Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. *Agriculture, Ecosystems and Environment*, 106: 261-274.

Sperry, J. S. (2000) Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology*, 104:13–23.

Sperry, J. S., Adler, F. R., Campbell, G. S., Comstock, J. P. (1998) Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant, Cell and Environment,* 21: 347–359.

Sperry, J. S., Sullivan, J. E. (1992) Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. *Plant Physiology*, 100 (2): 605-613.

Steele, K. A., Price, A. H., Witcombe, J. R., Shrestha, R., Singh, B. N., Gibbons, J. M., Virk, D. S. (2013) QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. *Theoretical and Applied Genetics*, 126 (1): 101-108.

Strasserf, R. J., Srivastava, A. (1995) Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria*. Photochemistry and Photobiology*, 61 (1): 32-42.

Strasser, R. J., Srivastava, A., Tsimilli-Michael, M. (2004) Analysis of the chlorophyll a fluorescence transient. *In*: Papageorgiou, G. Govindjee (eds.) *Chlorophyll fluorescence: a Signature of photosynthesis*. Dordrecht: Springer, p. 321– 362.

Strasser, R. J., Tsimilli-Michael, M. (2001) Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-test. *Chimie Nouvelle*, 75: 3321–3326.

Strauss, A. J., Kruger, G. H. J., Strasser, R. J., Van Heerden, P. D. R. (2006) Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll a fluorescence transient O-J-I-P. *Environmental and Experimental Botany*, 56: 147–157.

Sussmilch, F. C., Roelfsema, M. R. G., Hedrich, R. (2019) On the origins of osmotically driven stomatal movements. *New Phytologist*, 222 (1): 84-90.

Takahashi, S., Murata, N. (2008) How do environmental stresses accelerate photoinhibition? *Trends Plant Science*, 13: 178–182.

Tardieu, F. (2013) Plant response to environmental conditions: assessing potential production, water demand, and negative effects of water deficit. *Frontiers in Physiology*, 4 (17): 1-11.

Tenhunen, J. D., Pearcy, R. W., Lange, O. L. (1987) Diurnal variations in leaf conductance and gas exchange in natural environments. *In*: Zeiger, E., Farquhar, G. D., Cowan, I.R. (eds.) *Stomatal function*. California: Stanford University Press, p. 323-351.

Terashima, I., Hanba, Y. T., Tholen, D., Niinemets, Ü. (2011) Leaf functional anatomy in relation to photosynthesis. *Plant Physiology*, 155 (1): 108-116.

Tesfaye, S. G., Ismail, M. R., Kausar, H., Marziah, M., Ramlan, M. F. (2013) Plant water relations, crop yield and quality in coffee ('*Coffea arabica*' L.) as influenced by partial root zone drying and deficit irrigation. *Australian Journal of Crop Science*, 7 (9): 1361-1368.

Theroux-Rancourt, G., Ethier, G. and Pepin, S. (2014) Threshold response of mesophyll CO_2 conductance to leaf hydraulics in highly transpiring hybrid poplar clones exposed to soil drying. *Journal of Experimental Botany*, 65: 741–753.

Tholen, D., Ethier, G., Genty, B., Pepin, S., Zhu, X. G. (2012) Variable mesophyll conductance revisited: theoretical background and experimental implications. *Plant, Cell & Environment*, 35 (12): 2087-2103.

Todorov, D. T., Karanov, E. N., Smith, A. R., Hall, M. A. (2003) Chlorophyllase activity and chlorophyll content in wild type and eti 5 mutant of *Arabidopsis* *thaliana* subjected to low and high temperatures. *Biologia Plantarum*, 46 (4): 633-636.

Tognetti, R., Raschi, A., Jones, M. B. (2000) Seasonal patterns of tissue water relations in three Mediterranean shrubs co-occurring at a natural CO_2 spring. *Plant Cell and Environment*, 11: 1341–1351.

Torres Netto, A., Campostrini, E., Oliveira, J. G., Bressan-smith, R. E. (2005) Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. *Scientia Horticulture*, 104: 199-209.

Trifiló, P., Raimondo, F., Savi, T., Lo Gullo, M. A., Nardini, A. (2016) The contribution of vascular and extra-vascular water pathways to drought-induced decline of leaf hydraulic conductance. *Journal of Experimental Botany*, 67 (17): 5029-5039.

Trueba, S., Pan, R., Scoffoni, C., John, G. P., Davis, S. D., Sack, L. (2019) Thresholds for leaf damage due to dehydration: declines of hydraulic function, stomatal conductance and cellular integrity precede those for photochemistry. *New Phytologist*, 223 (1): 134-149.

Tsimilli-Michael, M., Strasser, R. J. (2013) The energy flux theory 35 years later: formulations and applications. *Photosynthesis Research*, 117 (1): 289-320.

Tyree, M. T., Sperry, J. S. (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. *Plant Physiology*, 88 (3): 574-580.

Tyree, M. T., Sperry, J. S. (1989) Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Biology*, 40: 19–36.

Tyree, M. T., Zimmermann, M. H. (2002) *Xylem structure and the ascent of sap*. 2. ed. Berlin: Springer, 284p.

Vadez, V. (2014) Root hydraulics: the forgotten side of roots in drought adaptation. *Field Crops Research*, *165*: 15-24.

Vadez, V., Rao, J. S., Bhatnagar-Mathur, P., Sharma, K. K. (2013) DREB1A promotes root development in deep soil layers and increases water extraction under water stress in groundnut. *Plant Biology*, 15 (1): 45-52.

Valentini, R., Epron, D., De Angelis, P., Matteucci, G., Dreyer, E. (1995) In situ estimation of CO_2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. cerris* L.) leaves: diurnal cycle under different level of water supply. *Plant, Cell and Environmental*, 18: 631–640.

Van Kooten, O., Snel, J. F. (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research*, 25 (3): 147-150.

Venancio, L. P., Filgueiras, R., Mantovani, E. C., do Amaral, C. H., da Cunha, F. F., dos Santos Silva, F. C., Althoff, D., dos Santos, R. A., Cavatte, P. C. (2020) Impact of drought associated with high temperatures on *Coffea canephora* plantations: a case study in Espírito Santo State, Brazil. *Scientific Reports*, 10 (1): 1-21.

Venturin, A. Z., Guimarães, C. M., de Sousa, E. F., Machado Filho, J. A., Rodrigues, W. P., Serrazine, Í. A., Bressan-Smith, R., Marciano, C. R., Campostrini, E. (2020) Using a crop water stress index based on a sap flow method to estimate water status in conilon coffee plants. *Agricultural Water Management*, 241: 106343.

Walter, H., Stadelmann, E. J. (1968) The physiological prerequisites for the transition of autotrophic plants from water to terrestrial life. *Bioscience*, 18: 694–701.

Wasaya, A., Zhang, X., Fang, Q., Yan, Z. (2018) Root phenotyping for drought tolerance: a review. *Agronomy*, 8 (11): 241.

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S. C., Prasad, S. S., Rebetzke, G. J., Kirkegaard, J. A., Christopher, J., Watt, M. (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany*, 63 (9): 3485-3498.

Wellburn, A. R. (1994) The spectral determination of chlorophyll a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, 144: 307-313.

Weraduwage, S. M., Chen, J., Anozie, F. C., Morales, A., Weise, S. E., Sharkey, T. D. (2015) The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. *Frontiers in Plant Science*, *6*: 167.

Werner, C., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., Schmulling, T. (2003) Cytokinin-deficient transgenic arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *The Plant Cell,* 15: 2532–2550.

Whitehead, D. (1998) Regulation of stomatal conductance and transpiration in forest canopies. *Tree Physiology*, 18: 633–644.

Worbes, M., Blanchart, S., Fichtler, E. (2013) Relations between water balance, wood traits and phenological behavior of tree species from a tropical dry forest in Costa Rica—a multifactorial study. *Tree Physiology*, 33 (5): 527-536.

Wright, I. J., Dong, N., Maire, V., Prentice, I. C., Westoby, M., Díaz, S., Rachael V. Gallagher, R. V., Bonnie F. Jacobs, B. F., Robert Kooyman, R., Elizabeth A. Law, E. A., Michelle R. Leishman, M. R., Ülo Niinemets, U., Peter B. Reich, P. B., Sack, L., Villar, R., Wang, H., Wilf, P. (2017) Global climatic drivers of leaf size. *Science*, 357 (6354): 917-921.

Wright, I. J., Westoby, M. (2002) Leaves at low versus high rainfall: coordination of structure, lifespan and physiology. *New Phytologist*, 155 (3): 403-416.

Xie, X., Wang, Y., Williamson, L., Holroyd, G. H., Tagliavia, C., Murchie, E., Hetherington, A. M. (2006) The identification of genes involved in the stomatal response to reduced atmospheric relative humidity. *Current Biology*, 16 (9): 882-887.

Xiong, D., Nadal, M. (2020) Linking water relations and hydraulics with photosynthesis. *The Plant Journal*, 101 (4): 800-815.

Xiong, Y. C., Li, F. M., Zhang, T., Xia, C. (2007) Evolution mechanism of non-hydraulic root-to-shoot signal during the anti-drought genetic breeding of spring wheat. *Environmental and Experimental Botany*, 59 (2): 193-205.

Xu, D.Q., Shen Y. (1996) Midday depression of photosynthesis. *In*: Pessarakli, M. (Ed.) *Handbook of photosynthesis*. 2. ed. New York: Marcel Dekker Inc., p.451-459.

Xu, Z. Z., Zhou, G. S., Wang, Y. L., Han, G. X., Li, Y. J. (2008) Changes in chlorophyll fluorescence in maize plants with imposed rapid dehydration at different leaf ages. *Journal of Plant Growth Regulation*, 27: 83–92.

Yin, Z., Meng, F., Song, H., He, X., Xu, X., Yu, D. (2010) Mapping quantitative trait loci associated with chlorophyll a fluorescence parameters in soybean (*Glycine max* (L.) Merr.). *Planta*, 231 (4): 875-885.

Yokoyama, G., Yasutake, D., Tanizaki, T., Kitano, M. (2019) Leaf wetting mitigates midday depression of photosynthesis in tomato plants. *Photosynthetica*, 57 (3): 740-747.

Young, L. W., Wilen, R. W., Bonham-Smith, P. C. (2004) High temperature stress of *Brassica napus* during flowering reduces micro and mega gametophyte fertility, induces fruitabortion, and disrupts seed production. *Journal of Experimental Botany,* 55: 485–495.

Yue, B., Xue, W., Xiong, L., Yu, X., Luo, L., Cui, K., Jin, D., Xing, Y., Zhang, Q. (2006) Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. *Genetics,* 172 (2): 1213–1228.

Zhang, F. P., Sussmilch, F., Nichols, D. S., Cardoso, A. A., Brodribb, T. J., McAdam, S. A. (2018) Leaves, not roots or floral tissue, are the main site of rapid, external pressure-induced ABA biosynthesis in angiosperms. *Journal of Experimental Botany*, 69 (5): 1261-1267.

Zhang, F.J., Zhang, K.K., Du, C.Z., Li, J., Xing, Y.X., Yang, L.T., Li, Y.R. (2015) Effect of drought stress on anatomical structure and chloroplast ultrastructure in leaves of sugarcane. *Sugar Tech*, 17: 41-48.

Zhou, Y., Lam, H.M., Zhang, J. (2007) Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. *Journal of Experimental Botany*, 58: 1207–1217.

Zhu, S. D., Chen, Y. J., Ye, Q., He, P. C., Liu, H., Li, R. H., Cao, K. F. (2018) Leaf turgor loss point is correlated with drought tolerance and leaf carbon economics traits. *Tree Physiology*, 38 (5): 658-663.

Zinn, K. E., Tunc-Ozdemir, M., Harper, J. F. (2010) Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany*, 61 (7): 1959-1968.

SUPPLEMENTARY DATA A

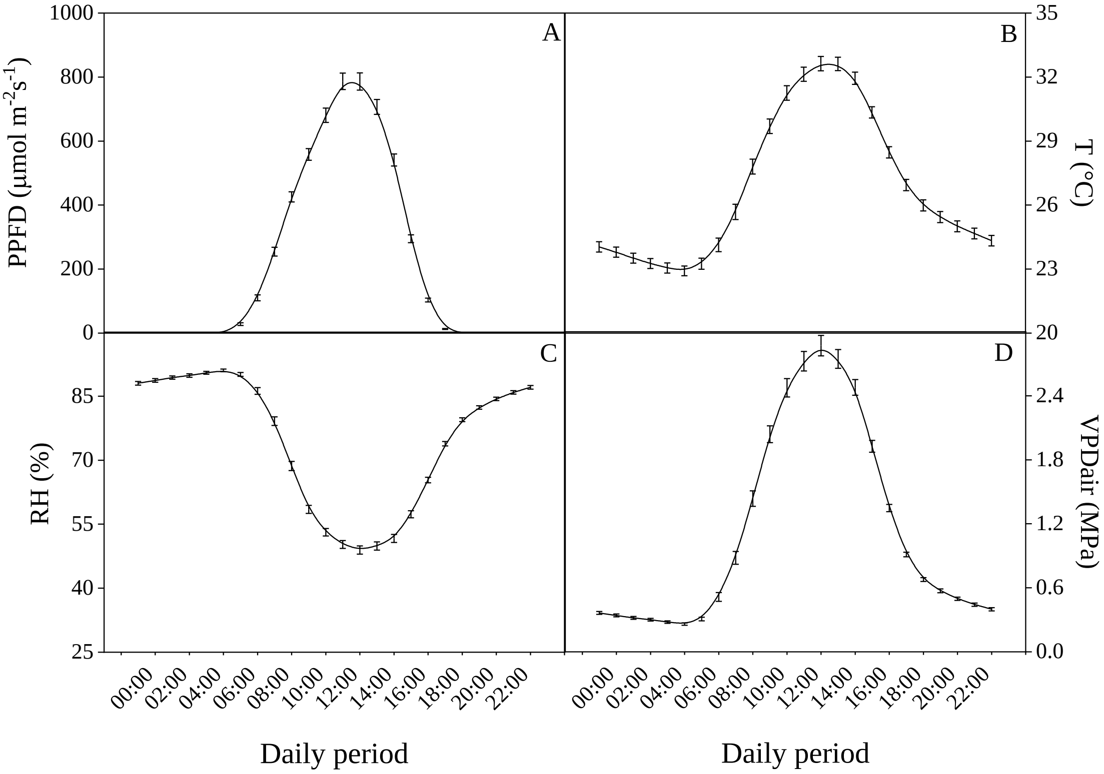

Figure S1. Horary medium values ± s.e. of photosynthetic photon flux density (PPFD, A), air temperature (T, B), relative humidity (RH, C), and air vapor pressure deficit (VPD_{air}, D) registered in a coffee growth period from May 2019 to April 2020.

SUPPLEMENTARY DATA B

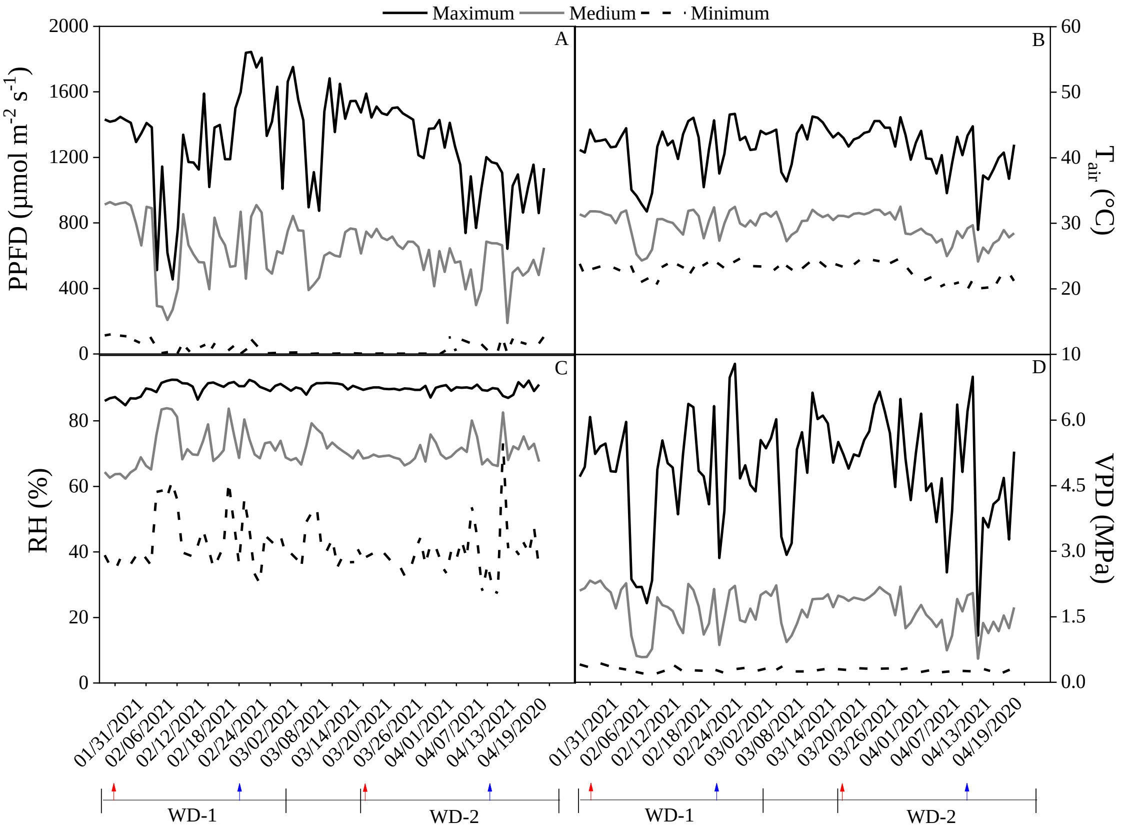

Figure S2. Maximum, medium and minimum values for photosynthetic active radiation (PPFD) (A), air temperature (T_{air}) (B), relative humidity (RH) (C), and air vapor pressure deficit (VPD_{air}) (D) registered among 7 a.m. to 6 p.m. in uninterrupted days, from beginning of the water withdrawn (January 26) until the end of the experiment (April 20).

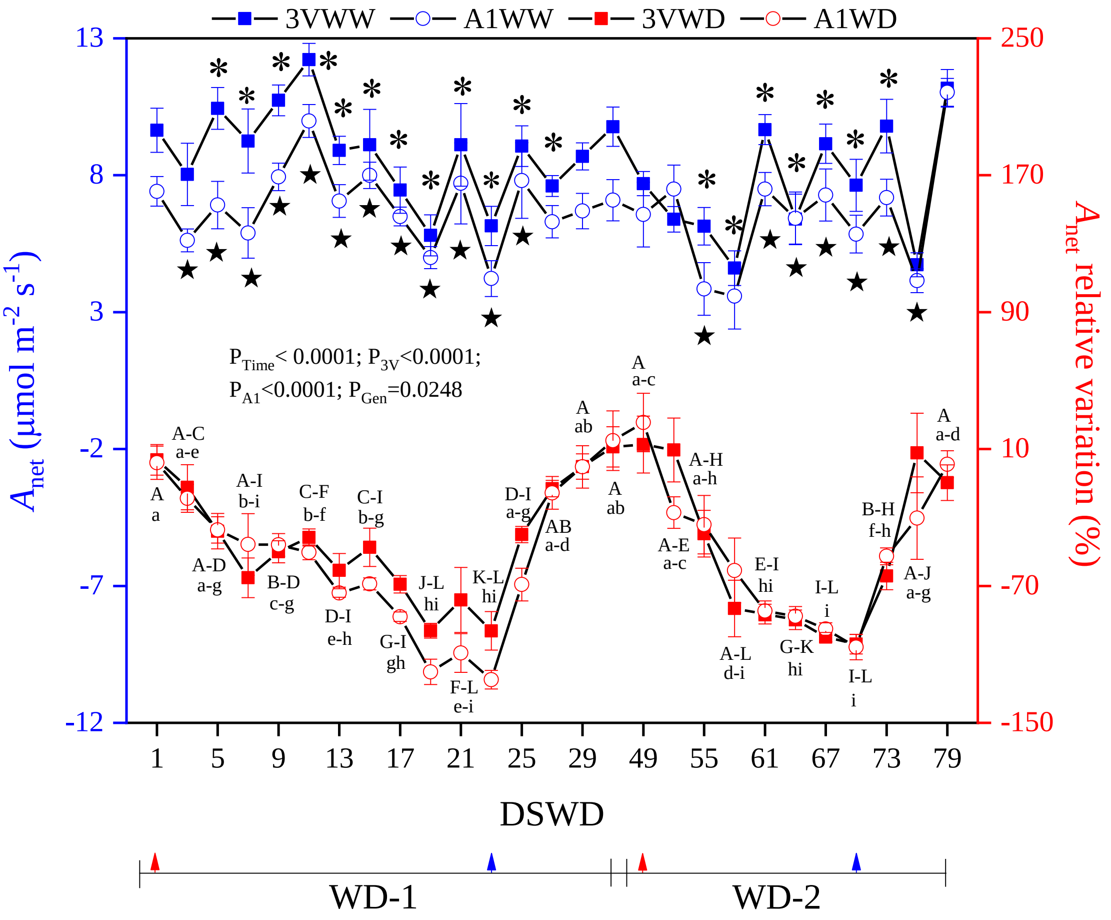

Figure S3. Medium values ± s.e. and ANOVA *P*-values for instantaneous net photosynthetic rate (A_{net}) (left y-axis) and A_{net} relative difference between WW (well-watered soil) and WD (soil water deficit) (right y-axis) in conilon coffee clones (3V and A1). The medium values followed by "*" and "*" express significant differences between soil water condition in '3V' and soil water condition in 'A1', respectively, at $p \leq 0.05$ using the Tukey test. The medium values followed by different lowercase (a,…,i) express significant difference between DSWD in '3VWD', whilst the medium values followed by different upper case (A,…,L) express significant difference between DSWD in 'A1WD' at $P \leq 0.05$ using the Tukey test. P_{Time}= express significance level in the DSWD; P_{3V}= express significance level for interaction between 3VWW:3VWD over the DSWD; P_{A1}= express significance level for interaction between A1WW:A1WD over the DSWD; P_{Gen}= express significance level for interaction between 3VWD:A1WD over the DSWD. Red arrow: beginning of soil water deficit; Blue arrow: rewatering.