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Physiological aspects, growth and yield of *Coffea* spp. in areas of high altitude

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Abstract

Low temperature and water deficit are the major climatic limitations for the coffee plant, affecting metabolic performance, development and yield. This work aims at evaluating the physiological responses of *Coffea* spp. genotypes grown in high-altitude (734 m) areas under field conditions, using 3-to-4-year-old plants of *C. arabica* cv. Catucaí Vermelho (785/15) and *C. canephora* cv. Encapa 8111 Clone 02 (02V). Predawn leaf potential water, net photosynthesis, stomatal conductance, internal-to-external CO_2 concentration ratio, and chlorophyll *a* fluorescence (JIP_{Test}) values were assessed in March (adequate temperature and water availability conditions), July (suboptimal temperature) and October (low water availability). The growth of orthotropic and plagiotropic branches and the number of nodes were monitored monthly for 13 months, whereas two years yield was evaluated. Low temperatures (July) affected photosynthesis of both genotypes, although 785/15 showed smaller after-effects by October and unaltered values of almost all fluorescence parameters throughout the year (reflecting a high functional stability), and a higher number of nodes with a potential positive impact on yield. The 02V plants also kept relevant photochemical functioning, maintaining the values of several JIP_{Test} parameters, a branch growth higher than 785/15, and a similar yield to that found for 02V in some low-altitude areas. Therefore, *C. canephora* was able to cope with moderate low temperature and water deficit constraints that prevail in areas of higher altitudes for part of the year, while maintaining yield performance. Therefore, this genotype shows considerable potential to be cultivated at higher altitudes than what is traditionally considered adequate for *C. canephora*.

Keywords: Coffee; Cold; Fast fluorescence kinetics; JIP_{Test}; Photosynthesis; Moderate water availability.
Abbreviations: *A*_net photosynthetic rate, ABS/CS_o_absorbed energy flux per cross section, ABS/RC_absorbed energy flux per reaction center, C_{Area}_estimate of the pool size of Q_A, Chl_chlorophyll, *Ci*/*Ca*_internal-to-ambient CO_2 concentration ratio, DI_o/RC_dissipation of energy flux per cross section, ET_o/ABS_quantum yield of electron transport flux from Q_A to Q_B, ET_o/CS_o_electron transport flux from Q_A to Q_B per cross section, ET_o/RC_specific energy flux for electron transport per reaction centre, ET_o/TR_o_efficiency/probability with which a PSII trapped electron is transferred from Q_A to Q_B, F_o_initial value of the fluorescence, F_J_fluorescence value at 2 ms, F_m_maximum fluorescence, F_v/F_m_ maximum quantum yield of PSII, g_s_stomatal conductance to water vapour, LHC_light harvesting complex, PAR_photosynthetically active radiation, PSII_photosystem II, Q_x_quinone, RC_reaction center, RC/CS_o_number of active reaction centers per cross section, TR_0/CS_o_energy flux trapped per cross-section, TR_o/RC_ energy flux trapped per reaction centre, Ψ_predawn leaf water potential.

Introduction

The genus *Coffea* includes at least 124 species, but *Coffea arabica* L. and *C. canephora* Pierre ex Froehner economically dominate the coffee trade (DaMatta and Ramalho, 2006; Davis et al., 2011). The world coffee bean production is somewhat above 8 million tonnes since 2010, most arising from South American and Asian countries, with Brazil leading with *ca*.2.7 million tonnes (ICO, 2015).

The main *C. canephora* genotypes (*e.g.*, Conilon and Robusta), usually grown at latitudes between 18° and 22° south, present the highest growth rates from September to

May, when the minimum and maximum temperatures are greater than 17 °C and less than 34 °C, respectively (Partelli et al., 2013). This species has an optimal average temperature range of approximately 22-26 °C (DaMatta and Ramalho, 2006; Rodrigues et al., 2016), showing clear reductions on both photosynthesis and growth when submitted to temperatures below *ca*. 18-20°C (Ramalho et al., 2003; Batista-Santos et al., 2011) and 17-18°C (DaMatta et al., 2007), respectively. Consequently, *C. canephora* has been considered to have poor acclimation ability to low temperatures, which are frequent at altitudes higher than 500 meters. On the other hand, *C. arabica* plants grown in latitudes between 16° and 20° south have their highest growth rates from September to March, when the minimum and maximum temperature averages are greater than 15 °C and less than 30 °C, respectively (Silva et al., 2004; Ferreira et al., 2013). Genotypes from this species can cope with short periods of temperatures as low as 4 °C (Ramalho et al., 2003; Partelli et al., 2009; Batista-Santos et al., 2011; Ramalho et al., 2014), therefore having a greater suitability to endure the somewhat lower temperatures found above 500 meters.

The coffee plant performance in terms of growth, development, biomass accumulation and productivity relies on their acclimation capability to environmental changes, which also depends on the quickness and duration of exposure, as well as the temperature range (Ramalho et al., 2014). As for other tropical species, both *C. arabica* and *C. canephora* present physiological and metabolic cold-related impacts, namely in net photosynthesis and in some sensitive key points of the photosynthetic apparatus (*e.g*., photosystem (PS) II), frequently accompanied by leaf tissue damage and shedding (Partelli et al., 2009; Batista-Santos et al., 2011). These impacts were associated with the cold-triggered oxidative stress, linked to an overproduction of excited molecules of oxygen and chlorophyll (Fortunato et al., 2010). Moreover, low temperatures can damage the coffee root system (Alonso et al., 1997; Queiroz et al., 2000). Still, some coffee genotypes showed relevant ability to cold acclimate to some extent. This was established to be related to significant reinforcement of antioxidative mechanisms, namely the photoprotective xanthophylls cycle and enzymes from the ascorbate-glutathione cycle (Ramalho et al., 2003; Fortunato et al., 2010), as well as to qualitative changes in the lipid matrix of chloroplast membranes (Partelli et al., 2011; Scotti-Campos et al., 2014) that maintained an adequate flexibility to membrane-related functions, such as thylakoid electron transport (Batista-Santos et al., 2011).

The *C. canephora* production strongly increased in the last decade, mostly by the increase production in Vietnam and the use of new genotypes in Brazil, where the Espírito Santo State congregates the largest production. That results from a significant increase in the demand for Robusta type of coffee (mostly obtained from *C. canephora* genotypes) of *ca*. 4% per year, mostly linked to the increased demand for soluble coffee (ICO, 2014), therefore increasing the importance of *C. canephora* in worldwide coffee trade. As a consequence,, the use of *C. canephora* cv. Conilon genotypes in areas of higher altitude increased considerably in recent years. This intended to couple Conilon's high productivity and vigour with the possibility of improved coffee bean quality at a higher altitude, as found for *C. arabica* (Abreu et al., 2012), in order to comply with an increasing consumer's demand.

Therefore, we aim at gathering knowledge of the impact on the photosynthetic apparatus, growth and yield under field conditions, in order to evaluate the possibility of the use of *C. canephora* selected genotypes in areas of high altitude, where it was submitted to moderate sub optimal temperatures and water availability limitation.

Results

Natural environmental conditions and leaf water status

The plants were monitored during three distinct periods of the year, in which were exposed to adequate temperature, high irradiance and unrestricted water supply (in March); to sub-optimal temperature and reduced water availability (in June-July); and to adequate temperature and reduced water supply (in October) (Fig. 1). In accordance to the seasonal rainfall, a gradual decrease of predawn leaf water potential (Ψ) was found from March until October in both genotypes. The highest and lowest Ψ values were observed in March (-0.47 MPa in 785/15; -0.33 MPa in 02V) and October (-1.38 MPa in 785/15; -0.99 MPa in 02V), respectively. Intermediate values were observed in July. *C. canephora* (02V) plants consistently showed somewhat higher values than *C. arabica* (785/15) plants, although significantly only in July (Fig. 2).

Assimilation and water loss control along the year

The highest net photosynthetic rates (*A*), reached *ca*. 9 µmol m^{-2} s^{-1} in both genotypes and were observed in March, confirming the suitability of the environmental conditions (related to air relative humidity and temperature, water availability and irradiance) for carbon assimilation (Fig. 3 A). In sharp contrast, the lowest annual *A* values (close to 3 µmol m^{-2} s^{-1} in both genotypes) were found in July, with significant decreases of *ca*. 70% (785/15) and 65% (02V), when compared to March values. Noteworthy, significant *A* differences were found between genotypes only in October, when 785/15 and 02V plants presented values close to 6 and 4 µmol m^{-2} s^{-1}, respectively. Such difference resulted from a partial recovery displayed mostly by 785/15 plants, although these values were still significantly below than those observed in March. The *A* changes were accompanied by a similar variation pattern in stomatal conductance to water vapour (g_s). Maximal absolute values for both genotypes (*ca*. 0.30 - 0.34 mol m^{-2} s^{-1}) were found in March (Fig. 3 B). Minimal g_s values (*ca*. 0.13 - 0.15 mol m^{-2} s^{-1}) were observed in July, with reductions of 57% (785/15) and 56% (02V) when compared to March values. Such close variation trends of *A* and g_s throughout the year (Fig. 3 A,B), on both genotypes, were implicated in the maintenance of the intercellular-to-external CO_2 concentration ratio (C_i/C_a) along the year (Fig. 3 C), and in the similar A/g_s best estimated fit, reflecting quadratic relationships (Fig. 4).

Photosynthetic apparatus performance of energy use

Regarding the JIP_{Test} parameters, the 785/15 plants showed a remarkable stability of all studied parameters, except for F_v/F_m, which presented a significant reduction in July. On the other hand, the 02V plants showed several significant modifications, therefore revealing changes in the pattern of light energy use/dissipation. Increases were observed in ABS/RC (13 and 40%), TR_o/RC (8 and 32%) and DI_o/RC (20 and 61%) in July and October, respectively, and significant decreases in the Q_A pool size (29%) in July, as well as in RC/CS_o in July (20%) and October (29%) (Table 1). For ET_o/RC, ET_o/TR_o, ET_o/ABS, TR_o/CS_o, ETo/CS_o and F_v/F_m the 02V plants did not show significant differences throughout the year. When comparing the genotypes, 785/15 showed higher ABS/RC, TR_o/RC, DI_o/RC, ABS/CS_o and

Table 1. Changes in the fluorescence parameters obtained from a JIP_{Test} analysis in March, July and October in *Coffea arabica* cv. Catucaí Vermelho 785/15 (785/15) and *Coffea canephora* cv. VitóriaIncaper 8142 Clone 02 (02V).

Genotype	March	July	October
ABS/RC [CV = 24.9%]			
785/15	1.09 ± 0.08 Aa	1.31 ± 0.09 Aa	1.26 ± 0.08 Aa
02V	0.93 ± 0.06 Ba	1.05 ± 0.05 ABb	1.30 ± 0.12 Aa
TR_o/RC [CV = 21.3%]			
785/15	0.83 ± 0.05 Aa	0.96 ± 0.05 Aa	0.95 ± 0.05 Aa
02V	0.74 ± 0.04 Ba	0.80 ± 0.03 ABb	0.98 ± 0.07 Aa
ET_o/RC [CV = 21.4%]			
785/15	0.39 ± 0.01 Aa	0.42 ± 0.01 Aa	0.46 ± 0.04 Aa
02V	0.40 ± 0.02 Aa	0.42 ± 0.03 Aa	0.45 ± 0.03 Aa
DI_o/RC [CV = 37.6%]			
785/15	0.26 ± 0.03 Aa	0.36 ± 0.04 Aa	0.31 ± 0.03 Aa
02V	0.20 ± 0.02 Ba	0.24 ± 0.02 ABb	0.32 ± 0.04 Aa
ET_o/TR_o [CV = 18.5%]			
785/15	0.47 ± 0.02 Ab	0.43 ± 0.03 Ab	0.49 ± 0.03 Aa
02V	0.55 ± 0.01 Aa	0.51 ± 0.02 Aa	0.47 ± 0.03 Aa
ET_o/ABS [CV = 20.8%]			
785/15	0.37 ± 0.02 Ab	0.32 ± 0.02 Ab	0.37 ± 0.03 Aa
02V	0.44 ± 0.01 Aa	0.40 ± 0.02 Aa	0.36 ± 0.03 Aa
ABS/CS_o [CV = 14.5%]			
785/15	8545 ± 372 Aa	8620 ± 331 Aa	9134 ± 298 Aa
02V	8294 ± 424 Aa	7514 ± 287 Ab	8088 ± 377 Ab
TR_o/CS_o [CV = 14.0%]			
785/15	6586 ± 305 Aa	6326 ± 200 Aa	6892 ± 163 Aa
02V	6558 ± 316 Aa	5814 ± 244 Aa	6164 ± 289Aa
ETo/CS_o [CV = 25.6%]			
785/15	3127 ± 256 Aa	2717 ± 170 Aa	3348 ± 210 Aa
02V	3630 ± 211 Aa	3030 ± 241 Aa	2971 ± 287 Aa
RC/CS_o [CV = 23.9%]			
785/15	8328 ± 735 Aa	6758 ± 300 Aa	7435 ± 312 Aa
02V	9211 ± 670 Aa	7358 ± 444 Ba	6565 ± 525 Ba
C_{Area} [CV = 29.8%]			
785/15	$1.04x10^6 \pm 0.11x10^6$ Aa	$0.82x10^6 \pm 0.07x10^6$ Aa	$0.95x10^6 \pm 0.06x10^6$ Aa
02V	$1.24x10^6 \pm 0.08x10^6$Aa	$0.88x10^6 \pm 0.08x10^6$ Ba	$1.00x10^6 \pm 0.09x10^6$ ABa
F_v/F_m [CV = 3.6%]			
785/15	0.79 ± 0.01 Aa	0.76 ± 0.01 Bb	0.77 ± 0.01 ABa
02V	0.80 ± 0.01 Aa	0.79 ± 0.01 Aa	0.78 ± 0.01 Aa

Each value represents the mean ± SE (n=12); different letters indicate significant differences along time within each genotype (A, B) or between genotypes for each data time (a, b), for a Tukey test at 5% probability. The ANOVA showed: significant differences between genotypes within the same month and between months for the same genotype for ABS/RC, TR_o/RC, DI_o/RC, DI_o/CS_o and F_v/F_m; significant differences only between genotypes within the same month for ET_o/TR_o, ET/ABS and ABS/CS_o; significant differences between months for the same genotype for RC/CS_o and C_{Area}. No significant differences were detected between genotypes within the same month and between months for the same genotype for ET_o/RC, TR_o/CS_o and ETo/CS_o.

Table 2. Average yield in 2011 and 2012 for *Coffea arabica* cv. Catucaí Vermelho 785/15 (785/15) and *Coffea canephora* cv. Vitória Incaper 8142 Clone 02 (02V), presented in volume (dm^3 $plant^{-1}$) and mass (g $plant^{-1}$). A conversion scale of 8 dm^3 coffee cherries per kg processed for 785/15 and 5.33 dm^3 coffee cherries per kg processed for 02V.

Genotype	Yield (dm^3 $plant^{-1}$)		Yield (g $plant^{-1}$)	
	2011	2012	2011	2012
785/15	4.15 b	6.17 a	562 b	771 a
02V	6.45 a	3.44 b	1210 a	647 b

Each value represents the mean (n=12). Different letters indicate significant differences between genotypes for each year for a Tukey test at 5% probability.

TR_o/CS_o values in July and ABS/CS_o values in October than did 02V. Conversely, 02V plants showed higher ET_o/TR_o, ET/ABS values in March and ET_o/TR_o, ET/ABS and F_v/F_m values than 785/15 in July.

Branch growth along the year and productivity

From December 2011 to November 2012, the 02V plants showed the greatest increase in orthotropic and plagiotropic branch lengths, with 5.34 cm and 3.36 cm $month^{-1}$, respectively, considering an average for the entire annual period. In this period, the 785/15 presented 2.75 and 2.06 cm $month^{-1}$ growth rates for the same parameters (Fig. 5). However, 785/15 produced a higher number of nodes per plagiotropic branch, with an average of 1.77 nodes $month^{-1}$ (with an annual total of 21.2 nodes/branch), as compared to 02V that produced 1.2 nodes $month^{-1}$ (with an annual total of 14.4 nodes/branch). Thus, for these spacing conditions, in one year, 785/15 produced more *ca*.6.8 nodes per plagiotropic branch than did 02V, which would increment its yield potential. Considering the first two annual yields, under non-irrigated conditions 785/15 produced 4.15 and 6.17 dm^3

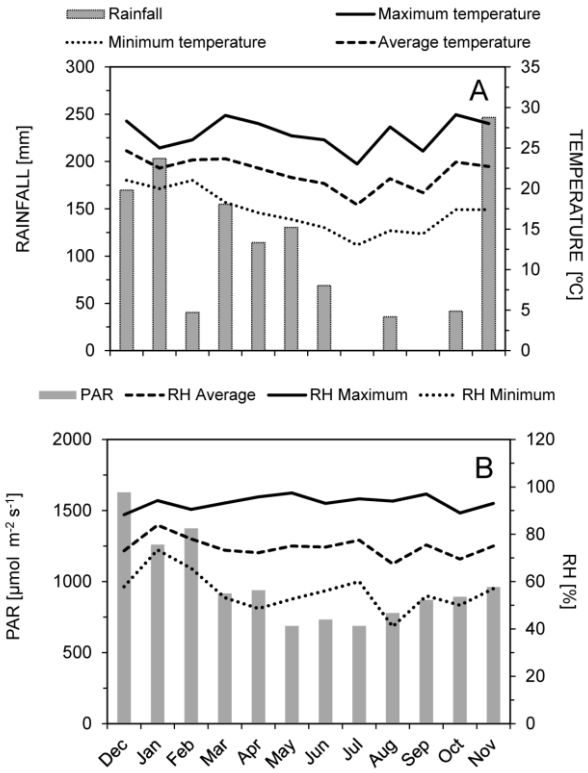

Fig 1. Monthly accumulated precipitation and temperature maximums, minimums and averages (A), the monthly relative-humidity, %RH, and the monthly average considering the daily maximum photosynthetic active radiation values, PAR (B). The data considered the period from December 2011 to November 2012 in high-altitude areas in Northwest Rio de Janeiro, Brazil.

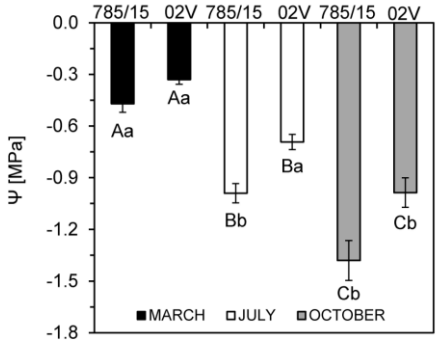

Fig 2. Variation of the predawn leaf water potential (Ψ) in March, July and October in *Coffea arabica* cv. Catucaí Vermelho 785/15 (785/15) and *Coffea canephora* cv. Vitória Incaper 8142 Clone 02 (02V) in high-altitude areas. Each value represents the mean ± S.E. (n=12). Below each data bar, different letters indicate significant differences between the months within each genotype (A, B, C) or between genotypes for each month (a, b). The ANOVA for Ψ showed significant differences between the genotypes within the same month and between months for the same genotype.

plant^{-1}, while 02V produced 6.45 and 3.44 dm^3 plant^{-1} in 2011 and 2012, respectively. These values, when converted to g plant^{-1} resulted in 562 and 771 in 785/15 and 1210 and 647 in 02V in 2011 and 2012, respectively, with an average yield of 666.5 g plant^{-1} for 785/15 and 928.5 for 02V (Table 2).

Discussion

Photosynthetic assimilation variation with temperature and water availability

In general, *C. arabica* growth rates are affected below 15 °C (Silva et al., 2004, Ferreira et al., 2013), although at 18 °C some *A* reduction can be already noted (Ramalho et al., 2003; Batista-Santos et al., 2011). On the other hand, *C. canephora* cv. Conilon showed impaired growth at minimum temperatures below 17 °C, but negative impacts on photosynthesis were reported to begin at 20 °C (Partelli et al., 2009; 2013), what agrees with the perception that *C. canephora* plants are less adaptable to lower temperatures than are *C. arabica* plants. In fact, only in the latter species were reported distinctive qualitative changes in the lipid matrix of chloroplast membranes, a stronger triggering and reinforcement of antioxidative mechanisms (Ramalho et al., 2003; Fortunato et al., 2010; Ramalho et al., 2014; Scotti-

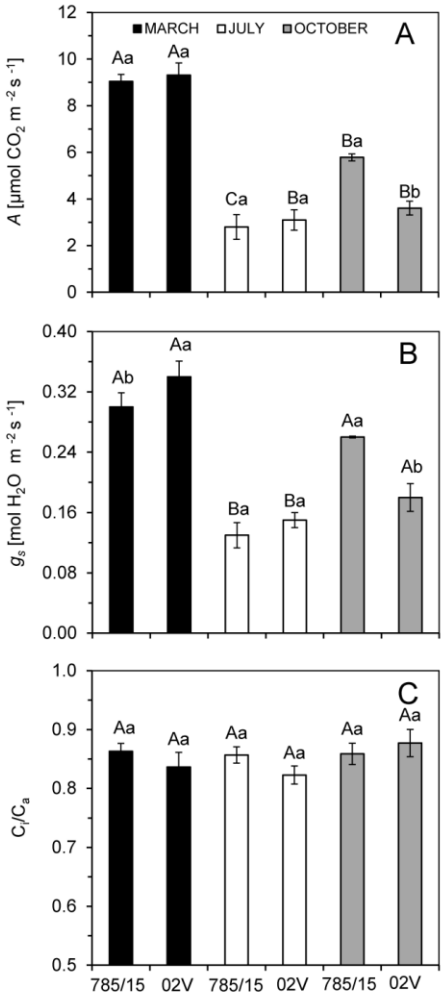

Fig 3. Changes in the CO_2 assimilation, *A*, (A) and stomatal conductance, g_s, (B) rates, as well as in the ratio of internal (C_i) to external (C_a) CO_2 concentration (C), as obtained in the middle morning period (9:00-10:30) in March (black bar), July (white bar) and October (grey bar) for *Coffea arabica* cv. Catucaí Vermelho 785/15 (785/15) and *Coffea canephora* cv. VitóriaIncaper 8142 Clone 02 (02V). Each value represents the mean ± S.E. (n=12). Above each data point, different letters indicate significant differences between the months within each genotype (A, B, C) or between genotypes for each month (a, b). The ANOVA for *A* and g_s showed significant differences between the genotypes within the same month and between months for the same genotype, while that for *Ci/Ca* showed no significant differences.

Campos et al., 2014), and a higher dynamics in some crucial mineral elements (Ramalho et al., 2013). Altogether, these responses work in tandem to better preserve the functioning of the photosynthetic apparatus, which will have a global metabolic positive impact. Our results showed significant reductions in *A* and g_s for both species (Fig. 3 A,B) in the coolest part of the year (July), when an average temperature of 18 °C was registered, but with minimum average of 13 °C. These *A* and g_s decreases could be related to the low night temperature and to reduced water availability (absence of rainfall in July, Fig. 1), as reflected by the Ψ reduction (Fig. 2). However, although the g_s decreases correlated well with those of *A* (Fig. 4), g_s maintained quite high values and would not constitute a key limitation to C-assimilation since the

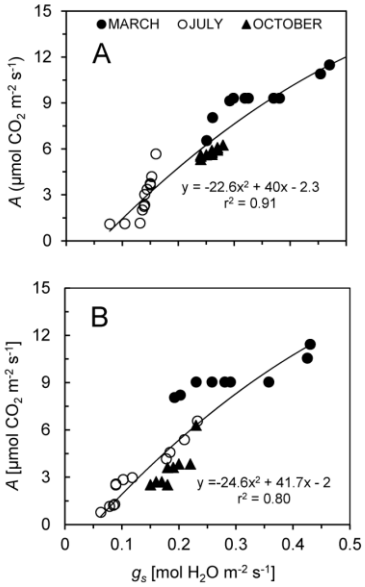

Fig 4. Best-fitting relationships between the CO_2 assimilation (*A*) and the stomatal conductance (g_s) rates in *Coffea arabica* cv. Catucaí Vermelho 785/15 (785/15) (A) and *Coffea canephora* cv. Vitória Incaper 8142 Clone 02 (02V) (B) using the individual values that were obtained in March (●), July (o) and October (*).

C_i/C_a ratio was not significantly changed (Fig. 3 C). Therefore, *A* decreases will be related to limitations, other than stomatal closure, possibly of biophysical and/or biochemical nature, configuring down-regulation events rather than mesophyll damages, as previously observed under moderate cold exposure of *Coffea* spp. plants (Ramalho et al., 2003; Partelli et al., 2009; Batista-Santos et al., 2011). However, the contribution of water shortage to *A* reduction through non-stomatal limitations cannot be discarded. In fact, in October, when the temperature values were already within a range that is considered adequate (Fig. 1) and the C_i/C_a value was unchanged, the net assimilation values were still clearly below those observed in March. Therefore, in October, when minimal Ψ values were reached, the low water availability would have been the main constraint to *A*. Notably these Ψ reductions to *ca*. -1.4 and -1.0 MPa in 785/15 and 02V, respectively, would configure only mild water deficit conditions, what allowed the maintenance of *A* relevant values, and further agrees with the reports of *A* reduction to negligible values only at predawn Ψ values close to -2.0 MPa (Lima et al., 2002). Our results further indicate that although they displayed a lower Ψ than the 02V plants did, the 785/15 plants showed in October a better cold recovery than the 02V plants, further agreeing with the findings of a faster and nearly complete recovery of *C. arabica* genotypes compared to *C. canephora* ones within two weeks after the end of chilling exposure and transfer to adequate temperature (Ramalho et al., 2003; Partelli et al., 2009; Batista-Santos et al., 2011).

Energy capture, use and dissipation by the photosynthetic machinery

Concomitant with the limitations at the biochemical level, non-stomatal effects are also frequently associated with

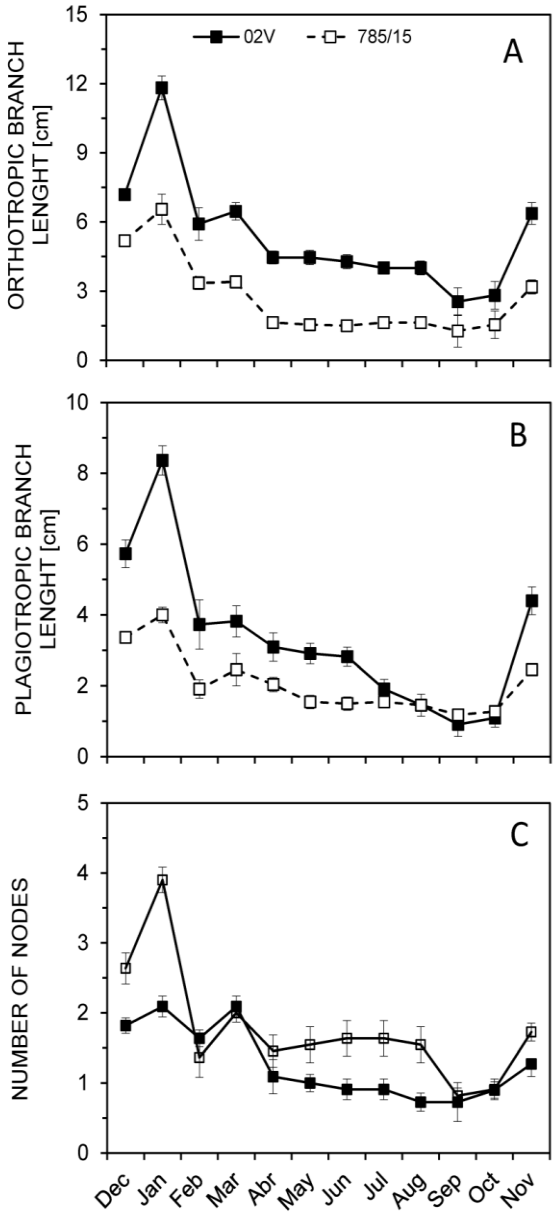

Fig 5. Monthly increment in orthotropic (A) and plagiotropic (B) branch lengths and the number of nodes per plagiotropic branch (C) from December 2011 to November 2012 in *Coffea arabica* cv. Catucaí Vermelho 785/15 (785/15) and *Coffea canephora* cv. Vitória Incaper 8142 Clone 02V (02V).Each value represents the mean ± S.E. (n=12).

impairments in the capture and driving of light energy to photochemical and non-photochemical events. In this view, 02V showed changes in several fluorescence parameters throughout the year, although the PSII maximal photochemical efficiency (F_v/F_m) was preserved (Table 1). In contrast, in the 785/15 plants a significant effect was noted only in the F_v/F_m ratio by July (Table 1), but the values were maintained between 0.75 and 0.85, which are considered to reflect a good efficiency level (Ramalho et al., 2003; Partelli et al., 2009). The JIP_{test} (Table 1) provided useful information, showing some impacts on the functioning of the thylakoid photosynthetic structures were present under moderate low temperature and water shortage (July) and upon rewarming but still with low water availability (October). These impairments were mostly related to the electron transport and energy dissipation events, particularly in 02V. In fact, in July, the reduction in the Q_A pool size (C_{Area}) was paralleled to a reduction in the number of active PSII reaction centres per cross section (RC/CS_o), as also observed in peach (Martinazzo et al.,.2012). These changes could reduce the probability of the photon energy, absorbed by Chl, to be used in the electron transport chain (Brestic and Zivcak, 2013). However, these decreases in 02V plants did not implicate a reduction in the electron transport flux from Q_A onwards (ET_o/RC) or an increase in the energy dissipation processes (DI_o/RC), in contrast to what was observed in *Lathyrus* genotypes under drought conditions (Silvestre et al., 2014), suggesting that in the coffee plants, the performance of the electron transport system was maintained. The gradual submission to low temperatures during a sufficiently long period permits the triggering of acclimation mechanisms involving a wide range of metabolic, morphological and ultrastructural modifications, most of which are controlled by gene expression changes (Kratsch and Wise, 2000). It should be emphasised that *Coffea* spp. was reported to have the ability to reinforce their antioxidant defences upon cold stressful conditions. Such defences include several enzymatic and non-enzymatic antioxidant molecules (Fortunato et al., 2010), among them zeaxanthin, lutein and β-carotene, which act in tandem to perform chlorophyll de-excitation through thermal dissipation (Ramalho et al., 2003; Batista-Santos et al., 2011). These pigments, which are present in the LHC and in the reaction centres, showed higher amounts in some *C. arabica* than in *C. canephora* plants, agreeing with the present results. In fact, the dissipation processes (DI_o/RC) were maintained at a somewhat higher status in 785/15 than in 02V plants under cold (July) for similar *A* reductions and without significant changes in any of the JIP_{test} parameters in 785/15. This genotype showed a dissipation value (DI_o/RC) 38% higher in July, which was 50% higher than that of the 02V plants. Such enhancement of the dissipation capabilities further justified the F_v/F_m decrease in 785/15 to a value significantly lower than that in 02V, and was likely linked to the reinforcement of photoprotective energy dissipating pigments and not to damaging events, as previously found in coffee genotypes under moderate cold conditions (Partelli et al., 2009; Ramalho et al., 2014). Under mild water deficit (October), the C_{Area} values approached those observed in March in both genotypes. However, RC/CS_o was even more affected in 02V plants, although the absorbed photon flux per PSII reaction centre (ABS/RC) and the maximum trapped excitation flux per PSII (TR_o/RC) increased, whereas the absorbed photon flux per PSII reaction centre (ABS/RC) was maintained. This maintenance of the energy capture, concomitant with a reduction of the active PSII reaction centres, would increase the need for higher energy dissipation in 02V, agreeing with the *ca*. 60% enhancement of DI_o/RC compared to the March value. As observed in other species (Silvestre et al., 2014; Burssoti et al., 2010), these dissipation mechanisms were efficient in protecting the photosynthetic machinery and maintaining the photochemical efficiency of PSII, since ABS/CS_o, TR_0/CS_o, ET_0/CS_o and F_v/F_m ratio values were not affected throughout the year, most likely due to the moderate nature of the imposed water and cold stresses.

Annual plant branch growth pattern and yield performance

It is widely acknowledged for a large number of crop species

that morpho-agronomic traits studies are quite useful to evaluate genetic variability and differences in the ability to cope to specific environmental conditions (Ghosh et al., 2013; Roy et al., 2013), including for coffee (*e.g.*, Partelli et al., 2013; Rodrigues et al., 2014). The analysed growth parameters showed similar patterns in both genotypes, with the reduced growth phase being closely related to the dry season, as previously reported by Amaral et al. (2006). However, as the low water availability and the lower temperature periods are largely concomitant, both environmental variables could have contributed to the observed growth reduction. In fact, the average temperature in April did not explain the reduced growth *per se* since this value is within the range considered adequate for coffee (Sediyama et al., 2001). However, the minimum temperature, which correlates well with the branch growth reduction in *C. canephora* cv. Conilon (Partelli et al., 2013), was already below optimal values (Fig. 1). Additionally, growth might have been affected due to the ongoing fruiting stage between December and March/April, as fruit development imposes a strong competition for photoassimilates and minerals (Laviola et al., 2007), limiting the vegetative growth in favour of the development of reproductive organs (Amaral et al., 2006).

The 02V plants showed a higher growth of both orthotropic and plagiotropic (Fig.5 A,B) branches, even in these suboptimal temperature conditions. Yet, the number of nodes was higher in 02V as compared to that of 785/15 only during the December/January period, reversing between April and August, due to the fact that branch growth in *C. canephora* is affected below 17 °C (Partelli et al., 2013). Altogether, this resulted in a higher number of nodes in the 785/15 plants on a year basis. This is an important trait since the number of nodes is a good indicator of the amount of productive buds and, therefore, a major component of the potential productivity (Bonomo et al., 2004). Finally, it seems noteworthy that, in contrast to what is usually accepted for *C. canephora* genotypes, the 02V plants presented a good yield performance. In fact, the first two yields under these non-irrigated conditions resulted in an average production of 928.5 g plant^{-1}, which is higher than the average first two harvests reported for low altitude plantations for similar genotype (Bragança et al., 2001). Therefore, despite the observed impact on some photosynthetic related parameters, the 02V plants showed an interesting ability to cope with the environmental conditions of moderate lower temperature and water shortage.

Materials and Methods

Plant material and growth conditions

Three- to four-year-old plants from *C. arabica* L. cv. Catucaí Vermelho (785/15) planted from seeds and spaced 2.2 × 0.5 m, and *C. canephora* Pierre ex Froehner cv. Conilon Vitória Incaper 8142 Clone 02 (02V) according to Fonseca et al. (2004), planted from cuttings and spaced 3 × 1 m. This experiment was performed in northwest Rio de Janeiro, Brazil (20° 56'16" latitude and 41° 54'44" longitude), at an altitude of 734 m, with an oxisol type of soil. The plant species were managed according to soil analysis and phytosanitary control when needed, however without irrigation.

The climate conditions data were obtained from a meteorological station (*Thies Clima*, Adolf Thies GmbH and Co. KG, Hauptstraße, Göttingen) that was installed in the experiment area (Fig. 1). The determinations were carried out throughout the year or in three distinct periods: March (with adequate temperature and water availability conditions), July (with a temperature considered lower than adequate) and October (under rewarming conditions but with some water deficit).

Leaf water potential and gas exchange measurements

Leaf water potential (Ψ) was measured immediately after leaf excision at predawn in March, July and October 2012, using a pressure chamber (Model 1000, PMS Instrument Co., Albany, OR, USA), according to Schölander et al. (1965). Measurements were performed on twelve leaves (one per plant), which were adjacent to those used for gas exchange determinations.

The net photosynthetic (*A*) and stomatal conductance to water vapour (g_s) rates, as well as the internal-to-ambient CO_2 concentration ratio (C_i/C_a) were assessed in March, July and October 2012 using a portable open-system infrared gas analyser (LI-6200, Li-COR, Lincoln, NE, USA). The measurements were performed in the morning, beginning after *ca*. 3 hours of illumination (therefore, approximately 9-10:30, when preliminary work showed maximal photosynthetic rates) on full sunny days (with an irradiance of 1204 ± 39, 1020 ± 52 and 1253 ± 79 μmol m^{-2} s^{-1} in March, July and October, respectively). Determinations were carried out on the 3rd pair of recently mature leaves from several branches from the upper 3rd part of the plant (fully sun exposed), alternating between one side and the other of the planting line. During the measurements, the vapour pressure deficit between the leaf and the air was 2.74 ± 0.15 (March), 3.56 ± 0.09 (July) and 3.01 ± 0.11 kPa (October).

Chlorophyll a fluorescence analysis through the JIP$_{Test}$

Chlorophyll (Chl) *a* fluorescence was evaluated on the same non-detached leaves used for the gas exchange measurements, using a Pocket PEA fluorometer (Hansatech, King's Lynn, UK). The obtained values were used to perform a JIP$_{Test}$ analysis, which is based on a simple model of energy flow through PSII (Strasser et al., 2004). For this analysis, part of the sampled leaf was dark-adapted for *ca*. 30-40 min. using leaf clips (Hansatech) to turn the reaction centres into an "open" (oxidised Q_A) state (Bolhár-Nordenkampf et al., 1989). Thereafter, the leaf samples were exposed to saturating irradiance (3,500 μmol m^{-2} s^{-1}) to obtain the Chl *a* PSII fast fluorescence kinetic transients (OJIP). The signal was recorded at: 1) 50 μs, which is the initial point, F_0, (step **O**), indicating the minimal fluorescence signal that was immediately reached at the onset of illumination; 2) 300 μs (F300), which is required to calculate the initial slope of the relative variable fluorescence (V) kinetics M_0 (observed rate of Q_A reduction), where $M_0 = [(F_{300}-F_0)]/[(F_m-F_0)/0.25\ ms)]$; 3) 2 ms to obtain F_J (**J**-step); 4) 30 ms to obtain F_I (**I**-step); and 5) the maximum fluorescence value, F_m, (or **P** value), usually at *ca*. 0.5 s.

The JIP$_{Test}$ refers to a translation of the original fluorescence data into the calculation of the following biophysical parameters, all referring to time zero (onset of fluorescence induction). These quantify the PSII behaviour concerning:

1) the specific energy fluxes (per reaction centre, RC) a) for absorption (ABS/RC = $[M_o/((F_j - F_o)/(F_m - F_o))]/(F_v/F_m)$), reflecting the apparent antenna size of active PSII; b) for trapping (TRo/RC = $[1 - (F_o/F_m)]$; c) for electron transport ($ET_o/RC = [TR_o/RC] - M_o$); and d) for dissipation (DI_o/RC =

[ABS/RC] – [TR_o/RC]), reflecting the energy dissipation of the antenna system that is not used in the Q_A reduction;
2) the flux ratios or yields e) for the efficiency/probability with which a PSII trapped electron is transferred from Q_A to Q_B ($ET_0/TR_0 = 1 - [(F_j - F_o)/(F_m - F_o)]$), and f) the quantum yield of electron transport flux from Q_A to Q_B, reflecting the efficiency with which an absorbed photon results in the electron transport beyond Q_A^- ($ET_0/ABS = [(1 - F_o/F_m)\times ET_o/TR_o]$); 3) the phenomenological energy fluxes per excited cross-section (or density) (CS), g) for absorption ($ABS/CS_o = F_o$), h) trapping ($TR_0/CS_o = [F_v/F_m]\times[ABS/CS_o]$) and i) electron transport ($ET_0/CS_o = [F_v/F_m]\times[ET_o/TR_o]\times[ABS/CS_o]$). Furthermore, j) the number of active reaction centres per cross section ($RC/CS_o = [F_v/F_m \times \{[(F_j - F_o)/(F_m - F_o)]/M_o\}\times F_o]$), k) the pool size of Q_A through the complementary area (C_{Area} = area between fluorescence curve and F_m), and the l) maximum quantum yield of PSII (F_v/F_m) were also calculated (Force et al., 2003; Ripley et al., 2004; Strasser et al., 2004; Strauss et al., 2006; Bussotti et al., 2010; Stirbet and Govindjee, 2011; Silvestre et al., 2014).

Branch growth and productivity evaluation

Orthotropic and plagiotropic branch lengths were obtained using a graduated ruler (minimal division: 1 mm) from twelve branches (one per plant). The number of nodes per plagiotropic branch was obtained on a monthly basis from December 2011 to December 2012 by visual counting, alternating between one side and another in the planting line. The two first harvests were performed in May-June, both in 2011 and 2012. The yield volume was obtained in dm^3 $plant^{-1}$ and transformed into g $plant^{-1}$, considering a conversion factor of 8 dm^3 of coffee fruit per kg of processed grain for 785/15 (Carvalho et al., 2009) and 5.33 dm^3 of coffee fruits per kg of processed grain for 02V (Partelli et al., 2014).

Statistical analysis

A completely randomised design with 12 replicates in a factorial scheme of 3 × 2 with three seasons and two genotypes was used to evaluate the leaf gas exchanges, Chl *a* fluorescence and Ψ parameters, whereas for the growth data, a factorial scheme of 12 × 2 with 12 dates (13 months but one is subtracted to calculate monthly increment) and two genotypes was used. The various measured and calculated parameters were analysed through a two-way ANOVA ($P \leq 0.05$) (except when stated otherwise), to evaluate the differences between the dates or genotypes, followed by a Tukey test for mean comparisons among the dates for each genotype or between genotypes for each date. A 95% confidence level was used for all tests. For the sake of simplicity concerning the ANOVA results, only the significant differences (as related to the date, genotypes or the interaction date × genotype) were shown in the figure and table captions for each parameter.

Conclusions

Coffea arabica cv. Catucaí Vermelho 785/15 (785/15) and *Coffea canephora* cv. Encapa 8111 Clone 02 (02V) plants were grown in areas of high altitude (734 m). When subject to low positive temperatures (July), both genotypes showed large reductions in *A* and g_s, albeit maintaining most of the photochemical performance. After the end of the lower temperature period, and even under mild water deficit (October), *C. arabica* presented a somewhat better recovery of *A* and g_s than *C. canephora*. Such better, although partial, *A* recovery in 785/15 plants would be linked to the maintenance of a higher photochemical functional status reflected in the absence of significant changes in the almost all of the JIP_{Test} parameters. Concerning the 02V plants, despite significant impacts on some of the photosynthetic-related parameters under cold (July) and in the period thereafter (October), several JIP_{Test} parameters were maintained (ET_o/RC, ET_o/TR_o, ET_o/ABS, ABS/CS_o, ET_o/CS_o and F_v/F_m), reflecting the preservation of appreciable photochemical functioning under the experimental conditions. Furthermore, and most important, a similar yield to that in low-altitude areas was obtained for 02V. These results showed that JIP_{Test} analysis can be a useful probe for the evaluation of coffee photosynthetic performance, and that 02V genotype shows considerable potential to be cultivated at higher altitudes than what is traditionally considered adequate for *C. canephora*, therefore, opening new cultivation perspectives to this coffee species.

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