A Glimpse of Climate Change Impact on *C. arabica* L. and *C. canephora* Pierre ex A. Froehner Physiology – The Combined Effects of Enhanced Growth CO_2 and Temperature

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SUMMARY

The effective impact of climate changes on the coffee plant physiology, promoted by enhanced air [CO_2] and global warming remain to be fully elucidated through biological studies. Therefore, this work aims at linking important coffee physiological responses to environmental changes of enhanced growth [CO_2] and temperature on genotypes from the two major producing species. Potted plants from *C. arabica* cv. IPR 108 and of *C. canephora* cv. Conilon Clone 153 were grown under environmental controlled conditions, either at 380 or 700 μL CO_2 L^{-1} air, for 1 year, without water, nutrient or root development restrictions. After that the temperature was gradually increased from 25/20 ºC (day/night) up to 42/34 ºC. The long-term impacts of enhanced growth [CO_2] and enhanced temperature on the photosynthetic functioning were assessed at 25/20 ºC, 31/25 ºC, 37/30 ºC and 42/34 ºC, through leaf gas exchanges (rates of net photosynthesis, P_n, stomatal conductance, g_s, transpiration, T_r, and photosynthetic capacity, A_{max}), instantaneous water use efficiency (iWUE), fluorescence parameters (photochemical efficiency of the photosystem II under dark, F_v/F_m, and light, F_v'/F_m', conditions, as well as the photochemical, q_P, and non-photochemical, NPQ, quenchings, and quantum yield of the linear electron transport, ϕ_e), photosynthetic pigments (chlorophyll and carotenoids) and some molecules with antioxidant role (ascorbate and α-tocopherol). The results showed that enhanced [CO_2] stimulates photosynthetic functioning, without negative down-regulation. Minor impacts were found in the photochemical performance until 37 ºC, but extensive impacts were shown at 42 ºC, especially in IPR108.

Remarkable was the finding that enhanced [CO_2] preserved a higher functional status (P_n, A_{max}, F_o, F_v/F_m) at high temperatures (37 and 42 ºC), what seems quite relevant under the predicted climate changes and global warming scenarios.

INTRODUCTION

Coffee is one of the world's most traded agricultural products. Modeling studies have predicted that climate change and global warming will have a strong impact on the suitability of current cultivation areas and coffee biodiversity, but these studies did not anticipate potential mitigating effects of the increasing atmospheric [CO_2], as no information exists on the long-term effects of high [CO_2] and temperature on this plant.

The tolerance of the photosynthetic pathway is of crucial importance regarding plant acclimation to environmental variations, including changes in growth [CO_2], to which effects on photosynthesis and stomata were found in *Coffea* spp.. Under enhanced growth [CO_2], C_3 plants often presents 50% increases in the photosynthetic rate (P_n), even if a partial down-regulation (negative acclimation) of the photosynthetic apparatus occurs. The latter is often related to limitations on sink strength that prevents the plant from fully utilizing the higher photosynthate production. That may lead to an increase in non-structural carbohydrates (NSC) that in turn could depress gene expression and the amount/activity of photosynthetic enzymes, including RuBisCO, or reduce the levels of all components of the photosynthetic apparatus. Such impact also depends on the interactions with other environmental limitations. Nonetheless, an increase of P_n is commonly reported, due to the direct effect of a higher substrate (CO_2) availability and to a competitive inhibition of CO_2 over O_2 at the carboxylation sites of RuBisCO, reducing the photorespiration rate. In fact, in C_3 plants such photorespiration reduction under CO_2 enrichment is expected to enhance P_n to a greater degree at high than at low temperature, thereby, at least partially, offsetting the effects of supra-optimal temperatures on yield.

To our knowledge, we report here the first results concerning the physiological responses of the photosynthetic apparatus to elevated atmospheric [CO_2] and temperature in genotypes of the two major coffee producing species.

MATERIALS AND METHODS

Plant material and experimental design

Plants with *ca*. 1.5 years from *C. arabica* L. cv. IPR 108 (IPR108) and *C. canephora* Pierre ex A. Froehner cv. Conilon Clone 153 (CL153), were transferred into walk-in growth chambers (EHHF 10000, ARALAB, Portugal) and grown in 28 L pots under controlled conditions of temperature (25/20 ºC, day/night), irradiance (*ca*. 650-800 μmol m^{-2} s^{-1}), RH (75%), photoperiod (12 h), and either 380 μL CO_2 L^{-1} (380) or 700 μL CO_2 L^{-1} (700) air for 1 year, without water, nutrient or root development restrictions. Thereafter the temperature was increased from 25/20 ºC up to 42/34 ºC, at a rate of 0.5 ºC day^{-1}, with a 7 days temperature stabilization at 31/25, 37/30 and 42/34 ºC to allow analysis. Some parameters were analyzed 4 weeks later at 37/30 ºC to assess plant recovery. Analyses were performed on newly matured leaves.

Leaf gas exchanges

Gas exchanges were evaluated under both [CO_2], at *ca*. 650-800 μmol m^{-2} s^{-1} of irradiance, using an open-system infrared gas analyzer (Li-Cor 6400, LiCor, Lincoln, USA), and

included net photosynthesis (P_n), stomatal conductance (g_s),and transpiration (T_r) rates, as well as the instantaneous water use efficiency (iWUE, calculated as P_n/T_r). The photosynthetic capacity (A_{max}), expressing the photosynthetic apparatus potential under saturating light (900 μmol m^{-2} s^{-1}) and CO_2 (*ca*. 7%) and optimal temperature (25 ºC) conditions, was measured through O_2 evolution in a Clark-type O_2 electrode (LD2/2, Hansatech, UK), according to [3].

Chlorophyll a fluorescence parameters

The fluorescence parameters were determined on the same leaves used for the gas exchange evaluations, using a PAM-2000 system (H. Walz, Effeltrich, Germany), and included the minimal antennae fluorescence (F_o) and the maximal photochemical efficiency of the photosystem (PS) II (F_v/F_m) under dark-adapted conditions, as well as the actual PSII efficiency of energy conversion (F_v'/F_m'), the photochemical (q_P) and non-photochemical (NPQ) quenchings, and quantum yield of the linear electron transport (ϕ_e), all under photosynthetic steady-state conditions [8]. Growth irradiance for photosynthetic stimulation and 7500 μmol m^{-2} s^{-1} saturating flashes were used for these determinations.

Photosynthetic pigments

Total chlorophyll (Chl) and carotenoid contents were evaluated as in [3].

Non-enzyme antioxidants

Molecules with antioxidant role (ascorbate and α-tocopherol) were quantified in frozen samples (collected to liquid N_2 and kept at -80 ºC), as optimized for coffee.

RESULTS AND DISCUSSION

Leaf gas exchanges

Globally, it is known that *C. canephora* usually display better tolerance to higher temperatures than *C. arabica*, due to a higher temperature optimum, whereas the latter species can better cold acclimate, with the photosynthetic apparatus having a crucial role on the plant tolerance. The results showed that, in both genotypes, P_n presented significantly higher values when measured under enhanced [CO_2], irrespective of the temperature, probably linked to the inhibition of photorespiration related to the higher CO2 availability. Such increase was particularly clear in IPR108 that more than doubled the P_n rates found at the control temperature (25ºC). At 37ºC IPR108 showed maximal P_n values, for both [CO_2], whereas CL153 maintained the values observed already at 31ºC. Yet, both genotypes showed significant P_n reductions upon 42 ºC, especially in CL153-380 and IPR108-700. However, at this temperature the 700 plants still presented P_n values 140% (CL153) and 30% (IPR108) higher than their respective 380 μL CO_2 L^{-1} ones (Fig. 1).

The g_s increased at 37 and/or 42 ºC, particularly in the CL153-380 (200 and 50%) and IPR108-380 (210 and 217%) plants, although the maximal values were observed in IPR108-700. Such temperature impact on g_s provoked strong iWUE reductions on both genotypes and growth [CO_2], but the iWUE values were always higher in plants grown in enhanced [CO_2].

The A_{max} values showed somewhat higher absolute values for the plants grown at high [CO_2] at 25/20 ºC and 31/25 ºC in CL153, whereas IPR-700 plants presented similar values to those of IPR-380 for these temperatures. Also, IPR108 showed higher heat sensitivity at 37 ºC than

CL153, when comparing the plants grown under 380 µL CO_2 L^{-1}. For the extreme 42 ºC both genotypes were clearly affected, irrespective of [CO_2], but the 700 plants showed higher absolute values and lower impact than the 380 ones.

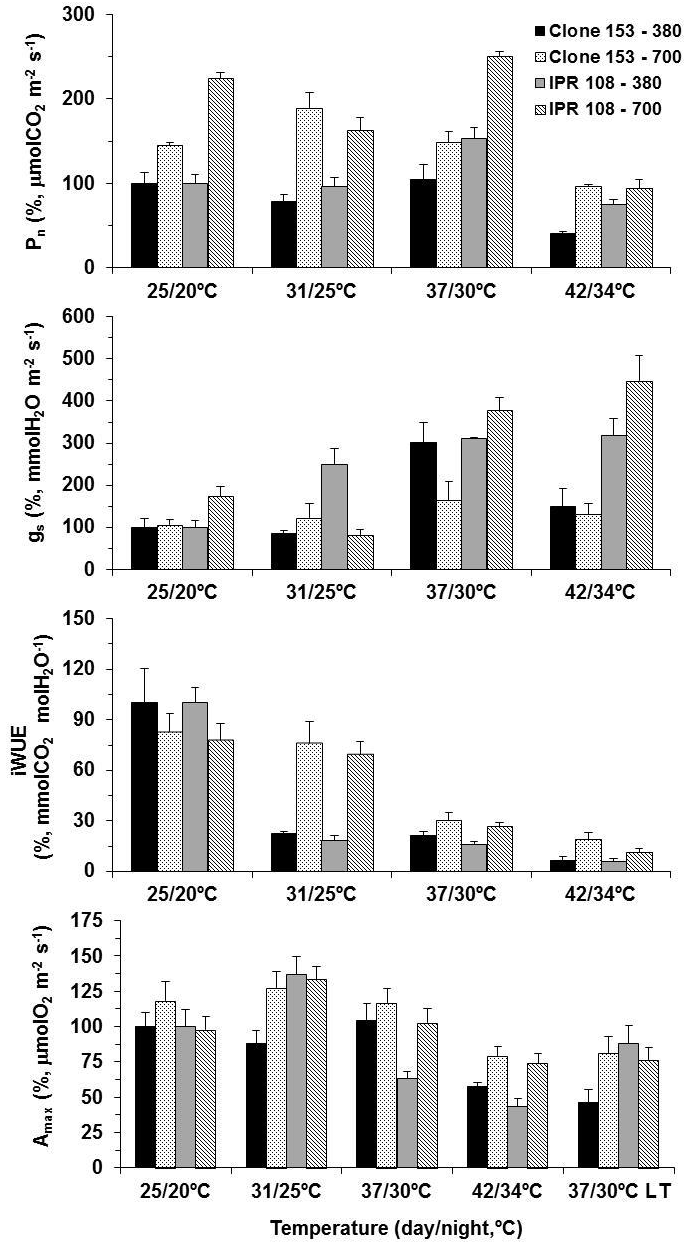

Figure 1. Changes (in %, within each genotype, relative to the 380 µL CO_2 L^{-1} plants at 25/20 ºC) for net photosynthesis (P_n), stomatal conductance (g_s), and photosynthetic capacity (A_{max}), rates, as well as to instantaneous water use efficiency (iWUE). Each value represents the mean ± SE (n=5-8).

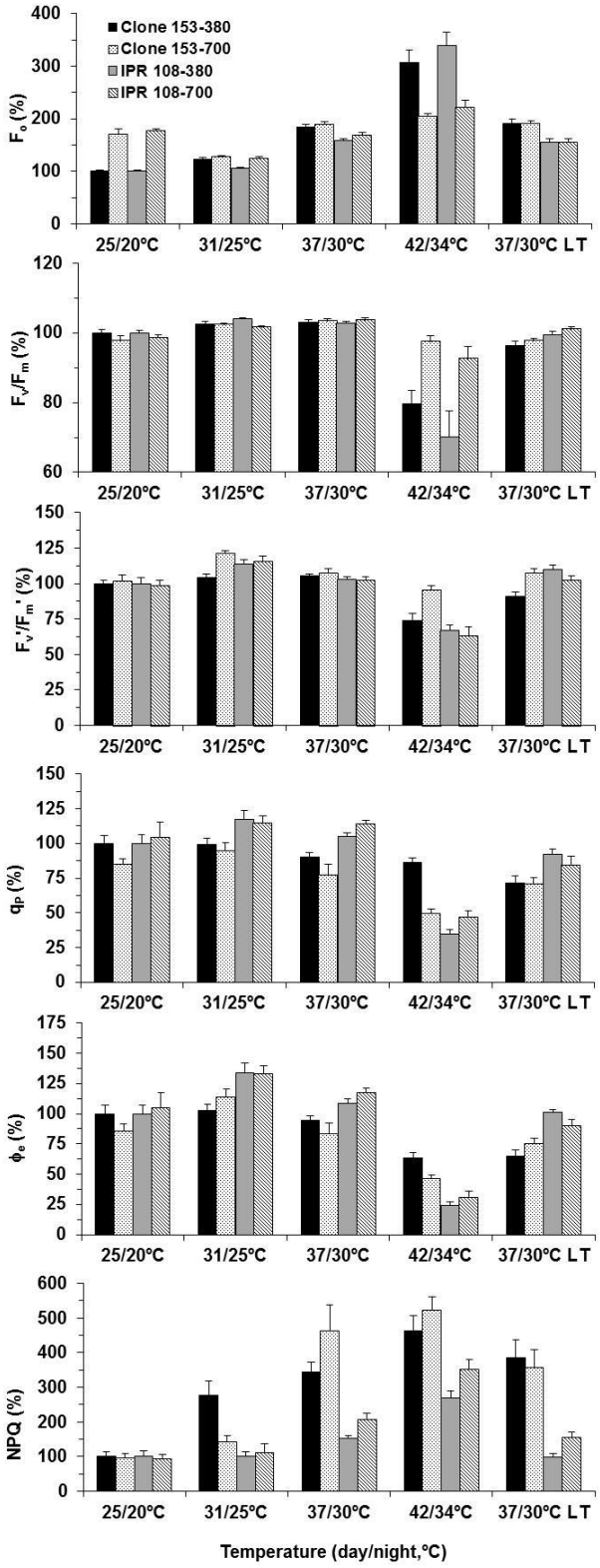

Figure 2. Changes (in %, within each genotype, relative to the 380 µL CO_2 L^{-1} plants at 25/20 ºC) for minimal fluorescence (F_o), maximal photochemical efficiency of the photosystem II (F_v/F_m), actual PSII efficiency of energy conversion (F_v'/F_m'), photochemical (q_P) and non-photochemical, (NPQ) quenchings, and quantum yield of the linear electron transport (ϕ_e). Each value represents the mean ± SE (n=5-8).

The results confirm the absence of photosynthetic down-regulation associated with high [CO_2], either at adequate or supra-optimal temperature. Also, as no stomatal closure occurred, the given P_n reductions would be linked to high temperature mesophyll impairments, as confirmed by the impact on A_{max}.

These were not-easily reverted as they were partially maintained upon the return to 37 ºC. Still, lower impacts were found at 37 ºC and, especially, at 42 ºC, in the plants grown at enhanced [CO_2], suggesting the maintenance of significantly higher functioning capability when compared to plants grown at normal [CO_2]. Moreover, the saturating irradiance to obtain A_{max} was always higher in plants under enhanced CO_2, suggesting that those plants might endure higher irradiance levels (data not shown).

Chlorophyll a fluorescence analysis

The stronger impact on the 380 plants at 42 ºC was further noted by the decline of F_v/F_m associated with an increase of F_o, the latter indicating that the threshold for irreversible damage was reached. This could be attributable to not readily reversible photoinhibitory impairments on PSII centers, possibly related to D1 protein loss, as suggested for coffee under high irradiance levels. Yet, such F_o rise could also be partially linked to the total chlorophyll increase (see below). Furthermore, part of the decrease in F_v/F_m and F_v'/F_m' might be related to photoprotective thermal energy dissipation processes, reflected in the moderate (for a maximum of 1.5) NPQ increase, although the highest NPQ rises occurred in the 700 plants that were less affected than the 380 ones (in F_v/F_m for both genotypes and in F_v'/F_m' for CL153). Negative effects at the extreme temperature of 42 ºC were further observed in q_P and ϕ_e, although without a clear tendency in relation to growth [CO_2].

Photosynthetic pigments and non-enzyme antioxidants

Both total Chl and total carotenoids (expressed on a dry weight basis) were maintained at similar or somewhat lower contents in the 700 plants than in 380 ones in the two genotypes (data not shown). Notably, both pigments tended to increase at higher temperatures, showing maximal values at 37 or 42 ºC, in what seemed to be a reinforcement of photosynthetic structures with heat exposure. Chl increased significantly only in IPR108 (*ca*. 35% at 37 ºC in both [CO_2]), whereas carotenoids showed significant rises in CL153-380 (19% at 42 ºC), IPR108-380 (37% at 37 ºC) and IPR108-700 (35% at 42%). Yet, the Chl (*a*/*b*) ratio followed an opposite trend reflecting a preferential Chl *b* synthesis, suggesting the occurrence of functional readjustments in the photosynthetic structures. These could include a higher proportional reinforcement of light harvesting chlorophyll-protein complex (LHCII) that contains the majority of Chl *b* (and a Chl *a*/*b* ratio around 1.4), instead of PSI that has a much higher Chl *a*/*b* ratio.

The higher functional status of the 700 plants, as compared to the 380 ones, could justify a lower need of antioxidant molecules. Yet, irrespective of genotype and [CO_2], the ascorbate content strongly decreased with the temperature rise, whereas α-tocopherol was increased in IPR108 and maintained in CL153 (data not shown), suggesting different roles in response to heat stress.

In conclusion, enhanced growth [CO_2] stimulates photosynthetic functioning, without down-regulation of photosynthesis. The photochemical functioning of coffee plants remained mostly unaffected until 37 ºC although considerable impacts were depicted at 42 ºC, especially in IPR108 when compared to CL153. Moreover, enhanced [CO_2] preserved a

higher functional status (*e.g.*, P_n, A_{max}, F_o, F_v/F_m) at high temperatures (37 and 42 ºC), what may constitute an important feature under the predicted climate changes and global warming scenarios.

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