Thylakoid Lipids Changes May Account for Photosynthetic Acclimation Ability of Two *Coffea* Species Subjected to Heat

P. SCOTTI-CAMPOS[1,2], I.P. PAIS[1,2], F.C. LIDON[2], L.D.MARTINS[3,4], M.A. TOMAZ[4], J.N. SEMEDO[1,2], A.P. RODRIGUES[6], A.F. FORTUNATO[3], I. PALOS[3], P. BATISTA-SANTOS[3], A.E. LEITÃO[2,3], E. LOPES[3], M.C. SIMÕES-COSTA[3], L. GOULAO[3], A.I. RIBEIRO-BARROS[2,3], M.J. SILVA[3], R. MAIA[5], C. MÁGUAS[5], F.H. REBOREDO[2], M.F. PESSOA[2], L.E. MORAIS[7], L.M. SANGLARD[7], W.L. ARAÚJO[7], R. GHINI[8], F.M. DAMATTA[7], J.C. RAMALHO[2,3*]

[1] Unid. Investigação em Biotecnologia e Recursos Genéticos, Inst. Nac. Inv. Agrária e Veterinária, I.P., Qta. Marquês, Av. República, 2784-505 Oeiras, Portugal
[2] CICEGe, Fac. Ciências Tecnologia, Univ. Nova Lisboa, 2829-516 Caparica, Portugal
[3] Grupo Interações Planta-Ambiente & Biodiversidade, Centro Ambiente, Agricultura e Desenvolvimento (BioTrop), Instituto Investigação Científica Tropical, I.P., Qta. Marquês, Av. República, 2784-505 Oeiras, Portugal
[4] Dept. Produção Vegetal, Centro Ciências Agrárias, Univ. Federal Espírito Santo, Alto Universitário, CP. 16, CEP 29500-000, Alegre, ES, Brazil
[5] Stable Isotopes and Instrumental Analysis Facility (SIIAF), Center for Environmental Biology, Fac. Sciences, Univ. Lisbon, Campo Grande, 1749-016 Lisboa, Portugal
[6] Centro de Estudos Florestais, DRAT, Inst. Sup. Agronomia, Univ. Lisboa, Tapada da Ajuda, 1349-017 Lisboa Portugal
[7] Dept. Biologia Vegetal, Univ. Federal Viçosa, 36570-000 Viçosa, MG, Brazil
[8]Embrapa Environment, Jaguariúna, SP, Brazil.

SUMMARY

Coffee is one of the world's most traded agricultural products, and its production could be threatened by global warming. The aim of this work was to evaluate the effects of heat on photosynthetic activity and thylakoid membrane lipid dynamics, on genotypes of the two major coffee producing species. Potted plants from *C. arabica* L. cv. IPR108 and *C. canephora* Pierre Ex A. Froehner cv. Conilon Clone 153 were grown for 1 year under controlled conditions of temperature (25/20ºC, day/night), irradiance (650-800 μmol m^{-2} s^{-1}), RH (75%), photoperiod (12 h), and 380 μL CO_2 L^{-1}. Thereafter, temperature was gradually raised to 42/34ºC (0.5ºC/ day), with a 7 days stabilization step at 31, 37 and 42ºC. Studies focused modifications of thylakoid lipid composition and photosynthetic performance. In CL153 photosynthetic capacity (A_{max}) was not affected until 42ºC (40% reduction). In IPR108 it was reduced 35 and 57% at 37ºC at 42ºC, respectively. Thylakoid electron transport rate for photosystems (PS) I and II increased (*ca*. 10-25%) up to 37ºC in both genotypes. At 42ºC only IPR108 presented depressed activities on PSII (15%) and PSI (18%). Under 37 and 42ºC, CL153 plants presented digalactosyldiacylglycerol (DGDG) (*ca*. 42%) and monogalactosyldiacylglycerol (MGDG) (28-34%) increases, while IPR108 showed higher MGDG at all temperatures. In CL153 less unsaturated DGDG and phosphatidylglycerol (PG) along with stable or increased DGDG/MGDG ratio, may have contributed to sustain thylakoid electron flow at 37ºC and even 42ºC. IPR108 displayed a strong PG rise at all temperatures, in accordance with enhanced PSs activity.

INTRODUCTION

According to modeling studies, global warming resulting from climate change may affect coffee production, one of the world's most traded agricultural products. Ambient temperature fluctuations have a direct impact on photosynthesis, namely through its effects on the thermally sensitive biochemical and physiological processes that occur in membranes. Beyond a critical temperature, heat induces thylakoid membrane destabilization and photosynthesis inhibition. Lipoperoxidation may also occur leading to photosynthesis impairment and leaf senescence. Plants cannot regulate their own temperature (poikilotherm organisms). Yet, membrane lipids may undergo quantitative and qualitative modifications upon stress exposure, ensuring an adequate fluidity under changing environmental conditions. Thylakoid membranes are mostly constituted by monogalactosyldiacilglicerol (MGDG), digalactosyldiacylglycerol (DGDG) and phosphatidylglycerol (PG) that contribute to photosynthetic structures stability and functioning. The aim of this work was to assess heat effects on thylakoid lipids and photosynthetic activity of two coffee genotypes.

MATERIALS AND METHODS

Plant material and experimental design

Plants with *ca*. 1.5 years from *C. arabica* L. cv. IPR108 (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 (25/20ºC, day/night), irradiance (*ca*. 650-800 μmol m^{-2} s^{-1}), RH (75%), photoperiod (12 h) and 380 μL CO_2 L^{-1} for 1 year, without water, nutrient or root development restrictions. Temperature was then increased from 25/20ºC up to 42/34ºC, at a rate of 0.5ºC day^{-1}. After 7 days stabilization at 31/25, 37/30 and 42/34ºC., analyses were performed on newly matured leaves.

Photosynthetic capacity

The photosynthetic capacity (A_{max}), representing light (*ca*. 900 μmol m^{-2} s^{-1}) and CO_2 (*ca*. 7%) saturated rate of photosynthesis under optimal temperature (25ºC), was measured with a Clark-type O_2 electrode (LD2/2, Hansatech, UK) according to [7].

Thylakoid electron transport rates

To obtain sub-chloroplast fractions and determine the *in vivo* electron transport rates associated with both PSI ($DCPIPH_2$→MV) and PSII, including (H_2O→DCPIP) or excluding (DPC→DCPIP) the oxygen evolving complex (OEC), measured polarographically with an LW2 O_2 electrode (Hansatech, UK) at 25 °C, optimized methods for coffee were followed [7].

Chloroplast membrane lipids

Lipid extraction and analysis were performed according to [8]. Lipid classes were separated by thin layer chromatography using two solvent systems. Lipid bands were scraped off, saponified and methylated for individual FAs analysis by gas-liquid chromatography (Varian CP-3380, U.S.A.). Individual FAs and lipid classes were identified by comparison with known standards (Sigma, Supelco). Total fatty acids (TFAs) values are the sum of individual FAs. The double bond index (DBI) was calculated as DBI = [(% monoenes + 2 x % dienes + 3 x % trienes / (% saturated FAs)].

RESULTS AND DISCUSSION

In CL153 A_{max} remained unaltered until 37ºC and was reduced 40% only at 42ºC (Fig. 1), whereas IPR108 presented reductions already at 37ºC (35%), and also at 42ºC (57%).

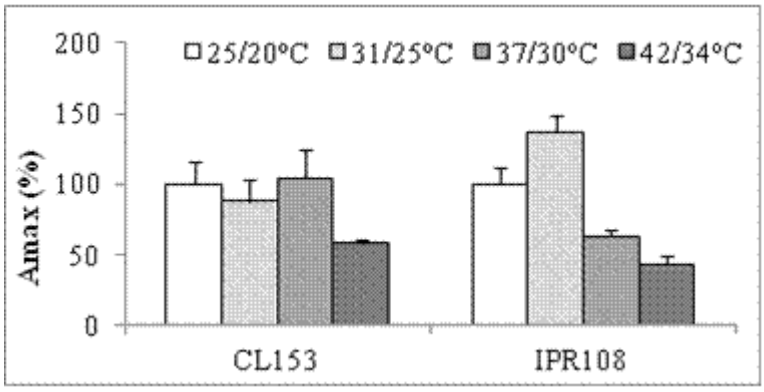

Figure 1. Changes (in % of µmol O_2 m^{-2} s^{-1}), within each genotype, relative to plants at 25/20ºC) for photosynthetic capacity (A_{max}) rates). Each value represents the mean ± SE (n=5-8).

As regards the thylakoid electron transport at photosystem (PS) I and II levels, increases at 37ºC (*ca*. 9-16%) were found in CL153 (Fig. 2). At 42ºC the values were similar to control. In IPR108 PSI and II activities strongly rised at 31ºC (34-40%) and 37ºC (20-26%), but at 42ºC fell 18% (PSI) and 15% (PSII). Oxygen evolving complex (OEC) did not contribute to changes in PSII activity (Fig. 2). Results denote a strong tolerance of such photosynthetic membrane bound events to high temperature in coffee. That agrees with the findings that light absorption, energy transfer and charge separation associated with PS II and I are quite insensitive to temperature in the biologically relevant range of 0-50ºC.

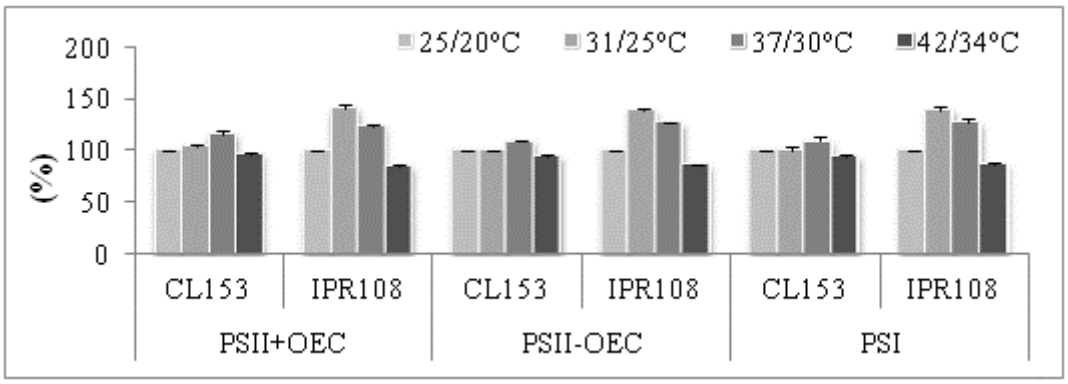

Figure 2. Changes (in %, from results expressed in µmolO_2 m^{-2} s^{-1}, within each genotype, relative to plants at 25/20ºC) for the *in vivo* electron transport rates associated with PSII, including (PSII+OEC) or excluding (PSII-OEC) the oxygen evolving complex (OEC), and PSI. Each value represents the mean ± SE (n=4-5).

Heat induces hyperfluidization of membranes, affecting lipid-protein interactions and causing various disturbances, such as, phase transitions of lipids and conformational changes, therefore altering their functions, namely in thylakoid PSII reactions. Such thylakoid

structural rearrangements are usually reversible when temperature returns to control values [10]. These short-term adaptive processes take place within minutes or hours and rapidly modify the thermal sensitivity of the photochemical apparatus of photosynthesis. Additionally, long-term acclimation may trigger other processes to high temperature that involves *de novo* protein and lipid synthesis. In CL153 chloroplast lipid, evaluated through total fatty acid (TFA) content, slightly decreased (12-16%) at 31 and 37ºC, while in IPR108 strong increases were found (Fig. 3), particularly at 37ºC (105% rise). At 42ºC the TFA contents approach control levels in both genotypes. Furthermore, the *de novo* synthesized lipids showed unaltered unsaturation (DBI) at 37ºC, but a decrease was found at 42ºC (Fig. 3).

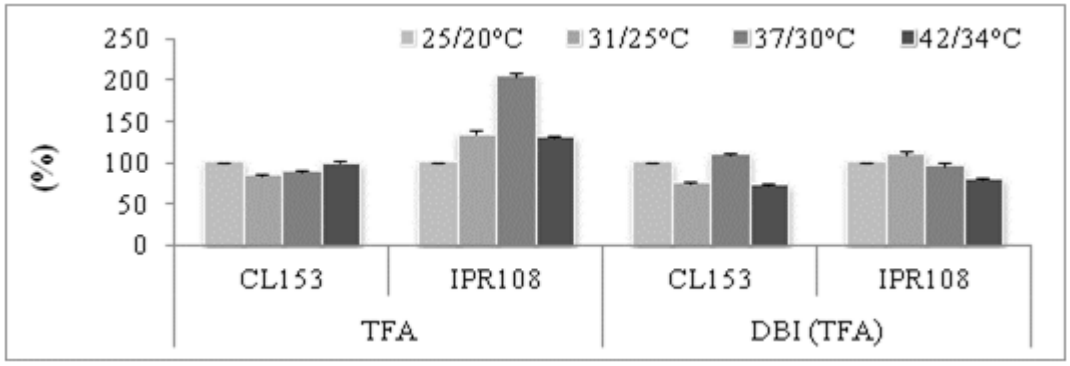

Figure 3: Changes (in %, within each genotype, relative to plants at 25/20ºC) for total fatty acids (TFA) and double bond index (DBI). Each value represents the mean ± SE (n=3-4).

Increased lipid content requires gross membrane biogenesis rather than modification of existing molecules, and may result in altered proportions of individual phosphoglycerides, which could indicate potential adaptive changes. As regards the galactolipids MGDG and DGDG (Fig. 4), CL153 plants subjected to high temperatures (37 and 42ºC) depicted significant DGDG (*ca*. 42%) and MGDG (28-34%) increases. In IPR108 DGDG decreased (32% at 37ºC), while MGDG increased at all temperatures (53, 12 and 39% at 31, 37 and 42ºC. Galactolipids ratio (DGDG/MGDG) was unaltered in CL153 (Fig. 4) except at 37ºC (10% increase). That ratio decreased in IPR108 with temperature (37% at 31 and 37ºC, and 31% at 42ºC), mainly due to a higher proportion of MGDG at all temperatures above control (Fig. 4). Synthesis of MGDG and DGDG may contribute for drought tolerance.

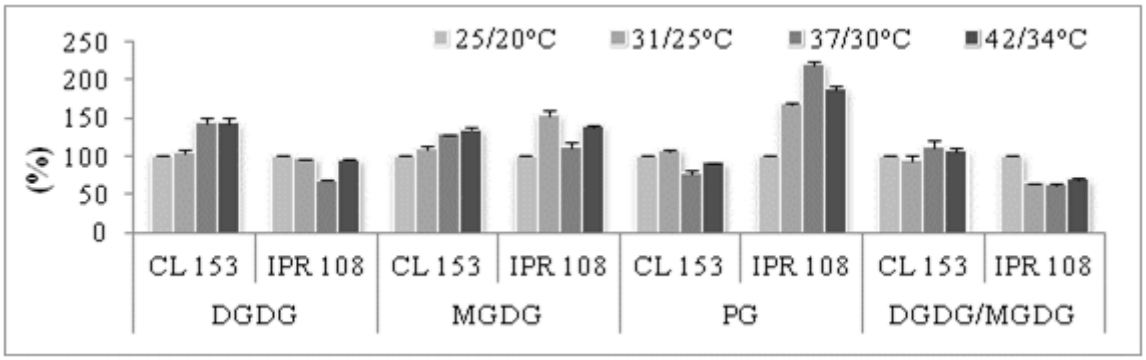

Figure 4: Changes (in %, within each genotype, relative to plants at 25/20ºC) for monogalactosyldiacylglicerol (MGDG), digalactosyldiacilglycerol (DGDG), phosphatidylglycerol (PG) and the ratio DGDG/MGDG. Each value represents the mean ± SE (n=3-4).

The packing arrangement of MGDG in the thylakoid outer monolayer is closely related to the rate of photo and dark-phosphorylation, being required for the functioning of the ATP synthase. Moreover, DGDG is a bilayer-forming lipid that helps to preserve thylakoid stability. Furthermore, variation in the proportion of galactolipids allows membrane structural and functional changes and the rise in DGDG/MGDG ratio due to enhanced DGDG synthesis was related to heat tolerance in other species, what agrees with the higher heat tolerance of *C. canephora* when compared to *C. arabica*.

In CL53 PG decreased at 37 and 42ºC (23 and 10%), while IPR showed a strong rise at all temperatures (67, 120 and 88% at 31, 37 and 42ºC) (Fig. 4). PG is a major chloroplast phospholipid, which plays an important role in photosynthetic structures stability and functioning [6]. In the inner monolayer PG and DGDG are needed for the formation, stability and functioning of photosynthetic complexes, namely of LHCII trimers.

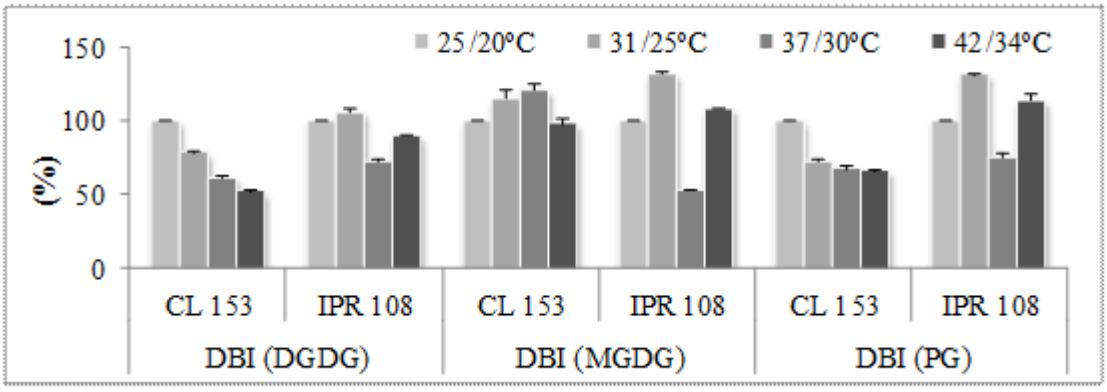

Figure 5: Changes (in %, within each genotype, relative to plants at 25/20ºC) for the double bond index (DBI) of monogalactosyldiacylglicerol (MGDG), digalactosyldiacilglycerol (DGDG) and phosphatidylglycerol (PG). Each value represents the mean ± SE (n=3-4).

Both genotypes tended to display higher TFA saturation (lower DBI) at 42ºC (Fig. 3). In CL153 this was due to less unsaturated DGDG and PG (Fig. 5). This feature, along with stable or increased DGDG/MGDG ratio, may have contributed to sustain thylakoid electron flow at 37ºC and even 42ºC (Fig. 2). IPR108 displayed a strong PG increase at all temperatures, which might be related to the enhanced thylakoid functioning and A_{max}. Yet, at 42ºC photosynthetic activity was strongly impaired. In fact, at both 37 and 42ºC, other limitations, linked, *e.g.*, to lower enzyme activity of RuBisCO, were likely involved in the reduction of photosynthetic performance.

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