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Lipid profile adjustments may contribute to warming acclimation and to heat impact mitigation by elevated [CO_2] in *Coffea* spp

Paula Scotti-Campos[a,b,1], Isabel P. Pais[a,b,1], Ana I. Ribeiro-Barros[b,c,1], Lima D. Martins[d], Marcelo A. Tomaz[d], Weverton P. Rodrigues[e], Eliemar Campostrini[e], José N. Semedo[a,b], Ana S. Fortunato[c,2], Madlles Q. Martins[f], Fábio L. Partelli[f], Fernando C. Lidon[b], Fábio M. DaMatta[g], José C. Ramalho[b,c,*]

[a] Unidade de Investigação em Biotecnologia e Recursos Genéticos (UIBRG), Instituto Nacional de Investigação Agrária e Veterinária, I.P.(INIAV), Oeiras, Portugal
[b] GeoBioSciences, GeoTechnologies and GeoEngineering (GeoBioTec), Faculdade de Ciências e Tecnologia(FCT), UniversidadeNOVAde Lisboa(UNL), Monte de Caparica, Portugal
[c] Plant-Environment Interactions & Biodiversity Lab (PlantStress&Biodiversity), Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia (ISA), Universidade de Lisboa (UL), Oeiras, Portugal
[d] Dept. Produção Vegetal (DPV), Centro de Ciências Agrárias (CCA), UniversidadeFederal do Espírito Santo (UFES), Alegre, ES, Brazil
[e] Setor Fisiologia Vegetal, Centro de Ciências e Tecnologias Agropecuárias (CCTA), Univ. Estadual Norte Fluminense (UENF), Campos dos Goytacazes, RJ, Brazil
[f] Dept. de Ciências Agrárias e Biológicas, Centro Universitário Norte do Espírito Santo (CEUNES), Universidade Federal Espírito Santo (UFES), São Mateus, ES, Brazil
[g] Dept. Biologia Vegetal, Universidade Federal Viçosa (UFV), Viçosa, MG, Brazil

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ABSTRACT

An unexpected heat resilience, and the mitigation of heat impacts by elevated [CO_2] were recently reported in *Coffea* spp. Plants must maintain membrane fluidity and integrity to cope with temperature changes, which requires an adequate lipid dynamics. This work provides the lipid profile (galactolipids, GL; phospholipids, PL; sulfolipids, SL) of chloroplast membranes, and the expression of a set of genes related to lipid metabolism in *Coffea arabica* L. (cv. Icatu and IPR108) and *C. canephora* cv. Conilon CL153, under elevated [CO_2] (380 or 700 µL L^{-1}), heat (25/20, 31/25, 37/30 and 42/34 °C, day/night) and their interaction. Major membrane lipids alterations, different among genotypes, included: A) responsiveness of total fatty acids (TFAs) synthesis to [CO_2] (except IPR108) and heat (except CL153); stronger remodeling (unsaturation degree) in the 700-plants from 37/30 °C to 42/34 °C, coordinated at transcriptional level with the down-regulation of fatty acid desaturase *FAD3* gene (*C. arabica*) and up-regulation of lipoxygenase genes *LOX5A* (CL153 and Icatu) and *LOX5B* (Icatu) at the highest temperature; B) quantitative and qualitative modifications in GL (monogalactosyldiacylglycerol, MGDG; digalactosyldiacylglycerol, DGDG), PL (phosphatidylcholine, PC; phosphatidylglycerol, PG), and SL (sulfoquinovosyldiacylglycerol, SQDG) classes, prompted by heat, elevated [CO_2], and, especially, the interaction, in CL153 and Icatu. Overall membrane enrichment with MGDG and DGDG as a result of heat and [CO_2] interaction in these genotypes, but at the highest temperature only in Icatu the high [CO_2] maintained greater contents and unsaturation values of these GLs than in the 380-plants. C) Among PL classes, PG seems to play an active role in heat acclimation of *C. arabica* genotypes, increasing in 700-plants at 42/34 °C. Globally, Icatu often showed changes closer to those of heat tolerant cv. CL153 than to cv. IPR108. Overall, lipid profile adjustments in chloroplast membranes, from TFAs bulk until FA unsaturation within each class, are expected to contribute to long-term acclimation to climate changes in coffee plant.

Abbreviations: DBI, double bond index; DGDG, digalactosyldiacylglycerol; FA, fatty acid; FAD, fatty acid desaturase; GL, total galactolipids; LOX, lipoxygenase; LR, lipid residue; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, total phospholipids; PS, photosystem; PUFA, polyunsaturated fatty acid; SL, sulfolipids; SQDG, sulfoquinovosyldiacylglycerol; TFA, total fatty acids; C16:0, palmitic acid; C16:1*t*, 3-*trans*-hexadecenoic acid; C16:1, *c* + *t cis* + *trans* palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid

* Corresponding author at: Plant-Environment Interactions & Biodiversity Lab (PlantStress&Biodiversity), Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior Agronomia, Universidade de Lisboa, Av. República, Quinta do Marquês, 2784-505, Oeiras, Portugal.
E-mail addresses: cochichor@isa.ulisboa.pt, cochichor@mail.telepac.pt (J.C. Ramalho).
[1] These authors contributed equally to this work.
[2] Current Affilliation: Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVAde Lisboa, Oeiras, Portugal.

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1. Introduction

One of the most remarkable aspects of climate changes resides in the continuous increase in air CO_2 concentration ($[CO_2]$) over the last decades. Projected $[CO_2]$ might reach values between 421 and 936 μL L^{-1}, together with a temperature rise between 0.3 and 4.8 °C, by the end of this century relative to the period 1986–2005 (IPCC, 2013).

Temperature changes are expected to severely impact most crops, which will increasingly face new environmental challenges. To thrive under changing temperatures, plants must undergo metabolic and physiological adjustments, including dynamic changes in membrane composition to keep adequate fluidity and integrity (Niu and Xiang, 2018). Cellular membranes are considered primary targets of environmental stresses, but also the sites where environmental stimuli are perceived giving rise to acclimative responses (Osakabe et al., 2013; Ruelland et al., 2015; Niu and Xiang, 2018). Detection and responses to fluidity modulation are among the early events in plant acclimation to daily and seasonal temperature shifts (Leshem, 1992; Tovuu et al., 2013), involving the regulation of the activity of innumerous membrane-bound proteins and of their encoding genes (Los and Zinchenko, 2009). High air temperatures can directly promote changes in membrane properties, including fluidity, permeability to water and solutes, composition, domains configuration and lipid-protein interactions (Leshem, 1992; Niu and Xiang, 2018). An excessive fluidization can disrupt the lipid bilayer, and cause irreversible membrane damage (Leshem, 1992; Los and Zinchenko, 2009), likely impairing membrane-based events, *e.g.*, chloroplast light energy capture and electron transport. Also, heat can promote lipid degradation through the lipoperoxidation of polyunsaturated fatty acids (PUFAs), due to ROS production stimulation (Higashi et al., 2015; Niu and Xiang, 2018), and phospholipases and galactolipases action, which are also stimulated under stress conditions (Sahsah et al., 1998).

Chloroplast membranes lipid composition and physical state are of crucial importance for photosynthetic functioning (Niu and Xiang, 2018). They have a unique lipid composition, of highly unsaturated glyceroglycolipids, comprising major components such as monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) (both uncharged), sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG) (both negatively charged). In thylakoid membranes, the galactolipids (GL) MGDG and DGDG represent 70 to 80% of the total lipid matrix, whereas SQDG and PG account for by 5% to 12% (Shimojima et al., 2009; Dörmann and Hölzl, 2009; Kobayashi, 2016). Additionally to PG, the phospholipids (PL) phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidic acid (PA) are also present in chloroplast membranes (Shimojima et al., 2009; Scotti-Campos et al., 2014a; Higashi et al., 2015), but the information on their roles in photosynthesis is scarce (Dörmann and Hölzl, 2009). Chloroplast membrane lipid remodeling, through *de novo* synthesis or modification of pre-existing FAs acids, is a crucial part of plant acclimation to abiotic stresses such as cold (Wang et al., 2006; Partelli et al., 2011), heat (Iba, 2006; Higashi et al., 2015; 2018), and drought (Torres-Franklin et al., 2009). This includes changes in FAs unsaturation degree, and in the amount and balance of lipid classes (Zheng et al., 2011; Partelli et al., 2011; Scotti-Campos et al., 2014a, 2014b; 2016; Bajerski et al., 2017), which constitute potential heat tolerance biomarkers.

Coffea arabica L. and *C. canephora* Pierre ex A. Froehner account for nearly 99% of the world coffee production from about 80 tropical countries, supporting the livelihoods of *ca.* 25 million smallholder farmers, while involving *ca.* 100-125 million people worldwide in the coffee chain of value (Ramalho et al., 2018a,b; DaMatta et al., 2019). Temperature and water availability are the most important limiting environmental variables for the coffee crop (DaMatta and Ramalho, 2006; Ramalho et al., 2018b), and the growing number of extreme events of severe drought spells combined with increased temperatures, are already affecting coffee yields and sustainability (Craparo et al., 2015; van der Vossen et al., 2015). Severe impacts are expected in *C. arabica* that is considered more sensitive to heat than *C. canephora* (DaMatta and Ramalho, 2006), and future warming is estimated to promote the extinction of at least 60% of all coffee species (Davis et al., 2019). Nevertheless, recent studies conducted by our research teams have demonstrated a a remarkable heat resilience to high temperatures (up to 37 °C) than expected, although at 42 °C a threshold for deleterious effects is reached. Most importantly, elevated $[CO_2]$ mitigated the heat impact on coffee photosynthesis, with membrane related events (*e.g.,* electron transport at PSI and II) being much less affected than biochemical events (*e.g.*, enzymes activity) (Martins et al., 2016; Rodrigues et al., 2016). This positive effect of elevated [CO2] was related to the triggering protective mechanisms, which favoured ROS control, and reduced photosystem (PS) II photoinhibition at 42 °C. Additionally, high $[CO_2]$ strengthened photochemical efficiency and biochemical functioning at all temperatures, from 25 to 42 °C (Martins et al., 2016; Rodrigues et al., 2016), while preserved mineral homeostasis (Martins et al., 2014) and bean quality (Ramalho et al., 2018a) at high temperature, disputing the idea that coffee is highly sensitive to warming (Semedo et al., 2018; DaMatta et al., 2019).

Chloroplast membranes are considered particularly sensitive to supra-optimal temperatures, but membrane based functions in *Coffea* spp., such as the thylakoid electron transport, showed a great heat tolerance, in sharp contrast with the high sensitivity of enzymes related to the energy metabolism (including RuBisCO) at 42 °C (Rodrigues et al., 2016). Besides, it is recognized the key role of membrane lipid adjustments to a successful long-term acclimation of *Coffea* spp. plants to cold (Partelli et al., 2011; Ramalho et al., 2014; Scotti-Campos et al., 2014a), and high irradiance (Ramalho et al., 1998). Despite such crucial role of membrane lipid remodeling in plant stress acclimation, how plants respond to single or combined impacts of supra-optimal temperatures and high $[CO_2]$ is poorly understood, without any information for the coffee plant. In this context, this work is a step forward to previous studies (Ramalho et al., 2013; Martins et al., 2014, 2016; Rodrigues et al., 2016; DaMatta et al., 2018), aiming at improving our knowledge regarding coffee crop performance under future warming scenarios. To reach this goal, we provide a comprehensive analysis relating chloroplast membrane lipid dynamics (from bulk TFAs content, until fine tuned changes in FA composition and saturation within each lipid class) to heat, enhanced air $[CO_2]$, and their interaction in three cropped genotypes belonging to the two main producing *Coffea* species.

2. Material and methods

2.1. Plant material and growth conditions

Following the experimental design described in Rodrigues et al. (2016), plants from three cropped coffee genotypes from the two main producing species were used: *Coffea arabica* L. cv. Icatu (an introgressed variety from *C. canephora* Pierre ex A. Froehner), *C. arabica* L. cv. IPR108, and *C. canephora* Pierre ex A. Froehner cv. Conilon Clone 153 (CL153). Plants of 1.5 years in age were grown for 10 months in 28 L pots in walk-in growth chambers (EHHF 10000, ARALAB, Portugal), under controlled temperature (25/20 °C, day/night), irradiance (*ca.* 700–800 μmol m^{-2} s^{-1}), relative humidity (75%), photoperiod (12 h), and either 380 μL CO_2 L^{-1} (380-plants) or 700 μL CO_2 L^{-1} (700-plants). Thereafter, temperature was increased from 25/20 °C to 42/34 °C, at a rate of 0.5 °C day^{-1}, with 7 days of stabilization at 31/25, 37/30 and 42/34 °C for sampling and evaluations. Unless otherwise stated, our control conditions refer to plants grown at 25/20 °C and 380 μL CO_2 L^{-1}. For analysis, the youngest, fully expanded leaves from plagiotropic and orthotropic branches of the upper (illuminated) part of the plant (5–8 plants per treatment), were collected after *ca.* 2 h of light. For membrane selectivity and lipid analysis the material was immediately used, while for gene expression studies, samples were flash frozen in liquid N_2 and stored at −80 °C until use. The plants were maintained without restriction of nutrients, space for root growth (Ramalho et al., 2013),

and water (predawn water potential above -0.25 MPa at 25/20 °C).

2.2. Electrolyte leakage

The cellular membrane integrity evaluation followed Matos et al. (2010). Ten leaf discs (0.5 cm^2 each) were rinsed (3x) and left to float on 15 mL of deionized water at 20 °C, for 24 h, when conductivity was measured (Crison GLP31, Crison Instruments S.A., Spain). Thereafter, total conductivity was obtained after exposing the samples at 90 °C for 2 h, followed by cooling to 20 °C. For each sample, electrolyte leakage was expressed as the percentage of total conductivity.

2.3. Chloroplast lipid analysis

2.3.1. Chloroplast membranes and lipids extraction

Enriched chloroplast membranes fractions were obtained from 3 to 4 g FW of leaf tissue, as optimized for *Coffea* spp. (Partelli et al., 2011; Scotti-Campos et al., 2014a). Briefly, freshly cut leaf material was immediately homogenized in 25 mL of a cold 50 mM MES buffer (pH 6.4), containing 0.4 M D-sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA, 1 mM $MnCl_2$, 0.4% (w/v) BSA, and 2 mM Na-ascorbate, filtered (with 8 layers of cheesecloth), and centrifuged (3000 *g*, 5 min, 4 °C). The obtained chloroplast pellet was used for lipid extraction, being mixed with 9 mL of a chloroform/methanol/water (1/1/1, v/v/v) solution, and centrifuged (4500 *g*, 10 min, 4 °C). Lower chloroform phase was evaporated to dryness under N_2 flux, and the lipid residue ressuspended in 1.5 mL of an ethanol:toluene (1:4) mixture, to be used in the next steps.

2.3.2. Total fatty acids and lipid class analysis

For FA analysis, a 50 µL aliquot of the previous lipid resuspension was saponified and methylated with BF_3. To quantify FAs, heptadecanoic acid (C17:0) was added to each sample as an internal standard. FAs methyl esters (FAME) were analyzed by GC-FID (Varian, CP-3380, USA), with a DB-Wax capillary column (J & W Scientific, 0.25 mm i.d. x 30 m, 0.25 µm). Column temperature was programmed to rise from 80 to 200 °C at 12 °C min^{-1}, after 2 min at the initial temperature. Injector and detector temperatures were 200 °C and 250 °C, respectively. Carrier gas was hydrogen with a flow rate of 1 mL min^{-1}, at a split ratio of 1:50 of the sample. Individual FAs were identified by comparison with a standard mixture (FAME Mix, Restek). Total fatty acids (TFA) value corresponds to the sum of individual FAs.

For lipid class separation and analysis another aliquot (150 µL) of the lipid resuspension was applied on TLC plates (G60 silicagel, Merck), followed by sequential elutions using chloroform/acetone/methanol/acetic acid/water (50/20/10/10/5, v/v/v/v), and subsequently in petroleum ether/diethyl ether/acetic acid (70/30/0.4, v/v/v). After spraying with 0.01% primuline (in 80% acetone) and visualization under UV, the lipid bands were scraped off, saponified and methylated as described above. The separation of PC from SQDG (not achieved through the above described method) was further performed by a two dimension TLC on G60 silicagel plates (Merck). After the first direction run in chloroform/methanol/water (75/25/2.5, v/v/v) the plate was left sufficient time for drying, and a second dimension run was performed in chloroform/methanol/acetic acid/water (80/9/12/2, v/v/v/v). Further band detection and analysis procedure was performed as described above for the other lipid classes. Individual lipid classes were identified by comparison with authentic Sigma standards. The unsaturation degree of TFA and lipid classes was calculated as the double bond index (DBI) according to Mazliak (1983) (DBI = [(%monoenes + 2 x %dienes + 3 x %trienes) / %saturated FAs]).

Trace amounts (< 1%) of the phospholipid class phosphatidylethanolamine (PE) were found. Given that (i) PE accounts for up to *ca*. 17% of total lipid classes in cell membranes of coffee leaves (Campos et al., 2003), and (ii) is absent from chloroplast membranes (Joyard et al., 1998; Li-Beisson et al., 2013) only a negligible contamination of extrachloroplastidial membranes occurred in our samples.

2.4. Gene expression analysis

Total RNA from coffee leaves was isolated and quantified according to Martins et al. (2017). One microgram of DNA-free total RNA was used to synthesize first-strand cDNAs using oligo-$(dT)_{18}$ primers and the SuperScriptIIfirst-strand synthesis system (Invitrogen, USA). The expression of four genes related to lipid metabolism, encoding three linoleate lipoxygenases (LOX3, *LOX5A, LOX5B*) and one ω-fatty acid desaturase (FAD3) was analysed by qRT-PCR using Malate dehydrogenase (*MDH*) and Ubiquitin-conjugating enzyme E2 (UBQ2) for data normalization (Martins et al., 2017). For each gene, specific primers were designed using Primer3 software, according to the following parameters: 62–65 °C melting temperature (Tm), 45–60 % GC content, 19–20 bp primer length, 80–150 bp amplicon length. Genes list and primer sequences, size of amplicon and gene accession number are presented in Table 1.

The qRT-PCR reactions were performed in 96-well plates using qPCR SYBR® Green Supermix (BioRad, USA) in a iQ™ 5 Real-Time Detection System (BioRad), using the following parameters: hot start activation of the Taq DNA polymerase at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, elongation at 72 °C for 30 s; and plate read. To verify the specificity of each amplification, and the absence of primer dimers, dissociation curves were obtained for each amplicon at the end of the PCR run, by continuous fluorescence measurement from 55 to 95 °C, with sequential steps of 0.5 °C for 15 s. Three technical replicates were used for each biological replicate, and the mean Ct used for data analyses. Full sample set was always included in each technical replicate to exclude any artifacts derived from between-run variations. No signals were detected in non-template controls run in parallel for each primer set. Two negative controls were included for each primer pair, in which cDNA was replaced by water or total RNA. Reaction efficiency was calculated for each primer.

2.5. Statistical analysis

Except for gene expression, data was analyzed using two-way

Table 1
Primer sequences and amplicon characteristics of the four coffee genes involved in lipid metabolism.

Gene symbol	Gene description	Primer sequence (5'–3')	Accession number[a]	Amplicon size (bp)
LOX3	Linoleate 13S-lipoxygenase 3-1, chloroplastic	F:F: GCGTCGTGTGCAATGATAG R: GATGCAAGCCAGATGAGAG	Cc00_g30760 (http://coffee-genome.org/Cc00_g30760)	153
LOX5A	Linoleate 9S-lipoxygenase 5	F: CCGCAGATTATCCAAGAGG R: CTTGGCTGCCGTAGACTTT	Cc02_g33320 (http://coffee-genome.org/Cc02_g33320)	152
LOX5B	Linoleate 9S-lipoxygenase 5, chloroplastic	F: GGCATACCCTCATCAATGC R: CTGGAACTGCGATTCCTCT	Cc03_g03580 (http://coffee-genome.org/Cc03_g03580)	155
FAD3	ω-3 fatty acid desaturase, chloroplastic	F: CGAGAAGCTGCCTTGGTATC R: GAGGGATTTGTGGGAAGAG	Cc02_g06400 (http://coffee-genome.org/Cc02_g06400)	147

[a] According to http://www.coffee-genome.org/ (Denoeud et al., 2014).

ANOVA ($P < 0.05$) to evaluate the differences related to air [CO_2], temperature, and their interaction, followed by a Tukey's test for mean comparison, always independently for each genotype. Overall, temperature *vs.* [CO_2] interaction was significant for most parameters.

The relative expression ratio of each target gene was computed based on its qRT-PCR efficiency and the crossing point (CP) difference of a target sample *vs.* the control (25/20 °C, 380 μL CO_2 L^{-1}) for each genotype. Data analysis for gene expression was performed using the Relative Expression Software Tool (REST 2009, available at http://www.genequantification.de/rest-2009.html). A 95% confidence level was adopted for all tests.

3. Results

3.1. Membrane selectivity

Cellular membrane leakage gradually increased with temperature regardless of [CO_2] and genotype, peaking at 42/34 °C (with the exception of CL153 plants grown under normal [CO_2] which was unresponsive to heat) (Fig. 1). At 42/34 °C, elevated [CO_2] had no impact on CL153, but increased leakage in Icatu and decreased it in IPR108, as compared to normal [CO_2]. However, the absolute leakage values were moderately low, even at the highest temperature.

3.2. Bulk analysis of chloroplast membranes lipids

3.2.1. Total fatty acids and unsaturation degree

Under control temperature total fatty acid (TFA) contents decreased under elevated [CO_2] in CL153 and Icatu (16% and 24%, respectively), remaining unaltered in IPR108 (Fig. 2A).

High temperature did not significantly affect TFA amounts in CL153 plants. However, mostly irrespective of [CO_2], rising temperatures promoted a gradual TFA increase until 37/30 °C in Icatu and IPR108, when maximal values represented significant increases of 41% and 82% in Icatu, and 105% and 83% in IPR108, for 380- and 700-plants, respectively, when compared to their controls at 25/20 °C. Exposure to 42/34 °C led to significant TFA reductions only in Icatu 380-plants (27%) and IPR108 380- and 700-plants (36% for both), as compared to 37/30 °C values. However, at that extreme temperature TFA values were still close (CL153 and Icatu 380-plants) or above (IPR108 and Icatu 700-plants) those of their controls (25/20 °C).

Under temperature and CO_2 control conditions the unsaturation degree of the bulk TFA in CL153 and Icatu presented the highest and the lowest DBI values (Fig. 2B), related to the highest C18:3 and lowest C16:0 percentages in CL153, and an opposite pattern in Icatu (data not shown). Under elevated air [CO_2] only IPR plants showed an altered DBI (17% increase).

With temperature increase, some qualitative changes occurred, mainly regarding C18:3, and C16:0, somewhat differently between [CO_2] conditions. The exposure to 37/30 °C did not modify DBI in any genotype, which was largely related to the maintenance of C18:3. However, in the 700-plants a higher unsaturation tendency (compared to the 380-plants) reflected increases in highly unsaturated C18:3 and reductions in the saturated C16:0. Further rise to 42/34 °C promoted a global DBI reduction, greater in the 700-plants of all genotypes, due to clear increases of C16:0 and reductions of C18:3, but only Icatu maintained DBI values similar to those of control plants, irrespective of [CO_2], suggesting a high membrane stability.

3.3. Lipid class composition of chloroplast membranes

The amount and relative weight of the main lipid classes (Table 2), as well as the FA composition within each class (Tables 3–9) were assessed. For sake of result presentation, the plant responses to temperature rise will hereafter focus on the 37/30 °C (cell functioning still preserved) and 42/30 °C (tolerance limit have been surpassed) (Rodrigues et al., 2016).

Under control conditions, CL153 presented the highest total galactolipid (GL) and total phospholipid (PL) contents (Table 2), in line with its largest TFA amounts.

At 25/20 °C, the impact of the elevated [CO_2] changed GL and PL contents, and their balance in a genotype dependent manner. GL was kept in CL153, but strongly increased in Icatu (110%) and decreased in IPR108 (17%). On the other hand, PL was reduced by 61% in CL153 and 30% in Icatu, but increased in IPR108 (66%), leading to GL/PL ratio increases of 1.6 and 2 fold in 700-plants of CL153 and Icatu, respectively, being halved in IPR108 (Table 2).

Temperature alone (380-plants) increased GL in all genotypes until 37/30 °C, but at 42/34 °C *C. arabica* plants showed a partial reduction. PL values varied moderately in Icatu and CL153 at 37/30 °C, sharply increased (271%) in IPR108, and strongly dropped from 37/30 to 42/34 °C in all genotypes, thus resulting in GL membrane enriched at the two highest temperatures (except IPR108 at 42/34 °C).

Considering the [CO_2] and heat interaction, different responses were found at the two highest temperatures. At 37/30 °C, CL153 did not show differences between [CO_2] conditions in GL, but a strong PL reduction was observed in 700-plants, whereas for IPR108 GL increased and PL decreased. For these two genotypes the 700-plants presented lower GL values than the 380-plants at 42/34 °C, although close to the initial control values. PL was reduced in CL153 and increased in IPR108. Notably, only Icatu-700-plants maintained high GL, PL, and GL/PL values at the highest temperature (and partly at 37/30 °C) than their 380-plants counterparts.

The impact within GL and PL was further resolved regarding their classes (Table 2). Under high [CO_2] at 25/20 °C, the GL maintenance in CL153 has hidden an 18% reduction in MGDG (although maintaining its relative weight) and a 26% rise in DGDG, reducing MGDG/DGDG ratio by *ca.* 33%. The large PL decrease in CL153 was due to reductions

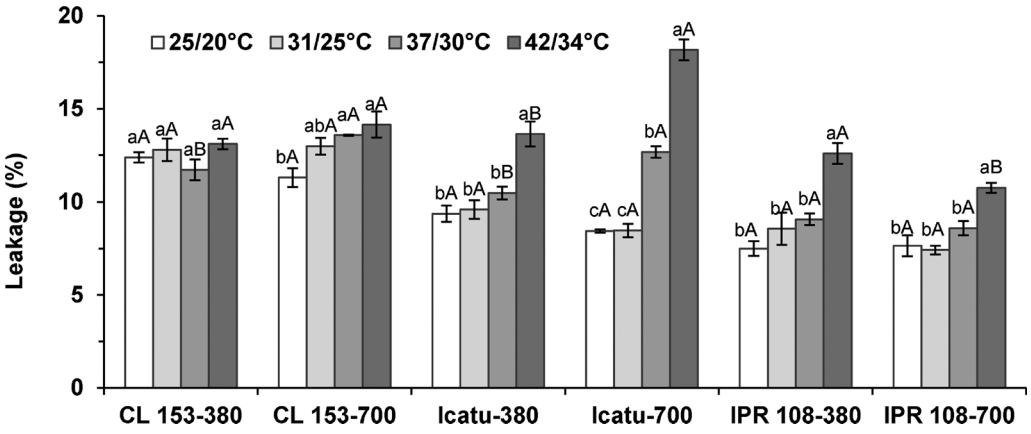

Fig. 1. Evaluation of membrane integrity, assessed through electrolyte leakage from leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25, 37/30 and 42/34 °C. For each parameter, the mean values ± SE (n = 5–6) followed by different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

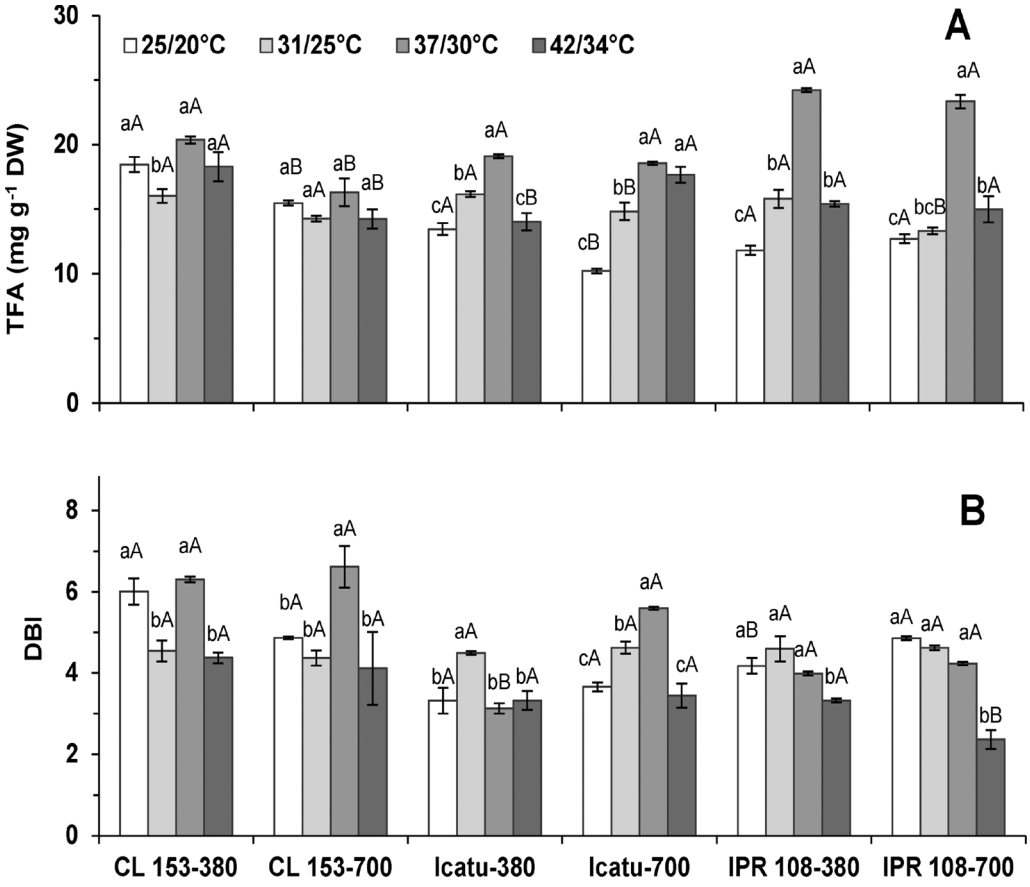

Fig. 2. Total fatty acid (TFA) content (A), and their double bon index (DBI) (B), from chloroplast membrane lipids of leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25, 37/30 and 42/34 °C. The mean values ± SE (n = 3) followed by different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

in PC (83%) and PG (41%). In Icatu, the increases in MGDG (84%) and, especially, DGDG (132%) justified the doubled GL content rise and the MGDG/DGDG decline, whereas the PL reduction was almost exclusively related to PC reduction (62%), partly compensated for PG increase (18%). In IPR108 700-plants the MGDG increase (19%) did not compensate the 35% DGDG decline, whereas the PL increases rely on PC (337%) and PG (20%) rises.

Under control conditions SGDG class represented between 1% (IPR108) and 5% (CL153 and Icatu) of all lipid classes (Table 2). Elevated [CO_2] altered SQDG contents in 700-plants, with reductions in CL153 (43%) and Icatu (44%), and increase (6 fold) in IPR108 (Table 2).

Temperature alone (380-plants) increased GL, with rises in all genotypes at 37/30 °C for MGDG (34, 40, 116%) and DGDG (7, 52, 31%), respectively in CL153, Icatu, and IPR108, and higher MGDG/DGDG values in CL153 and IPR108 (Table 2). MGDG and DGDG were reduced from 37/30 °C to 42/34 °C in Icatu and IPR108, although keeping similar or higher values than those observed at 25/20 °C. The CL153 380-plants showed maximal GL value at 42/34 °C relative to control, due to a further 14% DGDG increase from 37/30 to 42/34 °C.

Despite the stability of total PL in Icatu and CL153, and the strong rise in IPR108 at 37/30 °C, PG increased in CL153 (11%), Icatu (96%), and IPR108 (327%). With further temperature rise, PG values tended to decrease, but maintained similar (CL153, Icatu) or higher (IPR108) values than at 25/20 °C. Among the other PL classes, it stands out PC reduction at 37/30 °C (CL153 and Icatu), and 42/30 °C (all genotypes). PI and PA classes showed mostly fluctuations.

In the 380-plants, temperature rise resulted in SQDG reduction (54%) in CL153, and increase in *C. arabica* genotypes (138% in Icatu; 12 fold in IPR108) at 37/30 °C. Only the *C. arabica* plants showed no negative impact on SQDG at 42/30 °C as compared to the control temperature.

The interaction of elevated [CO_2] and heat promoted different adjustments. In CL153 700-plants, MGDG increased (38%) at 37/30 °C, but consistently maintained lower levels than their 380-counterparts. In contrast, the *C. arabica* genotypes showed higher contents (and weight) of MGDG in 700-plants at 37/30 °C (19% in Icatu; 35% in IPR108). At 42/34 °C, Icatu 700-plants where the only ones that further increased MGDG content in comparison to 37/30 °C. For DGDG, at 37/30 °C, 700-plants of all genotypes showed higher contents and weight than 380-plants, although only IPR108 showed increases when compared to the control (25/20 °C) (Table 2). Again, at 42/34 °C only the 700-plants of Icatu maintained higher (56%) DGDG values than the 380-plants, reflecting a 121% rise when compared to the respective 25/20 °C 380-plants. Despite these GL changes, the values of MGDG/DGDG ratio were quite similar between the two [CO_2] at the two highest temperatures (except CL153 at 37/30 °C).

Among PL classes, PG showed a distinct pattern among genotypes. PG contents and weight were mostly stable on CL153 700-plants (and below to the 380-plants values) along the experiment. For *C. arabica* genotypes, a progressive reinforcement of PG content (and weight) was found in 700-plants, peaking at 42/34 °C, when the values were higher than those of 380-plants. This reflected increases of 54% (Icatu) and 300% (IPR108) when compared to the 700-plants at 25/20 °C, thus showing one of the clearest [CO_2] *vs.* heat interaction among lipid classes adjustments. In fact, at the highest temperature PG became the more represented PL class in all genotypes.

PC and PI contents were lower in the 700-plants at 37/30 °C (except PI in Icatu) than in their 380-counterparts. This was particularly evident for PC, with reductions of 84, 60 and 67% in CL153, Icatu and IPR108, respectively, leading to severe drops of this class weight in all genotypes. At 42/34 °C, PC and PI values were kept or were even more reduced in the 700-plants, but did not differ from the values of 380-plants in most cases. PA usually represented less than 1% of lipid

Table 2
Total galactolipid (GL) and phospholipid (PL), sulpholipids, and individual lipid classes content (mg g^{-1} DW), as well as their relative weight (in parentheses), and ratios (GL/PL, MGDG/DGDG), from chloroplast membranes of leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 µL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25 °C, 37/30 °C, and 42/34 °C. MGDG: monogalactosyldiacylglycerol; DGDG: digalactosyldiacylglycerol; PC: phosphatidylcholine; PG: phosphatidylglycerol; PI: phosphatidylinositol; PA: phosphatidic acid; SQDG: sulfoquinovosyldialcylglycerol. For each parameter, the mean values ± SE (n = 3) followed by different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

		Genotype	[CO_2] (µL L^{-1})	Temperature (day/night, °C)			
				25/20 °C	31/25 °C	37/30 °C	42/34 °C
Galactolipids	**MGDG**	**CL 153**	**380**	7.48 ± 0.03bA (31)	6.86 ± 0.09 bA (32)	10.02 ± 0.28 aA (39)	9.98 ± 0.01 aA (40)
			700	6.10 ± 0.05 bB (31)	5.76 ± 0.01 bB (33)	8.45 ± 0.96 aB (40)	7.07 ± 0.04 abB (38)
		Icatu	**380**	3.91 ± 0.20 bB (30)	4.34 ± 0.10 bA (24)	5.46 ± 0.08 aB (30)	4.00 ± 0.14 bB (29)
			700	7.19 ± 0.14 aA (34)	3.80 ± 0.11 bA (25)	6.51 ± 0.34 aA (34)	7.08 ± 0.02 aA (34)
		IPR 108	**380**	3.11 ± 0.05 dB (28)	5.89 ± 0.07 bA (37)	6.72 ± 0.09 aB (29)	5.20 ± 0.01 cA (34)
			700	3.70 ± 0.13 bA (32)	3.78 ± 0.25 bB (26)	9.09 ± 0.12 aA (35)	3.81 ± 0.13 bB (29)
	DGDG	**CL 153**	**380**	7.96 ± 0.51 bB (33)	6.87 ± 0.11 bA (32)	8.51 ± 0.07 bA (33)	11.21 ± 0.13 aA (45)
			700	10.04 ± 0.04 aA (51)	7.83 ± 0.05 cA (44)	9.99 ± 1.30 aA (47)	8.47 ± 0.05 bcB (46)
		Icatu	**380**	4.57 ± 0.16 cB (35)	7.63 ± 0.23 aA (43)	6.94 ± 0.23 abB (38)	6.46 ± 0.08 bB (48)
			700	10.58 ± 0.10 aA (50)	7.92 ± 0.46 cA (52)	9.02 ± 0.14 bA (47)	10.10 ± 0.21 aA (49)
		IPR 108	**380**	6.24 ± 0.17 cA (55)	7.46 ± 0.03 abA (46)	8.17 ± 0.28 aB (35)	7.14 ± 0.00 bA (46)
			700	4.03 ± 0.05 cB (35)	7.13 ± 0.06 bA (50)	11.34 ± 0.12 aA (44)	4.84 ± 0.31 cB (37)
	MGDG/ DGDG	**CL 153**	**380**	0.94 ± 0.06 bA	1.00 ± 0.03 abA	1.19 ± 0.03 aA	0.89 ± 0.01 bA
			700	0.61 ± 0.01 bB	0.74 ± 0.01 abB	0.85 ± 0.07 abB	0.84 ± 0.01 aA
		Icatu	**380**	0.86 ± 0.01 aA	0.57 ± 0.03 bA	0.79 ± 0.01 aA	0.62 ± 0.03 bA
			700	0.68 ± 0.01 aB	0.48 ± 0.04 bA	0.72 ± 0.03 aA	0.70 ± 0.02 aA
		IPR 108	**380**	0.50 ± 0.02 bB	0.79 ± 0.01 aA	0.82 ± 0.01 aA	0.73 ± 0.00 aA
			700	0.92 ± 0.05 aA	0.53 ± 0.04 bB	0.80 ± 0.03 aA	0.79 ± 0.08 aA
	Total GL	**CL 153**	**380**	15.44 ± 0.48 bA (64)	13.73 ± 0.01 bA (65)	18.53 ± 0.20 aA (72)	21.19 ± 0.12 aA (84)
			700	16.15 ± 0.01 abA (82)	13.59 ± 0.04 bA (77)	18.44 ± 2.26 aA (87)	15.54 ± 0.01 abB (84)
		Icatu	**380**	8.48 ± 0.36 cB (64)	11.98 ± 0.13 aA (67)	12.39 ± 0.31 aB (68)	10.46 ± 0.06 bB (77)
			700	17.77 ± 0.24 aA (85)	11.72 ± 0.35 cA (77)	15.53 ± 0.49 bA (80)	17.19 ± 0.19 aA (83)
		IPR 108	**380**	9.35 ± 0.13 dA (83)	13.35 ± 0.10 bA (83)	14.89 ± 0.36 aB (64)	12.34 ± 0.01 cA (80)
			700	7.73 ± 0.08 dB (66)	10.91 ± 0.19 bB (76)	20.42 ± 0.00 aA (79)	8.65 ± 0.18 cB (65)
Phospholipids	**PC**	**CL 153**	**380**	4.27 ± 0.22 aA (18)	3.45 ± 0.06 bA (16)	2.78 ± 0.00 cA (11)	0.68 ± 0.01 dA (3)
			700	0.72 ± 0.04 aB (4)	0.57 ± 0.05 aB (3)	0.44 ± 0.19 aB (2)	0.43 ± 0.03 aB (2)
		Icatu	**380**	2.11 ± 0.08 aA (16)	2.45 ± 0.09 aA (14)	1.67 ± 0.03 bA (9)	0.53 ± 0.2 cA (4)
			700	0.80 ± 0.02 aB (4)	0.77 ± 0.17 aB (5)	0.67 ± 0.12 aB (3)	0.54 ± 0.03 aA (3)
		IPR 108	**380**	0.41 ± 0.08 bB (4)	0.53 ± 0.04 bB (3)	2.90 ± 0.05 aA (12)	0.24 ± 0.01 bA (2)
			700	1.79 ± 0.01 aA (15)	0.83 ± 0.02 bA (6)	0.97 ± 0.42 bB (4)	0.43 ± 0.06 bA (3)
	PG	**CL 153**	**380**	2.06 ± 0.01 abA (9)	1.86 ± 0.07 bA (9)	2.28 ± 0.03 aA (9)	1.85 ± 0.09 bA (7)
			700	1.22 ± 0.00 aB (6)	1.16 ± 0.06 aB (7)	1.39 ± 0.06 aB (7)	1.14 ± 0.12 aB (6)
		Icatu	**380**	0.99 ± 0.00 bA (8)	1.16 ± 0.03 bA (7)	1.94 ± 0.08 aA (11)	1.42 ± 0.01 abA (10)
			700	1.17 ± 0.03 bA (6)	1.27 ± 0.02 abA (8)	1.69 ± 0.33 abA (9)	1.80 ± 0.00 aA (9)
		IPR 108	**380**	0.64 ± 0.04 cA (6)	1.33 ± 0.10 bA (8)	2.73 ± 0.01 aA (12)	1.45 ± 0.03 bB (9)
			700	0.77 ± 0.02 dA (7)	1.25 ± 0.01 cA (9)	2.38 ± 0.06 bA (9)	3.08 ± 0.21 aA (23)
	PI	**CL 153**	**380**	0.91 ± 0.03 bA (4)	0.85 ± 0.02 bA (4)	1.23 ± 0.04 aA (5)	0.78 ± 0.01 bA (3)
			700	0.89 ± 0.05 aA (4)	0.82 ± 0.05 aA (5)	0.68 ± 0.16 aB (3)	0.65 ± 0.02 aA (4)
		Icatu	**380**	0.78 ± 0.02 aA (6)	0.39 ± 0.02 bB (2)	0.60 ± 0.03 aB (3)	0.56 ± 0.00 abA (4)
			700	0.74 ± 0.04 aA (4)	0.67 ± 0.02 aA (4)	0.80 ± 0.09 aA (4)	0.52 ± 0.0 bA (3)
		IPR 108	**380**	0.66 ± 0.01 bA (6)	0.37 ± 0.03 cB (2)	0.97 ± 0.00 aA (4)	0.45 ± 0.02 cB (3)
			700	0.35 ± 0.03 cB (3)	0.87 ± 0.01 aA (6)	0.76 ± 0.00 bB (3)	0.80 ± 0.02 bA (6)
	PA	**CL 153**	**380**	0.13 ± 0.01 cA (0.5)	0.10 ± 0.00 cA (0.5)	0.19 ± 0.00 bA (0.7)	0.23 ± 0.00 aA (0.9)
			700	0.09 ± 0.00 bcB (0.4)	0.11 ± 0.00 abA (0.6)	0.07 ± 0.01 cB (0.3)	0.14 ± 0.01 aB (0.8)
		Icatu	**380**	0.15 ± 0.01 abA (1.1)	0.10 ± 0.00 cB (0.6)	0.16 ± 0.01 aA (0.9)	0.12 ± 0.00 bcA (0.9)
			700	0.11 ± 0.01 abB (0.5)	0.13 ± 0.01 aA (0.9)	0.08 ± 0.02 bB (0.4)	0.14 ± 0.00 aA (0.7)
		IPR 108	**380**	0.09 ± 0.00 aA (0.8)	0.09 ± 0.01 aA (0.5)	0.12 ± 0.01 aB (0.5)	0.15 ± 0.03 aA (1)
			700	0.10 ± 0.00 bA (0.9)	0.05 ± 0.00 bA (0.4)	0.26 ± 0.03 aA (1)	0.06 ± 0.00 bB (0.4)
	Total PL	**CL 153**	**380**	7.37 ± 0.20 aA (31)	6.26 ± 0.04 bA (30)	6.49 ± 0.07 bA (26)	3.54 ± 0.10 cA (14)
			700	2.91 ± 0.00 aB (15)	2.66 ± 0.04 aB (15)	2.59 ± 0.30 aB (12)	2.36 ± 0.15 aB (13)
		Icatu	**380**	4.04 ± 0.11 aA (31)	4.09 ± 0.04 aA (23)	4.38 ± 0.01 aA (24)	2.63 ± 0.03 bB (19)
			700	2.83 ± 0.10 aB (14)	2.85 ± 0.07 aB (19)	3.23 ± 0.13 aB (17)	3.01 ± 0.04 aA (15)
		IPR 108	**380**	1.81 ± 0.05 bB (16)	2.31 ± 0.12 bB (14)	6.71 ± 0.04 aA (29)	2.29 ± 0.06 bB (15)
			700	3.00 ± 0.04 bA (26)	3.00 ± 0.02 bA (21)	4.37 ± 0.51 aB (17)	4.36 ± 0.30 aA (33)
Total (GL/PL)		**CL 153**	**380**	2.10 ± 0.12 bB	2.19 ± 0.01 bB	2.85 ± 0.11 bB	5.99 ± 0.21 aA
			700	5.54 ± 0.00 bA	5.11 ± 0.06 bA	7.14 ± 0.22 aA	6.60 ± 0.43 aA
		Icatu	**380**	2.10 ± 0.15 cB	2.93 ± 0.06 bB	2.84 ± 0.12 bB	3.97 ± 0.06 aB
			700	6.30 ± 0.30 aA	4.11 ± 0.23 bA	4.80 ± 0.20 bA	5.72 ± 0.02 aA
		IPR 108	**380**	5.19 ± 0.21 aA	5.79 ± 0.35 aA	2.23 ± 0.12 bB	5.38 ± 0.15 aA
			700	2.58 ± 0.06 cB	3.64 ± 0.08 bB	4.67 ± 0.17 aA	1.99 ± 0.09 cB
Sulpholipids	**SQDG**	**CL 153**	**380**	1.15 ± 0.06 aA (5)	1.19 ± 0.04 aB (6)	0.53 ± 0.00 bA (2)	0.45 ± 0.00 bA (2)
			700	0.66 ± 0.05 bB (3)	1.44 ± 0.05 aA (8)	0.28 ± 0.03 cB (1)	0.57 ± 0.01 bA (3)
		Icatu	**380**	0.63 ± 0.01 bA (5)	1.71 ± 0.04 aA (10)	1.50 ± 0.01 aA (8)	0.48 ± 0.03 bA (4)
			700	0.35 ± 0.00 bB (2)	0.69 ± 0.06 aB (5)	0.57 ± 0.12 abB (3)	0.46 ± 0.02 bA (2)
		IPR 108	**380**	0.13 ± 0.00 cB (1)	0.38 ± 0.03 cA (2)	1.70 ± 0.07 aA (7)	0.74 ± 0.03 bA (5)
			700	0.91 ± 0.00 aA (8)	0.48 ± 0.01 bA (3)	1.01 ± 0.21 aB (4)	0.21 ± 0.03 bB (2)

Table 3
Fatty acids composition (mol %) and unsaturation degree (DBI) of monogalactosyldiacylglycerol (MGDG) from chloroplast membranes of leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25, 37/30 and 42/34 °C. For each parameter, the mean values ± SE (n = 3) followed by different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

	Genotype	[CO_2] (μL˜ L^{-1})	Temperature (day/night, °C)			
			25/20°C	31/25°C	37/30°C	42/34°C
< C16:0	CL 153	380	0.82 ± 0.22 aA	0.35 ± 0.17 bB	0.07 ± 0.01 bA	0.17 ± 0.02 bA
		700	0.42 ± 0.03 bB	0.91 ± 0.09 aA	0.27 ± 0.02 bA	0.25 ± 0.02 bA
	Icatu	380	1.86 ± 0.35 aA	0.40 ± 0.00 bB	0.50 ± 0.15 bA	0.43 ± 0.04 bA
		700	0.42 ± 0.12 bB	1.17 ± 0.10 aA	0.35 ± 0.03 bA	0.32 ± 0.00 bA
	IPR 108	380	0.77 ± 0.12 aA	0.35 ± 0.01 bA	0.32 ± 0.00 bA	0.25 ± 0.01 bB
		700	0.80 ± 0.30 abA	0.35 ± 0.10 bcA	0.22 ± 0.02 cA	1.05 ± 0.05 aA
C16:0	CL 153	380	5.87 ± 0.54 aA	4.68 ± 0.06 aA	5.86 ± 0.56 aA	5.45 ± 0.17 aA
		700	4.18 ± 0.11 bB	5.46 ± 0.09 aA	3.74 ± 0.10 bB	6.07 ± 0.16 aA
	Icatu	380	8.53 ± 0.35 cA	7.37 ± 0.26 dB	11.37 ± 0.21 bA	13.45 ± 0.07 aA
		700	6.25 ± 0.21 cB	10.44 ± 0.23 abA	9.74 ± 0.14 bB	10.73 ± 0.00 aB
	IPR 108	380	10.79 ± 0.15 bA	7.97 ± 0.27 cA	17.58 ± 0.26 aA	10.27 ± 0.12 bB
		700	9.43 ± 0.43 cA	8.86 ± 0.03 cA	12.17 ± 0.12 bB	18.62 ± 1.13 aA
C18:0	CL 153	380	1.84 ± 0.16 cA	2.39 ± 0.04 bB	3.08 ± 0.02 aA	2.67 ± 0.01 bB
		700	1.92 ± 0.04 cA	2.71 ± 0.04 bA	3.09 ± 0.10 aA	3.27 ± 0.00 aA
	Icatu	380	2.69 ± 0.39 bA	2.77 ± 0.05 bB	3.75 ± 0.25 aA	3.01 ± 0.02 bA
		700	1.95 ± 0.05 bB	3.80 ± 0.02 aA	2.59 ± 0.04 bB	2.15 ± 0.00 bB
	IPR 108	380	2.83 ± 0.14 bA	2.86 ± 0.12 bB	4.77 ± 0.13 aA	2.05 ± 0.03 cB
		700	2.82 ± 0.06 bcA	3.50 ± 0.04 bA	2.94 ± 0.06 cB	4.46 ± 0.43 aA
C18:1	CL 153	380	0.91 ± 0.04 bA	0.89 ± 0.01 bA	0.70 ± 0.03 cA	1.12 ± 0.01 aA
		700	0.85 ± 0.02 aA	0.66 ± 0.01 bB	0.61 ± 0.00 bA	1.03 ± 0.01 aB
	Icatu	380	0.96 ± 0.01 cB	1.38 ± 0.03 cB	2.63 ± 0.19 bA	2.83 ± 0.04 aA
		700	1.56 ± 0.09 bcA	1.74 ± 0.10 bA	1.17 ± 0.04 cB	2.38 ± 0.00 aB
	IPR 108	380	1.24 ± 0.06 bA	1.11 ± 0.25 bA	2.98 ± 0.04 aA	2.38 ± 0.02 aB
		700	1.33 ± 0.05 bA	1.34 ± 0.01 bA	1.54 ± 0.15 bB	4.24 ± 0.29 aA
C18:2	CL 153	380	2.74 ± 0.09 d B	3.66 ± 0.04 cB	5.19 ± 0.02 bA	10.07 ± 0.06 aA
		700	3.75 ± 0.01 dA	4.63 ± 0.02 cA	5.42 ± 0.03 bA	9.54 ± 0.08 aB
	Icatu	380	4.63 ± 0.06 dA	5.28 ± 0.05 cB	14.38 ± 0.18 bA	21.81 ± 0.04 aA
		700	3.54 ± 0.07 dB	6.72 ± 0.01 cA	13.17 ± 0.10 bB	17.30 ± 0.00 aB
	IPR 108	380	5.60 ± 0.14 dA	7.42 ± 0.01 cA	14.21 ± 0.02 bA	17.29 ± 0.08 aA
		700	3.96 ± 0.08 dB	6.07 ± 0.10 cB	10.53 ± 0.11 bB	22.16 ± 0.39 aA
C18:3	CL 153	380	87.82 ± 0.48 aA	88.03 ± 0.15 aA	85.10 ± 0.60 bB	80.53 ± 0.18 cA
		700	88.88 ± 0.20 aA	85.63 ± 0.18 bB	86.86 ± 0.21 bA	79.84 ± 0.10 cA
	Icatu	380	81.34 ± 0.46 aB	82.80 ± 0.34 aA	67.37 ± 0.98 bB	58.46 ± 0.21 cB
		700	86.28 ± 0.45 aA	76.13 ± 0.24 bB	72.98 ± 0.19 cA	67.12 ± 0.00 dA
	IPR 108	380	78.78 ± 0.37 aB	80.30 ± 0.14 aA	60.13 ± 0.07 cB	67.75 ± 0.11 bA
		700	81.65 ± 0.75 aA	79.88 ± 0.02 bA	72.60 ± 0.42 cA	49.47 ± 0.08 dB
DBI	CL 153	380	32.76 ± 2.29 abB	37.63 ± 0.52 aA	29.87 ± 2.13 bB	31.87 ± 0.57 bA
		700	44.23 ± 1.41 aA	30.54 ± 0.32 bB	39.47 ± 1.23 aA	27.23 ± 0.57 bA
	Icatu	380	20.41 ± 0.88 bB	25.03 ± 0.98 aA	15.06 ± 0.74 cB	13.18 ± 0.13 cB
		700	31.84 ± 1.01 aA	16.10 ± 0.51 cB	19.68 ± 0.14 bA	18.14 ± 0.00 bcA
	IPR 108	380	17.76 ± 0.32 bB	23.33 ± 0.90 aA	9.38 ± 0.15 cB	19.17 ± 0.31 bA
		700	19.91 ± 1.26 aA	20.22 ± 0.01 aB	15.67 ± 0.13 bA	8.23 ± 0.33 cB

classes. Still, enhanced [CO_2] reduced PA in CL153 and Icatu; heat promoted an increase in 380-plants of CL153 (under the two higher temperatures) and IPR108 (42/30 °C), whereas under the interaction the 700-plants showed a consistent tendency to lower content and/or weight at the two highest temperatures (except IPR at 37/30 °C) than the 380-plants.

As regards SQDG, the interaction of conditions promoted significant reductions of content and weight at 37/30 °C in all genotypes, although at 42/34 °C differences persisted only in IPR108.

3.4. Fatty acid composition and saturation degree of complex chloroplast lipids

After noticing global changes in TFAs, and main lipid classes, a more fine level of responses can be addressed, including the FA composition within each class.

GL were by far the most unsaturated classes due to their large C18:3 amounts. Under control conditions C18:3 represented as much as 79 to 88% in MGDG (Table 3), and 43 to 70% in DGDG (Table 4), leading to DBI values of 18–33, and 4–8, in the same class order.

Elevated [CO_2] at 25/20 °C led to rises in C18:3, and reductions in C18:2 and C16:0 of MGDG, in both Icatu and IPR108, whereas in CL153 a slight C18:2 increase and a C16:0 reduction were found (Table 3). These modifications implicated significant increases of MGDG unsaturation degree of 700-plants of CL153 (35%), Icatu (56%), and IPR108 (12%).

In response to heat (37/30 °C), major MGDG changes were detected in *C. arabica* 380-plants, related to large reductions of C18:3, and increases in all the other FAs, mainly C18:2 and C16:0. Similar (but much more moderate) changes were observed in CL153 for C18:3, C18:2 and C18:0. Hence, heat reduced DBI in all genotypes, strongly in Icatu and IPR108. At 42/34 °C the changes were maintained or amplified in Icatu and, partly, CL153, but often reverted in IPR108.

Taking into account the opposite impacts on DBI promoted by high [CO_2] (increase) and heat (decrease), the simultaneous exposure to both conditions led to an intermediate behavior in 700-plants of all genotypes, with a somewhat lower levelof short FAs chain (C16:0). At 37/30 °C DBI was reduced in the 700-plants as compared with the values at 25/20 °C, but kept higher values than the 380-plants, usually related as well to intermediate changes of main FAs. At 42/34 °C stronger declines of C18:3, and rises of C16:0, C18:2, and C18:1 further reduced DBI in 700-plants, mainly in IPR108, which showed the lowest

Table 4
Fatty acids composition (mol %) and unsaturation degree (DBI) of digalactosyldiacylglycerol (DGDG) from chloroplast membranes of leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25 °C, 37/30 °C and 42/34 °C. For each parameter, the mean values ± SE (n = 3) followed by different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

	Genotype	[CO_2] (μL L^{-1})	Temperature (day/night, °C)			
			25/20 °C	31/25 °C	37/30 °C	42/34 °C
< C16:0	CL 153	380	0.92 ± 0.10 aA	0.62 ± 0.12 aA	0.23 ± 0.0 1bA	0.25 ± 0.03 bA
		700	0.48 ± 0.03 aB	0.76 ± 0.29 aA	0.25 ± 0.01 aA	0.26 ± 0.01 aA
	Icatu	380	1.27 ± 0.12 aA	0.66 ± 0.00 bB	0.59 ± 0.03 bA	0.36 ± 0.08 bA
		700	0.45 ± 0.05 bB	1.12 ± 0.03 aA	0.40 ± 0.02 bA	0.47 ± 0.01 bA
	IPR 108	380	0.64 ± 0.10 aB	0.58 ± 0.10 aA	0.43 ± 0.00 aA	0.42 ± 0.01 aB
		700	1.07 ± 0.03 aA	0.40 ± 0.03 bA	0.25 ± 0.07 bA	0.97 ± 0.05 aA
C16:0	CL 153	380	17.82 ± 0.03 dB	22.33 ± 0.71 cB	24.89 ± 0.50 bA	27.92 ± 0.13 aA
		700	23.41 ± 0.34 bA	27.00 ± 0.92 aA	23.03 ± 0.20 bB	26.85 ± 0.62 aA
	Icatu	380	30.79 ± 0.14 cA	28.31 ± 0.54 cB	34.40 ± 0.32 bA	38.55 ± 0.04 aA
		700	29.90 ± 0.90 bA	31.85 ± 0.95 bA	34.13 ± 0.59 aA	34.20 ± 0.22 aB
	IPR 108	380	33.74 ± 0.27 bA	31.25 ± 1.02 cA	41.01 ± 0.42 aA	35.69 ± 0.08 bB
		700	31.77 ± 0.37 bcA	30.48 ± 0.72 cA	33.71 ± 0.70 bB	41.61 ± 0.06 aA
C18:0	CL 153	380	8.04 ± 0.06 bA	8.46 ± 0.52 bB	11.28 ± 0.41 aB	11.37 ± 0.12 aB
		700	8.33 ± 0.24 cA	11.01 ± 0.07 bA	12.66 ± 0.23 aA	12.77 ± 0.29 aA
	Icatu	380	6.83 ± 0.11 bA	7.26 ± 0.18 abA	9.34 ± 0.26 aA	7.99 ± 0.02 abA
		700	6.66 ± 0.02 bA	8.07 ± 0.30 abA	9.95 ± 1.30 aA	7.97 ± 0.12 abA
	IPR 108	380	6.25 ± 0.10 cA	8.05 ± 0.20 aA	7.99 ± 0.09 aA	7.07 ± 0.05 bB
		700	6.76 ± 0.27 cA	8.17 ± 0.11 aA	7.51 ± 0.13 bA	8.62 ± 0.13 aA
C18:1	CL 153	380	1.05 ± 0.09 bB	0.86 ± 0.02 bB	0.95 ± 0.00 bB	2.43 ± 0.00 aA
		700	2.05 ± 0.17 abA	1.97 ± 0.03 abA	1.67 ± 0.04 bA	2.33 ± 0.03 aA
	Icatu	380	1.07 ± 0.02 cA	1.78 ± 0.01 bA	1.64 ± 0.12 bA	2.77 ± 0.05 aA
		700	1.28 ± 0.07 cA	1.43 ± 0.08 cB	2.08 ± 0.06 bA	2.83 ± 0.02 aA
	IPR 108	380	1.83 ± 0.02 bA	1.32 ± 0.14 cA	1.34 ± 0.02 cA	2.46 ± 0.00 aB
		700	0.89 ± 0.01 cB	1.34 ± 0.06 bA	1.51 ± 0.07 bA	2.87 ± 0.02 aA
C18:2	CL 153	380	2.57 ± 0.27 cB	5.03 ± 0.59 bB	4.52 ± 0.24 dB	8.86 ± 0.01 aA
		700	16.07 ± 0.12 bA	18.95 ± 0.34 aA	9.64 ± 0.15 cA	9.13 ± 0.07 cA
	Icatu	380	5.43 ± 0.87 cB	3.00 ± 0.13 dB	9.79 ± 0.43 bB	13.58 ± 0.10 aA
		700	12.11 ± 0.14 aA	13.42 ± 0.13 aA	13.30 ± 0.04 aA	12.88 ± 0.02 aA
	IPR 108	380	14.72 ± 0.10 aA	11.71 ± 0.07 cB	8.30 ± 0.01 dB	12.20 ± 0.03 bB
		700	2.28 ± 0.05 cB	12.71 ± 0.22 bA	12.89 ± 0.41 abA	13.55 ± 0.01 aA
C18:3	CL 153	380	69.59 ± 0.19 aA	62.69 ± 0.91 bA	58.12 ± 0.26 cA	49.17 ± 0.21 dA
		700	49.66 ± 0.22 bB	40.30 ± 0.40 cB	52.74 ± 0.20 aB	48.66 ± 0.24 bA
	Icatu	380	54.61 ± 0.98 bA	58.98 ± 0.22 aA	44.24 ± 0.08 cA	36.76 ± 0.20 dB
		700	49.59 ± 0.73 aB	44.12 ± 0.47 bB	40.14 ± 1.94 cB	41.65 ± 0.07 bcA
	IPR 108	380	42.82 ± 0.19 bB	47.09 ± 0.71 aA	40.93 ± 0.47 bB	42.17 ± 0.00 bA
		700	57.24 ± 0.60 aA	46.90 ± 0.36 bA	44.12 ± 1.36 cA	32.38 ± 0.12 dB
DBI	CL 153	380	8.16 ± 0.01 aA	6.41 ± 0.11 bA	5.06 ± 0.09 cA	4.25 ± 0.04 dA
		700	5.74 ± 0.01 aB	4.20 ± 0.14 cB	5.00 ± 0.01 bA	4.18 ± 0.06 cA
	Icatu	380	4.61 ± 0.05 aA	5.15 ± 0.08 aA	3.48 ± 0.27 bA	3.00 ± 0.01 bB
		700	4.73 ± 0.19 aA	3.96 ± 0.10 bcB	3.38 ± 0.08 cA	3.61 ± 0.01 cA
	IPR 108	380	3.96 ± 0.02 abB	4.19 ± 0.16 aA	2.86 ± 0.08 cB	3.56 ± 0.00 bA
		700	4.54 ± 0.14 aA	4.32 ± 0.11 abA	3.85 ± 0.12 bA	2.49 ± 0.01 cB

value in this experiment.

The impact of elevated [CO_2] on the unsaturation degree of DGDG was rather diverse in CL153 (30% reduction), Icatu (maintainance) and IPR108 (15% increase). In fact, the major FA (C18:3) was reduced in CL153 (29%) and Icatu (9%), and increased (34%) in IPR108, with an opposite variation on C18:2. Higher C16:0 and C18:1 values were found only in CL153 (Table 4).

Similarly to MGDG, heat (37/30 °C) induced DBI decreases in DGDG regardless of genotypes. This resulted from reductions in C18:3, and increases in C16:0, C18:0 and C18:2 (the latter absent in IPR108), with earlier responses (31/25 °C) in CL153 380-plants. At 42/34 °C the DBI values tended to a further reduction in CL153 and Icatu, due to stronger reduction of C18:3, and increases of C16:0 and C18:1 and C18:2.

Contrasting with MGDG, elevated [CO_2] did not modify the heat response (37/30 °C) in DGDG, showing close DBI values under both [CO_2] in CL153 and Icatu. However, these similar DBI values resulted from lower abundance of C18:3, and higher abundance of C18:1 and C18:2 in 700-plants than in 380-plants. Notably, at 42/34 °C only Icatu 700-plants showed higher DBI values than the 380-plants for both GL classes, with an opposite pattern found in IPR108.

In addition to quantitative (and weight) modifications (Table 2), PL classes were qualitatively altered in their FA composition and unsaturation (Tables 5–8), often distinct among genotypes.

The large increase of PC in IPR108 plants under enhanced [CO_2] alone did not change the DBI (Table 5), despite the strongly altered FA composition, with significant increases in C18:3 (61%) and C16:0 (8%), and reduction of C18:2 (41%), and C18:1 (20%). By contrast, PC decreased in the 700-plants of CL153 and Icatu (Table 2), with DBI reductions of 46% and 57%, respectively. This resulted mainly from an enrichment in the more saturated FAs C16:0 (32% and 49%), and C18:0 (84% and 49%), and falls in C18:2 (69% and 79%) in CL153 and Icatu, respectively, what in parallel reduced C-chain length of FAs (large C16:0 increases).

Heat alone, also reduced PC amount at 37/30 °C (CL153 and Icatu), and, especially,at 42/34 °C (all genotypes). DBI was declined, mainly related with C16:0 increases, coupled to C18:2 reductions. The strong PC increase in IPR108 380-plants (6.1 fold) at 37/30 °C did not modify the DBI despite the changes in most FAs, including C16:0 increase and C18:3 reduction.

The [CO_2] *vs*. heat interaction amplifyed the impact on PC, which showed the strongest reductions at 37/30 °C in all genotypes. Still, in the 700-plants DBI values did not differ from those at 25/20 °C,

Table 5
Fatty acids composition (mol %) and unsaturation degree (DBI) of phosphatidylcholine (PC) from chloroplast membranes of leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25 °C, 37/30 °C and 42/34 °C. For each parameter, the mean values ± SE (n = 3) followed by different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

	Genotype	[CO_2] (μL L^{-1})	Temperature (day/night, °C)			
			25/20 °C	31/25 °C	37/30 °C	42/34 °C
< C16:0	CL 153	380	1.54 ± 0.15 aA	0.86 ± 0.01 bA	0.33 ± 0.12 cA	1.01 ± 0.02 bA
		700	1.75 ± 0.12 aA	1.18 ± 0.01 bA	0.56 ± 0.08 cA	1.07 ± 0.07 bA
	Icatu	380	2.01 ± 0.22 aA	0.95 ± 0.11 aB	0.56 ± 0.01 aA	1.07 ± 0.05 aA
		700	2.47 ± 0.31 bA	4.48 ± 0.91 aA	0.92 ± .02 cA	1.08 ± 0.34 bcA
	IPR 108	380	1.14 ± 0.00 cB	2.15 ± 0.41 bA	1.30 ± 0.28 cA	3.72 ± 0.03 aA
		700	1.89 ± 0.08 aA	0.98 ± 0.03 bB	0.81 ± 0.17 bA	1.44 ± 0.07 abB
C16:0	CL 153	380	32.67 ± 0.06 bB	34.69 ± 1.26 bB	35.02 ± 1.19 bB	57.41 ± 0.24 aA
		700	43.01 ± 1.51 bA	47.60 ± 0.17 aA	48.33 ± 1.34 aA	50.40 ± 0.45 aB
	Icatu	380	35.87 ± 0.84 cB	39.08 ± 1.12 bcB	42.60 ± 1.19 bB	50.94 ± 0.31 aA
		700	53.27 ± 1.10 bA	58.70 ± 0.86 aA	53.18 ± 1.34 bA	48.45 ± 1.11 cA
	IPR 108	380	40.89 ± 0.00 bB	55.14 ± 0.88 aA	43.15 ± 1.29 bB	44.27 ± 0.99 bB
		700	44.30 ± 0.23 cA	54.74 ± 0.52 aA	49.16 ± 1.35 bA	57.47 ± 1.80 aA
C18:0	CL 153	380	8.10 ± 0.23 cB	10.37 ± 0.02 bcB	14.55 ± 0.94 aA	11.59 ± 0.76 bA
		700	14.93 ± 0.06 bA	18.42 ± 1.35 aA	11.96 ± 0.18 cB	11.77 ± 0.13 cA
	Icatu	380	9.34 ± 0.34 abB	10.37 ± 0.17 aA	9.27 ± 0.83 abA	7.40 ± 0.20 bB
		700	13.89 ± 0.55 aA	9.56 ± 0.62 bA	9.69 ± 0.03 bA	9.39 ± 0.93 bA
	IPR 108	380	12.32 ± 0.00 aA	11.27 ± 0.35 abA	9.16 ± 0.51 bcA	7.83 ± 0.22 cA
		700	10.46 ± 0.80 bB	12.86 ± 0.76 aA	9.36 ± 0.82 bA	8.47 ± 0.17 bA
C18:1	CL 153	380	4.17 ± 0.09 aA	4.02 ± 0.02 aA	4.59 ± 0.38 aA	5.24 ± 0.01 aA
		700	5.24 ± 0.10 abA	3.82 ± 0.83 bA	4.69 ± 0.24 abA	5.90 ± 0.20 aA
	Icatu	380	3.84 ± 0.08 bA	2.56 ± 0.06 cA	5.53 ± 0.33 aA	4.93 ± 0.14 aB
		700	4.38 ± 0.50 bcA	2.65 ± 0.11 cA	3.77 ± 0.12 bA	6.16 ± 0.27 aA
	IPR 108	380	4.32 ± 0.00 bcA	3.91 ± 0.42 cA	5.00 ± 0.13 bA	8.27 ± 0.33 aA
		700	3.45 ± 0.19 bB	3.72 ± 0.23 bA	4.16 ± 0.13 bB	5.71 ± 0.11 aB
C18:2	CL 153	380	31.60 ± 0.42 aA	31.99 ± 0.78 aA	27.19 ± 0.24 bA	7.17 ± 0.20 cAB
		700	9.89 ± 0.30 abB	8.80 ± 1.37 bB	11.76 ± 0.30 aB	10.63 ± 0.05 abA
	Icatu	380	31.76 ± 0.11 aA	29.49 ± 0.65 abA	26.94 ± 0.08 bA	14.82 ± 0.17 cA
		700	6.80 ± 0.17 bB	8.70 ± 1.34 bB	12.00 ± 0.61 aB	13.90 ± 0.82 aA
	IPR 108	380	26.06 ± 0.00 bA	8.96 ± 0.16 dA	29.74 ± 1.49 aA	17.78 ± 0.13 cA
		700	15.33 ± 0.99 aB	7.08 ± 0.37 cA	14.72 ± 0.19 abB	11.86 ± 0.86 bB
C18:3	CL 153	380	21.90 ± 0.36 aB	18.08 ± 0.44 bA	18.33 ± 0.30 bB	17.58 ± 0.76 bB
		700	25.19 ± 1.30 aA	20.18 ± 0.63 bA	22.70 ± 0.73 abA	20.23 ± 0.51 bA
	Icatu	380	17.19 ± 0.08 bA	17.55 ± 0.46 abA	15.10 ± 0.04 bB	20.84 ± 0.04 aA
		700	19.18 ± 0.67 abA	15.90 ± 1.16 bA	20.43 ± 0.64 aA	21.01 ± 1.82 aA
	IPR 108	380	15.27 ± 0.00 bB	18.57 ± 1.20 aA	11.65 ± 0.72 cB	18.13 ± 0.33 abA
		700	24.56 ± 0.32 aA	20.63 ± 1.10 bA	21.80 ± 0.37 abA	15.05 ± 0.59 cB
DBI	CL 153	380	3.20 ± 0.01 aA	2.70 ± 0.14 bA	2.29 ± 0.08 cA	1.04 ± 0.05 dB
		700	1.73 ± 0.12 aB	1.24 ± 0.08 bB	1.58 ± 0.03 aB	1.39 ± 0.02 abA
	Icatu	380	2.62 ± 0.05 aA	2.29 ± 0.11 abA	2.00 ± 0.08 bcA	1.63 ± 0.01 cA
		700	1.12 ± 0.07 bcB	0.94 ± 0.11 cB	1.40 ± 0.02 abB	1.67 ± 0.18 aA
	IPR 108	380	1.92 ± 0.00 aA	1.14 ± 0.07 bA	1.89 ± 0.05 aA	1.81 ± 0.06 aA
		700	1.94 ± 0.10 aA	1.18 ± 0.08 bA	1.67 ± 0.17 aA	1.11 ± 0.08 bB

although being lower than those of 380-plants in CL153. This was a consequence of higher weight of C16:0 and a strong reduction of C18:2 in the 700-plants.

PG amount was reduced at elevated [CO_2] and 25/20 °C only in CL153 (Table 2), without striking changes in DBI in all genotypes, and with changes in all individual FAs in IPR108 (Table 6). With temperature rise, PG amounts increased in the *C. arabica* plants up to 37/30 °C, in both [CO_2] (Table 2). From 25/20 to 37/30 °C, all plants except IPR108 700 tended to lower DBI values due to small FA variations, mainly related to C18:1 and C16:0 enrichment, and C18:3 reduction. At 42/34 °C, CL153 showed the lowest DBI values, irrespective of [CO_2].

Elevated [CO_2] at 25/20 °C did not alter the relative abundance of *trans*-hexadecenoic acid (C16:1*t*) in Icatu, but reduced it in CL153 (12%) and IPR108 (29%). The exposure to heat (37/30 °C and 42/34 °C) resulted in decreases of C16:1*t*, larger in CL153 and IPR108 than in Icatu. At 37/30 °C, the C16:1*t* weight values were similar among [CO_2] conditions, but at 42/34 °C the 700-plants of Icatu and IPR108 displayed lower values than their 380-counterparts, although this might have been compensated (at least partly) for the higher PG content in 700-plants (Table 2).

High [CO_2] at 25/20 °C did not modify the DBI of PI class in all genotypes despite the significant rise in C16:0 in the *C. arabica* genotypes and C18:0 reduction (Icatu) (Table 7), thus showing an enrichment in shorter C-chain length, as in PC. Heat alone increased PI amounts in CL153 and IPR108, but with an opposed impact on DBI, which was decreased or increased, respectively, at 37/30 °C. These changes were mostly related to C16:0 increase and C18:3 reduction (CL153), and C16:0, C18:2 and C18:3 increase, and a large C18:0 reduction (IPR108). Notably, even the DBI maintenance in Icatu at 37/30 °C included an enrichment in FA with a short C-chain (C16:0), and a reduction in C18:0, what was further exacerbated (together with a C18:3 rise) at 42/34 °C in all genotypes and both [CO_2]. Regarding the [CO_2] and heat interaction, no clear DBI trends were depicted, but the 700-plants of Icatu (37/30 °C) and IPR108 (42/34 °C) showed lower values than the 380-plants at these temperatures. This resulted mainly from a strong increase of C18:0 in the first case, while increases of the saturated (C16:0 and C18:0) and decreases in the unsaturated (C18:2 and C18:3) FAs for the second case (Table 7).

PA class did not show relevant DBI changes due to the single exposure to elevated [CO_2] or heat (37/30 °C) in Icatu and CL153 (Table 8). However, at 42/34 °C a global rise of DBI was found in all genotypes and both [CO_2] (except IPR108 700-plants), usually resulting

Table 6
Fatty acids composition (mol %) and unsaturation degree (DBI) of phosphatidylglycerol (PG) from chloroplast membranes of leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25 °C, 37/30 °C and 42/34 °C. For each parameter, the mean values ± SE (n = 3) followed by different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

	Genotype	[CO_2] (μL L^{-1})	Temperature (day/night, °C)			
			25/20 °C	31/25 °C	37/30 °C	42/34 °C
< C16:0	CL 153	380	4.10 ± 0.24 aA	1.24 ± 0.11 bB	0.54 ± 0.00 bA	0.67 ± 0.02 bB
		700	1.52 ± 0.42 abB	2.11 ± 0.56 aA	0.67 ± 0.06 bA	1.69 ± 0.14 abA
	Icatu	380	2.87 ± 0.21 aA	1.89 ± 0.07 abA	1.79 ± 0.83 abA	0.86 ± 0.08 bB
		700	2.03 ± 0.18 abA	2.63 ± 0.03 aA	0.99 ± 0.08 bA	2.05 ± 0.06 abA
	IPR 108	380	1.87 ± 0.19 aB	1.28 ± 0.02 abA	0.68 ± 0.17 bA	1.89 ± 0.26 aA
		700	3.39 ± 0.12 aA	0.98 ± 0.33 cA	0.61 ± 0.04 cA	1.95 ± 0.13 bA
C16:0	CL 153	380	41.13 ± 0.32 cA	48.64 ± 0.15 abA	44.66 ± 0.64 bcA	50.54 ± 1.50 aA
		700	43.15 ± 2.31 aA	47.48 ± 1.28 aA	44.94 ± 0.35 aA	47.06 ± 1.65 aA
	Icatu	380	34.30 ± 0.73 bA	29.30 ± 0.02 cB	34.42 ± 0.41 bA	41.45 ± 0.95 aA
		700	33.22 ± 0.25 cA	44.52 ± 0.62 aA	36.29 ± 0.41 bcA	38.11 ± 1.63 bB
	IPR 108	380	35.46 ± 0.57 bB	35.04 ± 0.18 bB	44.03 ± 0.60 aA	36.88 ± 0.38 bB
		700	41.99 ± 0.07 bA	37.74 ± 1.13 cA	36.60 ± 0.76 cB	46.16 ± 0.58 aA
C16:1 *t*	CL 153	380	19.88 ± 0.01 aA	15.89 ± 0.25 bA	13.17 ± 1.15 cA	9.56 ± 0.11 dA
		700	17.56 ± 0.23 aB	16.49 ± 0.42 aA	12.15 ± 0.42 bA	11.03 ± 0.41 bA
	Icatu	380	23.27 ± 0.10 aA	22.03 ± 0.51 abA	20.14 ± 0.86 bA	20.01 ± 0.21 bA
		700	23.27 ± 1.34 aA	15.67 ± 0.01 cB	20.14 ± 0.88 bA	14.93 ± .81 cB
	IPR 108	380	26.22 ± 1.19 aA	17.58 ± 0.52 bA	17.59 ± 1.00 bA	17.09 ± 0.54 bA
		700	18.67 ± 0.40 aB	18.90 ± 1.12 aA	18.21 ± 0.16 aA	14.19 ± 0.17 bB
C18:0	CL 153	380	10.16 ± 0.49 bA	11.94 ± 0.83 abA	13.46 ± 1.12 abA	13.06 ± 0.63 aA
		700	10.77 ± 0.21 cA	10.82 ± 0.30 cA	17.43 ± 0.75 aA	13.92 ± 0.12 bA
	Icatu	380	12.32 ± 0.81 aA	11.37 ± 0.12 aB	11.3 ± 0.19 aB	11.40 ± 0.04 aA
		700	12.05 ± 0.21 bA	14.52 ± 0.13 aA	14.08 ± 0.10 aA	8.98 ± 0.32 cB
	IPR 108	380	11.28 ± 0.28 aB	10.85 ± 0.24 aB	10.05 ± 0.21 abA	9.05 ± 0.01 bB
		700	13.27 ± 0.72 aA	13.56 ± 0.63 aA	9.26 ± 0.26 bA	10.51 ± 0.51 bA
C18:1	CL 153	380	12.58 ± 0.22 bA	13.26 ± 0.07 abA	16.24 ± 1.74 abA	16.66 ± 0.51 aA
		700	12.60 ± 0.42 bcA	11.35 ± 1.49 cA	15.91 ± 0.40 abA	17.34 ± 0.94 aA
	Icatu	380	12.27 ± 0.96 bcA	9.79 ± 0.50 cA	17.19 ± 0.98 aA	14.64 ± 0.29 abA
		700	11.65 ± 0.65 bcA	10.33 ± 0.14 cA	16.08 ± 0.52 aA	14.23 ± 1.00 abA
	IPR 108	380	13.16 ± 0.12 bA	13.13 ± 0.12 bA	17.78 ± 0.09 aA	17.63 ± 0.11 aA
		700	7.79 ± 0.14 dB	12.60 ± 0.36 cA	15.87 ± 0.35 bB	17.35 ± 0.04 aA
C18:2	CL 153	380	4.44 ± 0.34 abA	3.75 ± 0.02 bA	5.34 ± 0.64 aA	4.10 ± 0.16 abA
		700	5.25 ± 0.52 aA	3.33 ± 0.48 bA	4.14 ± 0.11 abB	3.80 ± 0.16 abA
	Icatu	380	5.01 ± 0.19 abA	3.84 ± 0.30 bA	5.09 ± 0.37 aA	6.15 ± 0.31 aA
		700	4.60 ± 0.03 bcA	3.42 ± 0.27 cA	5.78 ± 0.44 aA	6.75 ± 0.18 aA
	IPR 108	380	5.12 ± 0.08 bA	5.07 ± 0.03 bA	3.73 ± 0.19 cB	6.52 ± 0.22 aA
		700	3.01 ± 0.08 cB	4.47 ± 0.17 bB	5.41 ± 0.04 aA	5.10 ± 0.13 aB
C18:3	CL 153	380	7.72 ± 0.49 aA	5.29 ± 0.41 aB	6.59 ± 1.72 aA	5.43 ± 0.12 aA
		700	9.16 ± 1.39 aA	8.41 ± 0.59 abA	4.76 ± 0.22 cA	5.17 ± 0.16 bcA
	Icatu	380	9.96 ± 1.53 bA	21.78 ± 1.50 aA	7.29 ± 1.10 bA	5.48 ± 0.43 bB
		700	13.16 ± 2.66 aA	8.92 ± 0.11 bB	9.41 ± 1.17 abA	14.95 ± 0.75 aA
	IPR 108	380	6.88 ± 1.13 cB	17.05 ± 0.81 aA	6.15 ± 1.65 cB	10.93 ± 0.35 bA
		700	11.88 ± 0.08 bA	11.75 ± 1.42 bB	14.04 ± 0.23 aA	4.74 ± 0.05 cB
DBI	CL 153	380	1.21 ± 0.08 aA	0.87 ± 0.03 aA	1.02 ± 0.13 aA	0.79 ± 0.03 aA
		700	1.28 ± 0.16 aA	1.01 ± 0.10 abA	0.81 ± 0.02 bA	0.84 ± 0.05 bA
	Icatu	380	1.59 ± 0.07 bA	2.52 ± 0.08 aA	1.40 ± 0.05 bcA	1.19 ± 0.06 cB
		700	1.80 ± 0.14 aA	0.99 ± 0.02 bB	1.58 ± 0.06 aA	1.82 ± 0.05 aA
	IPR 108	380	1.51 ± 0.04 bA	1.97 ± 0.07 aA	1.13 ± 0.04 cB	1.71 ± 0.03 bA
		700	1.21 ± 0.01 bcB	1.46 ± 0.09 bB	1.87 ± 0.08 aA	0.96 ± 0.01 cB

from enrichments in C18:3 and C18:2, and reductions in C18:0 and C18:1. Notably, the 700-plants of CL153 and Icatu (37/30 °C) and CL153 and IPR108 (42/34 °C) showed lower PA content together with lower DBI when compared to their 380-plants counterparts.

At 25/20 °C, elevated [CO_2] led to decreases (CL153 and Icatu) or increase (IPR108) in SGDG, which become more or less saturated, respectively, due to corresponding changes in the saturated (C16:0), and unsaturated (C18:3, C18:2) FAs (Table 9). Thus, in CL153 and Icatu a tendency to a lower C-chain length FAs occurs under elevated [CO_2]. With temperature rise alone, SQDG dropped at 37/30 °C in CL153 accompanied by DBI reductions, which were related to marked increase of C16:0 and decrease of C18:3. In contrast, the SQDG increase in 380-plants of *C. arabica* genotypes at 37/30 °C (Icatu since 31/25 °C) paralleled a DBI rise. This resulted mainly from increases in C18:3 and C18:2, and reduction of C18:0. Despite the SQDG reduction induced by elevated [CO_2] at 37/30 °C in all genotypes, only Icatu showed a reduced DBI, but all genotypes (and both [CO_2]) showed enrichment in C16:0 when compared to the values at 25/20 °C. At 42/34 °C a further enrichment in the smaller C-chain length C16:0 was observed only in the 380-plants of CL153 and Icatu, while their 700-counterparts showed significantly lower values of C16:0. IPR108 showed a lower content and DBI in the 700-plants.

3.5. Gene expression analysis

To complement lipid analysis, we performed qRT-PCR gene expression studies related to three linoleate lipoxygenases (*LOX3, LOX5A, LOX5B*) and one ω-fatty acid desaturase (*FAD3*), all related to chloroplast lipid metabolism (Table 10).

Under control temperature, elevated [CO_2] resulted in a clear down-regulation of the transcriptional activity of LOX genes in all genotypes, with the exception for *LOX5B* in *C. arabica* genotypes, which expression

Table 7
Fatty acids composition (mol %) and unsaturation degree (DBI) of phosphatidylinositol (PI) from chloroplast membranes of leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25 °C, 37/30 °C and 42/34 °C. For each parameter, the mean values ± SE (n = 3) followed by different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

	Genotype	[CO_2] (μL L^{-1})	Temperature (day/night, °C)			
			25/20 °C	31/25 °C	37/30 °C	42/34 °C
< C16:0	CL 153	380	5.43 ± 0.53 aA	1.84 ± 0.14 bA	2.10 ± 0.27 bA	1.31 ± 0.02 bB
		700	2.25 ± 0.25 aA	2.24 ± 0.08 bB	1.49 ± 0.18 bA	2.85 ± 0.52 bA
	Icatu	380	5.97 ± 0.65 aA	3.49 ± 0.32 bB	2.86 ± 0.49 bA	1.99 ± 0.26 bA
		700	4.30 ± 0.53 aB	5.62 ± 0.22 aA	1.54 ± 0.11 bB	1.65 ± 0.07 bA
	IPR 108	380	7.62 ± 0.13 aA	4.72 ± 0.37 bA	1.62 ± 0.13 cA	2.56 ± 0.38 cB
		700	5.13 ± 0.32 aB	1.70 ± 0.32 cB	1.55 ± 0.02 cA	3.49 ± 0.23 bA
C16:0	CL 153	380	43.88 ± 0.89 cA	52.45 ± 0.39 bA	53.11 ± 0.45 bA	55.94 ± 0.32 aA
		700	43.82 ± 0.88 bA	51.77 ± 1.01 aA	51.17 ± 0.45 aA	49.94 ± 0.04 aB
	Icatu	380	41.56 ± 0.69 cB	55.37 ± 0.60 aB	46.97 ± 0.88 bA	52.64 ± 0.94 aA
		700	48.46 ± 1.97 bcA	59.54 ± 1.38 aA	45.96 ± 1.08 cA	50.73 ± 0.57 bA
	IPR 108	380	47.16 ± 1.03 cB	60.03 ± 0.71 aA	52.21 ± 0.93 bA	51.20 ± 0.97 bcB
		700	52.41 ± 0.92 bA	54.26 ± 2.25 bB	50.64 ± 0.03 bA	60.63 ± 0.16 aA
C18:0	CL 153	380	11.21 ± 0.03 aA	11.95 ± 0.18 aA	8.67 ± 0.69 abB	6.78 ± 0.95 bA
		700	12.27 ± 0.53 aA	9.80 ± 0.47 abA	12.59 ± 1.05 aA	7.82 ± 1.44 bA
	Icatu	380	19.61 ± 0.82 aA	15.18 ± 0.91 aA	15.68 ± 2.89 aB	7.12 ± 0.07 bA
		700	15.38 ± 0.13 bB	12.48 ± 0.64 bA	26.48 ± 2.25 aA	6.20 ± 0.19 cA
	IPR 108	380	20.66 ± 1.62 aA	11.25 ± 1.07 bB	9.89 ± 0.59 bA	6.34 ± 0.28 cB
		700	20.52 ± 1.11 aA	17.93 ± 0.72 aA	8.58 ± 0.11 bA	9.64 ± 0.08 bA
C18:1	CL 153	380	3.93 ± 0.04 aA	3.92 ± 0.39 aA	3.69 ± 0.42 aA	2.82 ± 0.41 aA
		700	4.03 ± 0.42 aA	3.41 ± 0.12 aA	2.75 ± 0.13 aA	3.08 ± 0.24 aA
	Icatu	380	4.56 ± 0.35 aA	4.19 ± 0.06 aA	5.05 ± 0.42 aA	5.10 ± 0.13 aA
		700	4.46 ± 0.46 aA	3.31 ± 0.32 aA	4.17 ± 0.04 aA	3.67 ± 0.12 aB
	IPR 108	380	5.16 ± 0.23 bB	3.32 ± 0.01 cA	6.41 ± 0.19 aA	4.82 ± 0.33 bA
		700	6.71 ± 0.48 aA	3.63 ± 0.11 bA	3.81 ± 0.05 bB	4.88 ± 0.37 bA
C18:2	CL 153	380	15.99 ± 0.34 aB	16.10 ± 0.02 aA	17.72 ± 1.06 aA	11.83 ± 0.35 bB
		700	18.50 ± 0.06 aA	14.84 ± 1.05 bA	14.99 ± 0.03 bB	14.73 ± 0.24 bA
	Icatu	380	12.67 ± 1.89 abA	10.82 ± 0.24 bA	15.84 ± 1.24 aA	12.10 ± 0.37 abA
		700	12.61 ± 0.58 aA	10.06 ± 1.09 aA	11.41 ± 0.94 aB	13.32 ± 0.04 aA
	IPR 108	380	10.30 ± 0.11 bA	11.95 ± 0.24 bA	16.21 ± 1.02 aB	12.34 ± 0.02 bA
		700	8.28 ± 0.33 bB	9.55 ± 0.21 bB	19.99 ± 0.24 aA	9.38 ± 0.82 bB
C18:3	CL 153	380	19.55 ± 1.10 aA	13.74 ± 0.06 bB	14.71 ± 0.23 bA	21.31 ± 0.50 aA
		700	19.13 ± 1.09 abA	17.94 ± 0.23 abA	17.01 ± 1.58 bA	21.57 ± 1.91 aA
	Icatu	380	15.62 ± 0.61 bA	10.95 ± 0.29 cA	13.60 ± 1.61 bcA	21.05 ± 0.76 aA
		700	14.79 ± 0.28 bA	8.99 ± 1.47 cA	10.44 ± 0.38 cA	24.43 ± 0.29 aA
	IPR 108	380	9.09 ± 0.12 cA	8.72 ± 0.26 cB	13.67 ± 1.30 bA	22.73 ± 0.76 aA
		700	6.95 ± 0.28 bA	12.92 ± 1.95 aA	15.43 ± 0.03 aA	11.98 ± 0.60 aB
DBI	CL 153	380	1.64 ± 0.09 aA	1.20 ± 0.01 bA	1.32 ± 0.11 abA	1.42 ± 0.08 abA
		700	1.74 ± 0.05 aA	1.39 ± 0.08 abA	1.31 ± 0.06 bA	1.63 ± 0.15 abA
	Icatu	380	1.23 ± 0.11 abA	0.83 ± 0.01 bA	1.22 ± 0.05 abA	1.52 ± 0.08 aA
		700	1.14 ± 0.07 bA	0.67 ± 0.10 bA	0.82 ± 0.18 bB	1.81 ± 0.03 aA
	IPR 108	380	0.73 ± 0.01 cA	0.74 ± 0.01 cA	1.28 ± 0.01 bA	1.67 ± 0.08 aA
		700	0.61 ± 0.02 cA	0.85 ± 0.10 bA	1.50 ± 0.04 aA	0.83 ± 0.01 bcB

was up-regulated (significantly in IPR108).

Upon temperature rise all LOX genes were down-regulated in CL153. For *C. arabica* genotypes *LOX3* and *LOX5A* transcript levels were also consistently lower than those from the control (except *LOX5A* in Icatu at 31/25 °C). However, *LOX5B* transcripts varied differently, with an accumulation in Icatu (31/25 °C and 37/30 °C) and IPR108 (37/30 °C), showing, respectively, *ca.* 3.5, 5.7 and 1.8 higher fold values than their respective controls.

The exposure to both enhanced [CO_2] and heat did not greatly modify the response pattern of *LOX3* in none of the genotypes. However, in 700-plants an increase in transcriptional activity was observed for *LOX5A* and *LOX5B* in Icatu at 42/34 °C (4.73 and 1.63 fold), and for *LOX5B* in IPR108 at 31/25 °C (4.48 fold) and 37/30 °C (3.21 fold). Furthermore, such up-regulated values in the 700-plants were always higher than those of the 380-plants at each temperature.

The pattern of *FAD3* expression showed as well variations among genotypes. Almost no significant changes were found in CL153 and Icatu for these environmental conditions. Still, CL153 consistently presented higher transcript accumulation under increased temperature alone (380-plants), whereas Icatu showed a significant reduction at 42/34 °C irrespective of [CO_2]. In contrast, the IPR108 plants showed strong down-regulation of *FAD3* transcription associated with i) elevated [CO_2] (at 25/20 °C), ii) heat, at the two highest temperature (380-plants), and iii) under the combined exposure of these conditions from 31/25 °C onwards.

4. Discussion

4.1. Membrane integrity and TFA changes under elevated [CO_2] and heat

Overall, the moderate low membrane leakage values (even at 42/34 °C) (Fig. 1) should reflect a good thermostability, likely related to the heat driven increase of antioxidative enzymes and other protective proteins, coupled with the upregulation of their coding genes (Martins et al., 2016), together with an altered lipid composition shown in this current study. Notably, TFA increased with heat, reaching maximal values at 37/30 °C, but at 42/34 °C only CL153 (both [CO_2]) and Icatu (700-plants) maintained their levels. Additionally, while the 380-plants from all genotypes maintained their DBI values at 37/30 °C, important increases in FA unsaturation were observed in Icatu and CL153 at elevated [CO_2]. This reflected qualitative changes of newly synthesized PUFAs, suggesting that a higher fluidity of chloroplast membranes was

Table 8
Fatty acids composition (mol %) and unsaturation degree (DBI) of phosphatidic acid (PA) from chloroplast membranes of leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25 °C, 37/30 °C and 42/34 °C. For each parameter, the mean values ± SE (n = 3) followed by different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

	Genotype	[CO_2] (μL L^{-1})	Temperature (day/night, °C)			
			25/20 °C	31/25 °C	37/30 °C	42/34 °C
< C16:0	CL 153	380	13.77 ± 0.40 aA	11.33 ± 0.64 abA	7.27 ± 2.76 bA	1.77 ± 0.16 cB
		700	14.27 ± 0.26 aA	13.65 ± 1.30 abA	8.38 ± 0.11 cA	9.26 ± 0.79 bcA
	Icatu	380	15.64 ± 0.66 aA	9.02 ± 0.09 cB	11.93 ± 1.21 bA	6.45 ± 0.44 dA
		700	5.96 ± 0.05 bB	16.94 ± 0.28 aA	7.09 ± 0.60 bB	5.09 ± 0.00 bA
	IPR 108	380	13.04 ± 0.49 aA	7.58 ± 0.71 bA	8.33 ± 0.46 bA	4.32 ± 0.11 cB
		700	5.84 ± 0.30 aB	3.75 ± 0.98 abB	1.51 ± 1.31 bB	6.47 ± 0.36 aA
C16:0	CL 153	380	29.16 ± 0.24 aA	26.13 ± 0.33 aB	26.69 ± 3.18 aB	25.92 ± 0.77 aA
		700	28.10 ± 1.04 bA	43.38 ± 2.57 aA	39.91 ± 4.06 aA	25.68 ± 2.39 bA
	Icatu	380	33.27 ± 1.28 cB	36.74 ± 0.71 bB	41.09 ± 1.53 aA	39.53 ± 0.54 abA
		700	36.05 ± 0.15 bA	47.21 ± 0.52 aA	38.38 ± 0.19 bB	38.53 ± 0.00 bA
	IPR 108	380	36.36 ± 1.18 bA	39.30 ± 3.05 abA	44.79 ± 1.40 aA	33.53 ± 1.17 bB
		700	36.78 ± 0.91 bA	43.42 ± 1.28 aA	25.80 ± 0.35 cB	39.33 ± 0.79 abA
C18:0	CL 153	380	23.53 ± 1.19 bcA	35.71 ± 0.88 abA	42.84 ± 8.17 aA	11.57 ± 0.26 cA
		700	21.41 ± 2.18 aA	20.12 ± 1.27 aB	17.65 ± 1.58 aB	17.27 ± 2.18 aA
	Icatu	380	21.23 ± 1.86 bB	26.96 ± 0.26 aA	15.45 ± 0.70 cB	15.01 ± 0.55 cA
		700	27.52 ± 1.06 aA	18.81 ± 0.26 bB	28.78 ± 0.13 aA	16.69 ± 0.00 bA
	IPR 108	380	22.23 ± 0.08 bB	28.47 ± 0.96 aB	26.70 ± 0.60 abA	13.40 ± 1.33 cB
		700	35.91 ± 0.54 aA	32.61 ± 0.24 aA	14.17 ± 0.29 cB	22.92 ± 3.71 bA
C18:1	CL 153	380	15.62 ± 0.12 aA	12.51 ± 0.30 aA	13.36 ± 1.92 aA	6.62 ± 0.58 bA
		700	11.35 ± 0.42 abB	7.55 ± 0.44 cB	13.51 ± 1.00 aA	8.51 ± 0.33 bcA
	Icatu	380	12.16 ± 0.75 aA	11.59 ± 0.33 aA	12.06 ± 1.19 aA	9.62 ± 0.40 aA
		700	12.81 ± 1.27 aA	7.24 ± 0.12 bB	14.13 ± 0.52 aA	7.70 ± 0.00 bA
	IPR 108	380	11.60 ± 0.52 abA	8.50 ± 3.04 bB	14.02 ± 0.87 aA	7.99 ± 0.3 bA
		700	12.61 ± 0.02 aA	13.64 ± 0.33 aA	6.22 ± 1.65 bB	8.16 ± 1.13 abA
C18:2	CL 153	380	7.85 ± 0.15 abA	3.23 ± 0.77 bA	3.60 ± 0.12 bA	16.81 ± 0.23 aA
		700	11.95 ± 1.59 aA	7.45 ± 2.55 aA	10.07 ± 6.07 aA	17.58 ± 1.47 aA
	Icatu	380	10.14 ± 1.08 aA	7.01 ± 0.37 bA	10.90 ± 0.56 aA	2.93 ± 0.66 cB
		700	7.86 ± 0.52 bB	4.27 ± 0.45 cB	5.71 ± 0.22 bcB	14.89 ± 0.00 aA
	IPR 108	380	8.78 ± 0.10 bA	8.61 ± 1.12 bA	4.00 ± 0.10 cB	21.36 ± 0.20 aA
		700	3.69 ± 0.15 cB	2.63 ± 0.18 cB	26.47 ± 0.27 aA	14.12 ± 2.88 bB
C18:3	CL 153	380	10.06 ± 0.52 bA	11.08 ± 1.64 bA	10.23 ± 4.03 bA	37.31 ± 0.01 aA
		700	12.92 ± 1.80 abA	7.85 ± 0.49 bA	10.48 ± 4.70 bA	21.70 ± 1.98 aB
	Icatu	380	7.55 ± 0.65 bA	8.67 ± 0.32 bA	8.56 ± 0.39 bA	26.47 ± 0.39 aA
		700	9.79 ± 1.91 bA	5.52 ± 0.58 cB	5.91 ± 0.62 cB	17.10 ± 0.00 aB
	IPR 108	380	7.99 ± 0.01 bA	7.53 ± 0.55 bA	2.15 ± 0.63 cB	19.38 ± 0.50 aA
		700	5.17 ± 0.53 cB	3.94 ± 0.57 cB	25.84 ± 0.01 aA	9.00 ± 0.85 bB
DBI	CL 153	380	1.03 ± 0.02 bA	0.83 ± 0.05 bA	1.05 ± 0.17 bA	3.97 ± 0.05 aA
		700	1.38 ± 0.22 bA	0.68 ± 0.10 bA	0.92 ± 0.04 bA	2.23 ± 0.31 aB
	Icatu	380	0.91 ± 0.12 bA	0.78 ± 0.03 bA	0.91 ± 0.04 bA	1.59 ± 0.06 aA
		700	0.88 ± 0.10 bA	0.43 ± 0.01 cB	0.63 ± 0.03 bcB	1.57 ± 0.00 aA
	IPR 108	380	0.92 ± 0.05 bA	0.76 ± 0.15 bcA	0.41 ± 0.01 cB	2.19 ± 0.01 aA
		700	0.53 ± 0.03 cB	0.41 ± 0.02 cB	3.30 ± 0.03 aA	0.99 ± 0.21 bB

needed to sustain the higher photosynthetic activity in these 700-plants (see Rodrigues et al., 2016). Membrane remodeling involving slow decreases in glycerolipid unsaturation and FA synthesis is essential for plant acclimation to heat stress (Higashi et al., 2015; Niu and Xiang, 2018). In fact, the fall of plastidial trienoic fatty acids, and the rise in saturated and monounsaturated FAs is crucial to maintain membrane fluidity and to improve heat tolerance (Penfield, 2008; Niu and Xiang, 2018). Furthermore, photosynthetic performance closely depends on increased FAs unsaturation to preserve lipid acyl motion in thylakoid membranes (Harwood, 1998; Siegenthaler and Trémolières, 1998) and to the integration of newly synthesized D1 (Kern and Zouni, 2009) under stressful conditions. Notably, PUFAs enrichment will increase membrane lipoperoxidation risk, which might be compensated by a somewhat reinforced antioxidative system (Martins et al., 2016), and, mostly, by the higher photosynthetic functioning (Rodrigues et al., 2016), which is, ultimately, the best mechanism to prevent ROS over production.

A tolerance threshold was crossed at 42/34 °C, especially in the 380-plants, as shown by the strong increase of the minimal fluorescence from the antennae (F_o), reflecting impairments at the light harvesting complexes (LHC) and PSII electron transport (Rodrigues et al., 2016) that was related to an over fluidity of chloroplast membranes (Tovuu et al., 2013). This could be related to the strong TFA reduction from 37/30 to 42/34 °Cin 380-plants of Icatu and both [CO_2] of IPR108. Still, FA changes resulted in minimum unsaturation in all treatments (Fig. 2B), in agreement with the strong repression of the chloroplastic ω-3 fatty acid desaturase (FAD3) gene expression in *C. arabica* genotypes (maintained in CL153) at 42/34 °C (Table 10), thus reducing membrane fluidity and compensating an over fluidity promoted by extreme high temperature.

The regulation of FA saturation by temperature seems to be controlled by FA desaturases (FAD) family at transcriptional and post-transcriptional levels (Penfield, 2008), with the inhibition of FAD activities by heat being related to reduced FA double bonds, altered membrane fluidity, and increased heat tolerance (Murakami et al., 2000; Higashi et al., 2015; Niu and Xiang, 2018). Notably, under elevated [CO_2] all genotypes showed a greater responsiveness in the unsaturation degree from 37/30 °C to 42/34 °C. In IPR108, DBI decreases occurred together with a strong TFA reduction, likely reflecting a controlled degradation process, since leakage values remained low, and relevant PSII activity was maintained (Rodrigues et al., 2016). Additionally, lipid remodeling was particularly evident in the 700-plants

Table 9
Fatty acids composition (mol %) and unsaturation degree (DBI) of sulfoquinovosyldialcylglycerol (SQDG) from chloroplast membranes of leaf samples of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25 °C, 37/30 °C and 42/34 °C. For each parameter, the mean values ± SE (n = 3) followed by different letters express significant differences between temperatures for the [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B), separately for each genotype.

	Genotype	[CO_2] (μL L^{-1})	Temperature (day/night, °C)			
			25/20 °C	31/25 °C	37/30 °C	42/34 °C
< C16:0	CL 153	380	3.18 ± 0.02 aB	1.78 ± 0.02 bB	1.75 ± 0.02 bB	1.53 ± 0.04 bB
		700	7.80 ± 0.42 aA	4.46 ± 0.22 bA	3.31 ± 0.28 cA	3.23 ± 0.14 cA
	Icatu	380	10.44 ± 0.07 aA	0.88 ± 0.09 bB	1.04 ± 0.05 bB	2.48 ± 0.09 bA
		700	6.06 ± 0.78 aB	3.68 ± 0.92 abA	2.38 ± 0.10 bA	2.57 ± 1.05 bA
	IPR 108	380	10.73 ± 0.00 aA	4.00 ± 0.85 bA	2.98 ± 0.78 bA	0.44 ± 0.03 cB
		700	2.68 ± 0.10 bB	2.03 ± 0.34 bB	1.27 ± 0.10 bA	6.33 ± 0.45 aA
C16:0	CL 153	380	46.95 ± 0.00 cB	61.55 ± 1.24 bA	66.92 ± 1.26 aA	70.33 ± 0.31 aA
		700	55.92 ± 1.04 bA	62.81 ± 1.09 aA	66.19 ± 1.35 aA	65.01 ± 0.57 aB
	Icatu	380	64.45 ± 0.74 cB	63.80 ± 1.12 cB	67.58 ± 1.07 bB	72.49 ± 0.33 aA
		700	74.38 ± 1.12 bA	71.22 ± 0.06 bA	78.85 ± 0.64 aA	64.35 ± 0.01 cB
	IPR 108	380	64.53 ± 0.00 bA	68.18 ± 0.34 aB	67.01 ± 0.55 abB	64.92 ± 0.98 bB
		700	62.59 ± 0.10 bA	71.72 ± 0.77 aA	69.90 ± 1.17 aA	71.70 ± 0.76 aA
C18:0	CL 153	380	13.67 ± 0.42 aB	9.05 ± 0.16 bB	11.27 ± 0.90 bA	7.62 ± 0.50 cB
		700	15.60 ± 0.19 aA	14.41 ± 0.85 aA	10.39 ± 0.23 bA	12.28 ± 0.14 bA
	Icatu	380	10.21 ± 0.49 aA	6.71 ± 0.03 aA	7.24 ± 0.74 aA	7.23 ± 0.21 bB
		700	8.76 ± 0.30 bA	7.48 ± 0.38 bA	7.53 ± 0.11 bA	11.43 ± 0.87 aA
	IPR 108	380	11.36 ± 0.00 aA	10.56 ± 0.44 aA	6.30 ± 0.62 bA	7.59 ± 0.27 bA
		700	7.00 ± 0.49 aB	5.40 ± 0.33 aB	6.97 ± 0.23 aA	7.08 ± 0.29 aA
C18:1	CL 153	380	3.10 ± 0.06 aA	3.25 ± 0.07 aA	2.75 ± 0.19 aA	1.11 ± 0.00 bB
		700	3.96 ± 0.14 aA	3.70 ± 0.75 aA	3.29 ± 0.19 abA	2.06 ± 0.07 bA
	Icatu	380	3.70 ± 0.12 aA	1.85 ± 0.06 cA	2.88 ± 0.21 bA	2.47 ± 0.07 bA
		700	2.62 ± 0.31 aB	1.90 ± 0.06 bA	2.24 ± 0.09 abB	2.42 ± 0.05 abA
	IPR 108	380	2.94 ± 0.00 aB	2.91 ± 0.28 aA	3.47 ± 0.01 aA	2.95 ± 0.14 aA
		700	3.52 ± 0.17 aA	2.76 ± 0.17 bA	2.12 ± 0.09 cB	2.99 ± 0.12 abA
C18:2	CL 153	380	3.07 ± 0.05 bA	3.08 ± 0.13 bA	2.95 ± 0.07 bA	4.60 ± 0.13 aA
		700	0.62 ± 0.03 cB	0.66 ± 0.11 cB	1.51 ± 0.05 bB	2.43 ± 0.01 aB
	Icatu	380	2.09 ± 0.03 cA	3.18 ± 0.11 bA	4.91 ± 0.12 aA	4.47 ± 0.05 aA
		700	0.39 ± 0.01 cB	0.78 ± 0.13 cB	1.76 ± 0.07 bB	4.54 ± 0.37 aA
	IPR 108	380	1.63 ± 0.00 cB	1.00 ± 0.03 cA	5.00 ± 0.36 bA	6.48 ± 0.09 aA
		700	3.46 ± 0.25 aA	0.84 ± 0.04 cA	1.84 ± 0.04 bB	3.13 ± 0.29 aB
C18:3	CL 153	380	30.01 ± 0.43 aA	21.30 ± 0.86 bA	14.35 ± 0.45 cA	14.81 ± 0.64 cA
		700	16.10 ± 1.10 aB	13.97 ± 0.63 aB	15.31 ± 0.60 aA	14.99 ± 0.38 aA
	Icatu	380	9.11 ± 0.15 cA	23.58 ± 0.89 aA	16.34 ± 0.15 bA	10.86 ± 0.04 cB
		700	7.81 ± 0.32 bA	14.93 ± 1.30 aB	7.24 ± 0.35 bB	14.69 ± 1.61 aA
	IPR 108	380	8.80 ± 0.00 cB	13.35 ± 1.01 bB	14.57 ± 1.22 bB	17.61 ± 0.45 aA
		700	20.75 ± 0.41 aA	17.25 ± 0.89 bA	18.56 ± 0.52 abA	8.77 ± 0.52 cB
DBI	CL 153	380	1.56 ± 0.03 aA	1.03 ± 0.06 bA	0.65 ± 0.04 cA	0.70 ± 0.03 cA
		700	0.71 ± 0.04 aB	0.61 ± 0.02 aB	0.71 ± 0.02 aA	0.67 ± 0.02 aA
	Icatu	380	0.45 ± 0.02 cA	1.12 ± 0.06 aA	0.82 ± 0.02 bA	0.55 ± 0.00 cB
		700	0.33 ± 0.03 bA	0.61 ± 0.05 aB	0.33 ± 0.02 bB	0.72 ± 0.09 aA
	IPR 108	380	0.44 ± 0.00 cB	0.57 ± 0.04 bcA	0.76 ± 0.03 abA	0.95 ± 0.03 aA
		700	1.03 ± 0.05 aA	0.72 ± 0.04 bA	0.80 ± 0.08 bA	0.45 ± 0.02 cB

of CL153 and Icatu, where the largest DBI reductions occurred without TFA decreases. Such strong unsaturation decline might be related to the up-regulation of *LOX5A* (CL153 and Icatu) and *LOX5B* (Icatu) at 42/34 °C (Table 10). In fact, PUFAs are released from chloroplast glycerolipids under heat conditions by lipase-catalyzed reactions as part of the lipid remodeling process (Higashi et al., 2018), being thereafter preferential substrates of 9- and 13-LOXs (Babenko et al., 2017). In summary, TFA unsaturation was highly responsiveness to both heat and elevated [CO_2] in all genotypes, but such remodeling dynamics was more evident under the heat and [CO_2] interaction.

4.2. Lipid classes adjustments and photosynthetic functioning preservation

Another "layer" of membrane adjustments to environmental changes regards the groups of complex lipids, GL, PL, and SL which have specific roles in the photosynthetic apparatus.

4.2.1. Galactolipid modifications

The exposure to enhanced [CO_2], and/or heat (37/30 and 42/34 °C) increased the GL/PL ratio in CL153 and Icatu, related to PL reductions (high [CO_2]), and/or GL increases (Table 2), since GL can partly replace PL in plastidial membranes (Dörmann, 2013). Notably, the GL/PL rise in 700-plants of CL153 and Icatu occurred occurred in parallel with TFA fall at 25/20 °C, and with the maintenance (CL153) or increased (Icatu) TFA synthesis at 37/30 and 42/34 °C, thus, clearly pointing to membrane remodeling, likely supporting these plants metabolic plasticity (Rodrigues et al., 2016) under the imposed conditions.

Both GL classes MGDG and DGDG were highly responsive to elevated [CO_2] at control temperature. These classes are integral constituents of different photosynthetic pigment-protein complexes in chloroplast membranes (Dörmann and Hölzl, 2009; Kern and Zouni, 2009), being involved, namely, in the stabilization of photosynthetic complexes, thylakoid grana formation, and enclosing an important fraction of protective system against ROS action, based on lipid soluble molecules (Spicher et al., 2016). In Icatu GL rise was related to the synthesis of both non-bilayer (MGDG) and bilayer (DGDG) lipids, whereas in CL153 only DGDG increased and total GL values were kept stable. In both genotypes, a reduction of the MGDG/DGDG ratio was due to a substantial enrichment of DGDG, likely contributing to support the greater photosynthetic activity in the 700-plants under 25/20 °C (Ramalho et al., 2013), because DGDG is involved in membrane stabilization, regulation of ionic permeability and preservation of

Table 10
Quantitative RT-PCR expression studies of genes encoding linoleate lipoxygenases (*LOX3, LOX5A, LOX5B*) and an ω- 3 fatty acid desaturase (*FAD3*) in coffee leaves from *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu and IPR108 plants, grown under 380 or 700 μL CO_2 L^{-1}, at control (25/20 °C, day/night) and supra-optimal temperatures of 31/25 °C, 37/30 °C and 42/34 °C. Separately for each genotype and gene, asterisks denote significant differences in gene expression when compared to the respective genotype control: 380 μL CO_2 L^{-1} at 25/20 °C (***P < 0.001; **P < 0.01; *P < 0.05).

Genotype	Temperature (day/night)	[CO_2] (μL L^{-1})	Gene expression			
			LOX3	*LOX5A*	*LOX5B*	*FAD3*
Clone 153	**25/20 °C**	**380**	1.000	1.000	1.000	1.000
		700	0.049	0.151	0.031*	0.985
	31/25 °C	**380**	0.017**	0.131	0.058	2.500
		700	0.024*	0.120	0.057	0.957
	37/30 °C	**380**	0.022*	0.144	0.024*	2.029
		700	0.023*	0.138	0.068	0.721
	42/34 °C	**380**	0.074	0.669	0.082	1.506
		700	0.115	1.690	0.269	0.872
Icatu	**25/20 °C**	**380**	1.000	1.000	1.000	1.000
		700	0.209**	0.791	1.861	1.456
	31/25 °C	**380**	0.507	1.536	3.515*	1.701
		700	0.135**	1.265	1.660	1.221
	37/30 °C	**380**	0.378**	0.417	5.730**	0.484
		700	0.495	0.745	0.256	0.907
	42/34 °C	**380**	0.767	0.178*	0.795	0.108*
		700	0.413*	4.730**	1.631	0.071*
IPR108	**25/20 °C**	**380**	1.000	1.000	1.000	1.000
		700	0.094***	0.403	2.750*	0.104***
	31/25 °C	**380**	0.450*	0.859	0.793	1.150
		700	0.231**	0.333	4.480**	0.112***
	37/30 °C	**380**	0.479*	0.289*	1.780*	0.481*
		700	0.221**	0.334	3.211*	0.266**
	42/34 °C	**380**	0.574*	0.062**	1.111	0.116***
		700	0.442*	0.172	0.276	0.228**

membrane protein activities (Siegenthaler and Trémolières, 1998).

Heat alone also increased GL classes, but at 37/30 °C the 700-plants of all genotypes could have strengthened chloroplast membranes resilience in association with enriched DGDG pool. It has been demonstrated that MGDG/DGDG reduction, linked to preferential DGDG synthesis, was linked to stable membrane bilayer structure under drought, retarded leaf senescence, and improved thylakoid thermostability (Scotti-Campos et al., 2013; Chen et al., 2018; Niu and Xiang, 2018). DGDG is crucial for the function and structural integrity and stability of PSI and II (Dörmann and Hölzl, 2009; Kern and Zouni, 2009), is required for photoprotection (Kobayashi, 2016), and is less prone to degradation by lipolytic enzymes than MGDG (Sahsah et al., 1998; Scotti-Campos et al., 2013), thus in line with better preservation of PSI and PSII activities in the 700-plants (Rodrigues et al., 2016). Moreover, at 42/34 °C Icatu 700-plants stoodout, since they were the only ones to maintain higher MGDG and DGDG contents than their corresponding 380-plants, which in turn were among the most impaired plants at this temperature (Rodrigues et al., 2016). Additionally, MGDG increases in all genotypes at 37/30 °C and both [CO_2], might have contributed to preserve thylakoid electron transport until this temperature (Rodrigues et al., 2016), since MGDG is involved in LHCII oligomerization, PSII and cytochrome (cyt) b_6f thylakoid structure and activities, linear electron transport (Kern and Zouni, 2009; Wu et al., 2013; Kobayashi, 2016), and in plant stress response and photoprotection (Zheng et al., 2011; Kobayashi, 2016; Goss et al., 2017).

Another critical factor deeply related to acclimation to varying temperatures is FA composition and saturation in lipid classes, which determines membrane fluidity and permeability (Spicher et al., 2016). Under [CO_2] and temperature control conditions, GL classes were within the range reported in *Coffea* sp. (Partelli et al., 2011), both showing the predominance of C18:3, particularly in MGDG (79–88%), turning this lipid classe the most unsaturated one.

Elevated [CO_2] alone induced significant unsaturation increases in MGDG, especially in Icatu and CL153. However, Icatu based this change on a strong *de novo* MGDG synthesis with altered composition in all FAs, whereas CL153 showed lower MGDG amount and small FA changes, suggesting the remodeling of pre-existing FAs (Tables 2,3). In the latter genotype, DBI was reduced in DGDG linked to strong changes in C16:0 and C18:2 *vs.* C18:3 in 700-plants, concomitantly to higher photosynthetic performance (Ramalho et al., 2013).

Heat reduces lipids unsaturation in a remodelling process that is well conserved among many plant species (Higashi et al., 2015, 2018; Spicher et al., 2016), releasing PUFAs (*e.g.*, C18:3) that are preferential substrates of LOX (Babenko et al., 2017). In fact, irrespective of [CO_2] all genotypes showed reduced unsaturation in MGDG and DGDG at 37/30 °C, as compared to 25/20 °C, usually related to lower C18:3 and C16:0 increases. These values where maintained in Icatu at 42/34 °C, but were further reduced in CL153 and IPR108 700-plants. This reduces membrane fluidity and lipoperoxidation susceptibility, likely contributing to keep thylakoid membranes performance by avoiding the uncoupling of electron transport under heat stress (Spicher et al., 2016), while improving thylakoid thermostability (Niu and Xiang, 2018).

Notably, elevated [CO_2] modified the heat response at 37/30 °C, by promoting higher DBI values in MGDG (all genotypes), and at 42/34 °C only in Icatu. Thus, although plants synthesized molecular species with higher ROS sensitivity, an adequate (higher) unsaturation degree (and fluidity) might be needed to support the greater photosynthetic activity in 700-plants (Rodrigues et al., 2016), and the repair processes related to D1 protein (from PSII) insertion, which is efficiently supported by unsaturated FAs (Kern and Zouni, 2009). Still, such higher ROS sensitivity and higher fluidity might have been (partly) compensated, respectively, by a reinforcement of antioxidative molecules (Martins et al., 2016), and a slight increase in the longer FA chains under elevated [CO_2] (25/20 °C and 37/30 °C). The latter suggests an increased melting point temperature, promoting better membrane stability under heat (Bajerski et al., 2017). Interestingly, in CL153 and Icatu at 37/30 °C, similar DBI values in DGDG hidden lower C18:3 (and higher C18:1 and C18:2) values in the 700-plants, suggesting that different lipid remodeling routes may results in similar saturation levels. Such lower C18:3 content in DGDG might be associated with a reduced heat impact (Matos et al., 2010).

4.2.2. Phospholipid modifications

Phospholipid classes (PL) are also crucial components of chloroplast membranes, with roles in photosynthetic performance and stress acclimation, and include PC, PG, PI, and PA (Wang et al., 2006; Wada and Mizusawa, 2009; Scotti-Campos et al., 2014a). Heat did not negatively affect PL content in all genotypes until 37/30 °C, but high [CO_2] reduced PL in CL153 and Icatu from 25/20 °C to 37/30 °C, with rise of GL/PL ratio in all genotypes. These modifications did not impair the metabolic functioning which was mostly preserved until 37/30 °C. Moreover, in 700-plants PL values were maintained (CL153 and Icatu), or increased (IPR108) from 37/30 to 42/34 °C, in contrast to 380-plants that also displayed an impaired photosynthetic performance at the highest temperature(Martins et al., 2016; Rodrigues et al., 2016).

PL reductions were closely related to PC changes, which was the most abundant PL at 25/20 °C and normal [CO_2], but one of the less representative under heat and/or elevated [CO_2] exposure. In CL153 and Icatu decreased PC pools were accompanied by a reduced unsaturation degree, and, thus reduced fluidity and ROS lipoperoxidation susceptibility, although the higher presence of C16:0 (with shorter chain length) at the two highest temperatures, might have increased membrane fluidity (Bajerski et al., 2017). PC is a building block of the outer membrane (Joyard et al., 1998), and a bilayer stabilizing lipid (Wang et al., 2006), likely integrating the Cyt b_6f complex (Wada and

Mizusawa, 2009), and increases in PC amount and unsaturation were associated with membrane restoration in *Coffea* spp. under cold stress (Partelli et al., 2011; Scotti-Campos et al., 2014a). Nonetheless, the 700-plants of CL153 and Icatu at 25/20 °C, and of all genotypes at 37/30 °C, had large PC decreases and lower PC values than the 380-plants (Table 2), while at 42/34 °C minimal PC contents were found for both [CO_2]. Taking all this information into account, and considering that 700-plants showed a better photosynthetic performance at all temperatures, and that elevated [CO_2] mitigated the heat impact on photosynthesis (Rodrigues et al., 2016), it is suggested that PC did not play a major role in photosynthetic functioning under these environmental conditions.

The bilayer forming lipid PG showed a totally different variation pattern than PC, rather diverse among species. PG stabilizes membranes (Murata and Siegenthaler, 1998) and its depletion compromises chloroplast development, dissociates PSII dimers and LHCII trimers, and impairs electron transport in PSII (but not in PSI) (Murata and Siegenthaler, 1998; Wada and Mizusawa, 2009), being required for cyclic electron transport around PSI, and for PSII reactivation of after photoinhibition (Kobayashi, 2016). Elevated [CO_2] alone slightly increased PG amounts in *C. arabica* genotypes, and decreased them in CL153. Under heat and at both [CO_2], PG was maintained in CL153, but gradually increased in *C. arabica* genotypes, with a higher content obtained at 42/34 °C in the 700-plants. At this temperature PG becomes the highest PL class, irrespective of [CO_2] or genotype (Table 2), suggesting that lipases related to PG turnover might have been repressed (Wang et al., 2017). Therefore, our data regarding PG increase in *C. arabica* genotypes is in line with the better preservation of PSI and PSII activities (Rodrigues et al., 2016), and lower PSII phoinhibition (Martins et al., 2016) in 700-plants, at 42/34 °C, pointing to a role in heat acclimation, as reported for cold in Icatu plants (Partelli et al., 2011).

C16:1*t* deserves a special attention given that it is an exclusive component of thylakoid PG with specific roles in the photosynthetic apparatus, having a lower low-melting point that contributes to a decrease the phase transition temperature of thylakoid lipids (Harwood, 1998), being associated with chloroplast membrane stability in *Coffea* spp. under chilling Partelli et al., 2011; Scotti-Campos et al., 2014a). Only Icatu maintained C16:1*t* values under enhanced [CO_2] (25/20 °C), and showed the smallest reduction at 37/30 °C (in both [CO_2]). In all cases, minimal C16:1*t* weight was found at 42/34 °C, denoting a clear heat impact that might have been compensated by the PG increase in *C. arabica* 700-plants.

PI, a structural component of the inner and outer chloroplast membranes and thylakoids, being required for membrane biogenesis, remodeling (Leshem, 1992; Joyard et al., 1998), and regulatory functions (Ruelland et al., 2015). PI content showed only small variations until 37/30 °C, although with some increase in IPR108 irrespective of [CO_2], but slight reductions were usually observed at 42/34 °C in all genotypes. With few exceptions, PI unsaturation remained largely unchanged, but an enrichment of FA with smaller C-chain length (C16:0) under elevated [CO_2] (25/20 °C) was found in *C. arabica* plants, and at 42/34 °C (both [CO_2]) in all genotypes. Therefore, PI role coffee heat acclimation, if existing, remains unveiled.

The less represented PL class, PA, is a biological active molecule involved in lipid signaling, and in key processes such as stomatal closure, induction of leaf senescence and cell death (Park et al., 2004; Ruelland et al., 2015). Though its role in stress response remains to be elucidated, PA is considered a stress metabolite linked to membrane damage (Wang et al., 2006; Zheng et al., 2011), and ROS accumulation (Park et al., 2004). Therefore, the higher PA values often observed under normal [CO_2] at 25/20 °C, and also at the two highest temperatures, might reflect the lower photosynthetic activity, and less robust protective mechanisms (and higher ROS production) in 380-plants (Martins et al., 2016). The presented results are in line with the observations in the cold sensitive *Coffea dewevrei*, which displayed greater PA levels associated with lipid degradation after low temperature exposure (Partelli et al., 2011), consistent with a higher ROS content, and a less efficient antioxidative system (Fortunato et al., 2010).

4.2.3. Sulpholipid class modifications

The SQDG class is the only sulphur-containing anionic glycerolipid, maintaining thylakoid fluidity, stabilizating the photosynthetic processes, namely of ATP synthesis, PSII functioning (Taran et al., 2000; Dörmann and Hölzl, 2009; Kobayashi, 2016), and supporting the protein-protein interactions (and, thus functioning) of the cytochromes b_6 and *f* (Kern and Zouni, 2009). SQDG was present in very small amounts in photosynthetic membranes of *Coffea* plants. As for PC, elevated [CO_2] lowered SQDG content in CL153 and Icatu, and increased the saturation and weight of FA with smaller C-chain length, which likely reduced and increased membrane fluidity, respectively (Bajerski et al., 2017). However, heat promoted SQDG accumulation in *C. arabica* (mainly in 380-plants) until 37/30 °C, which might have contributed to the PSI and PSII electron transport tolerance until this temperature (Rodrigues et al., 2016), in line with heat tolerance promoted in green algae (Sato et al., 2003), *Arabidopsis* (Higashi, et al 2015) and wheat (Taran et al., 2000). Although DBI decreased in CL153 and increased in *C. arabica* genotypes due to heat, all genotypes displayed enriched C16:0 pools at 37/30 °C, as compared to 25/20 °C, irrespective of [CO_2]. However, under elevated [CO_2] and at the highest temperature, CL153 and Icatu showed lower C16:0 and higher C18:0 contents than the 380-plants, potentially reducing membrane fluidity. Therefore SQDG might play a role in membrane lipid adjustments to heat, mostly until 37/30 °C.

5. Conclusions

A strong membrane resilience to heat and [CO_2] was observed in all genotypes until 37/30 °C, as pointed by moderate permeability changes in all conditions. High responsiveness to [CO_2] (except IPR108) and heat (except CL153) was reflected in TFA content and DBI, and regulated at the *FAD* and *LOX* transcriptional level. The lowest unsaturation degree of TFA bulk at 42/34 °C, and the stronger DBI reduction from 37/30 °C to 42/34 °C, suggests important remodeling especially under the heat and [CO_2] interaction, with CL153 and Icatu maintaining TFA contents at the highest temperature. Important dynamics of the lipid matrix was further found regarding quantitative and qualitative modifications of complex lipids in all environmental conditions. Modificationss were clearer in MGDG, DGDG, PC, PG, and SQDG, somewhat differently for CL153 and Icatu 700-plants, which cope better with heat. In short: 1) elevated [CO_2] promoted the enrichment in GLs, linked to *de novo* synthesis of MGDG (Icatu) and, markedly, DGDG (Icatu and CL153), and reduced MGDG/DGDG ratio. 2) In all genotypes, heat (two highest temperatures) increased GLs classes and their saturation degree (the latter except in IPR108 at 42/34 °C), reducing fluidity and lipoperoxidation sensitivity. 3) The heat (37/30 °C) and elevated [CO_2] interaction further raised MGDG and DGDG in *C. arabica* genotypes, but only Icatu maintained high GLs contents, and higher DBI at the highest temperature. This implies that a higher unsaturation degree could be needed to support the higher photosynthetic activity in the 700-plants (as at 25/20 °C). 4) PLs were negatively affected only at 42/34 °C in 380-plants, but were reduced by elevated [CO_2] at all temperatures. PC was the most reduced lipid class, particularly under elevated [CO_2] where the best photosynthetic functioning was previously observed, whereas PI was mostly stable, without consistent differences between [CO_2] or temperatures. Therefore, PC and PI seemed to have a limited role in the preservation of the photosynthetic performance under heat, or in the mitigation of high temperature impact by elevated [CO_2]. 5) In sharp contrast, PG in *C. arabica* plants was slightly increased by high [CO_2], but stoods out with strong rises in response to heat for both [CO_2] at 37/30 °C, and in 700-plants at the extreme 42/34 °C, when became the more represented PL class,

pointing to an active role in heat acclimation response. C16:1*t* was better preserved in *C. arabica* genotypes. 6) Lower PA was usually associated with better photosynthetic functioning under elevated [CO_2]. 7) SQDG was heat responsive in *C. arabica* plants, increasing until 37/30 °C, but was strongly reduced at 42/34 °C. 8) Regarding membrane fluidity, changes in the relative abundance of saturated FAs were observed in response to the imposed conditions. Modifications of short *vs*. long C-chain FAs (common in microorganisms) were also observed, with enrichment of FA with smaller C-chain length (C16:0) in PC (in all genotypes), PI (*C. arabica*) and SQDG (CL153 and Icatu) under elevated [CO_2] at 25/20 °C, as well as in GL classes (*C. arabica*), PC and SQDG (in all genotypes) at 37/30 °C irrespective of [CO_2], usually counteracting DBI impact on fluidity.

Globally, Icatu often showed lipid changes closer to those of heat tolerant *C. canephora* (CL153) than to the less heat tolerant *C. arabica* (IPR108), likely related to the fact that this *C. arabica* genotype is an introgressed variety from *C. canephora*. Overall, coffee plants showed a complex network of adjustments in their chloroplast lipid profile in response to altered air [CO_2] and temperature, from the TFA bulk until fine adjustments of FAs (and saturation) within each class, which were likely related to their different photosynthetic performance and tolerance ability. Therefore, this work configures a step forward to unveil the role of lipid membrane retailoring to photosynthetic long-term acclimation to ongoing climate changes.

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CRediT authorship contribution statement

Paula Scotti-Campos: Validation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Isabel P. Pais:** Validation, Formal analysis, Investigation, Writing - original draft, Visualization. **Ana I. Ribeiro-Barros:** Conceptualization, Validation, Formal analysis, Investigation, Writing - review & editing, Visualization, Supervision. **Lima D. Martins:** Investigation, Formal analysis. **Marcelo A. Tomaz:** Investigation, Supervision. **Weverton P. Rodrigues:** Investigation, Formal analysis. **Eliemar Campostrini:** Investigation, Supervision. **José N. Semedo:** Formal analysis. **Ana S. Fortunato:** Validation, Investigation, Formal analysis. **Madlles Q. Martins:** Investigation, Formal analysis. **Fábio L. Partelli:** Investigation, Supervision. **Fernando C. Lidon:** Conceptualization, Writing - review & editing, Project administration. **Fábio M. DaMatta:** Conceptualization, Writing - review & editing. **José C. Ramalho:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that there are not any potential conflicts of interest.

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