Environmental and Experimental Botany 74 (2011) 194–204

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Characterization of the main lipid components of chloroplast membranes and cold induced changes in *Coffea* spp.

F.L. Partelli[a,b,1], P. Batista-Santos[b,1], P. Scotti-Campos[c], I.P. Pais[c], V.L. Quartin[b,c], H.D. Vieira[d], J.C. Ramalho[b,*]

[a] Dept. Ciências Agrárias e Biológicas, Centro Univ. Norte Espírito Santo, Univ. Federal Espírito Santo, Rodovia BR 101 Norte, Km. 60, Bairro Litorâneo, CEP 29932-540, São Mateus, ES, Brazil
[b] Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal, Instituto Investigação Científica Tropical, I.P., Av. República, Quinta do Marquês, 2784-505 Oeiras, Portugal
[c] Instituto Nacional de Investigação Agrária, INRB, I.P., Av. República, Quinta do Marquês, 2784-505 Oeiras, Portugal
[d] Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Ciências e Tecnologias Agropecuárias, Av. Alberto Lamego 2000, Parque Califórnia, 28013-602, Campos dos Goytacazes, RJ, Brazil

ARTICLE INFO

Article history:
Received 29 April 2010
Received in revised form 1 June 2011
Accepted 3 June 2011

Keywords:
Acclimation
Chloroplast membranes
Coffee tree
Cold
Fatty acids
Lipid classes
Unsaturation

ABSTRACT

Low temperatures affect many plant physiological and biochemical components, amongst them the lipid phase of membranes. The present work aimed to characterize the lipid composition of chloroplast membranes of three *Coffea* genotypes, representing three agronomic valuable species (*Coffea arabica* cv. Icatu, *Coffea canephora* cv. Conilon clone 02 and *Coffea dewevrei*), under adequate environmental conditions and to relate its cold tolerance ability to the adjustments triggered during a gradual temperature decrease, after chilling exposure and upon a recovery period. Under adequate temperature (25/20 °C, day/night) the lipid composition of chloroplast membranes was fairly similar amongst the genotypes concerning the total fatty acid (TFA) content and individual FAs (both globally or within the classes), suggesting a close lipid composition amongst *Coffea* species, which can be considered as "C18:3" plants. Under cold exposure and subsequent recovery the genotypes undergo adjustments, some of them with acclimation potential. The genotypes displayed some ability to increase lipid synthesis, increasing their FA content. However, under cold exposure (even at 4 °C), Icatu and *C. dewevrei* plants performed qualitative adjustments, including preferential synthesis of phospholipids (especially PG) instead of galactolipids and increases in the unsaturation degree of DGDG and phospholipid classes (PG, PC and PI). Clone 02 maintained almost all lipid characteristics, what explains its higher cold sensitivity. Furthermore, differences that contribute to explain contrasting cold sensitivity in Icatu (more tolerant) and *C. dewevrei* emerged when analyzing PA content (taken as a stress metabolite) and the FA composition within MGDG and PG classes. *C. dewevrei* presented the higher increase, absolute value and relative weight of PA, while Icatu was the solely genotype to show a rise in the unsaturation degree of MGDG and PG, displaying as well the highest DBI values for these classes. We conclude that lipid qualitative and quantitative adjustments constitute a flexible mechanism that decisively contributes to cold acclimation in *Coffea* spp., working in tandem with others that minimize oxidative stress damages.

1. Introduction

To most plants, the exposure to low positive temperatures impairs growth and productivity, affecting several photosynthetic components. In fact, it is common to observe reductions of stomatal conductance, net photosynthetic rate, photochemical efficiency of the photosystem (PS) II, thylakoid electron transport, enzymatic activity, carbon fixation, as well as detrimental effects on the structure and composition of the photosynthetic pigment complexes (Allen and Ort, 2001; Krause, 1994; Morcuende et al., 1996) and in chloroplast membranes lipids (Bohn et al., 2007; Harwood, 1998; Öquist, 1982). Most of these effects were observed in coffee plants under cold, although with different intensities amongst species,

Abbreviations: DBI, double bond index; DGDG, digalactosyldiacylglycerol; DPG, diphosphatidylglycerol; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol; TFA, total fatty acids; C16:0, palmitic acid; C16:1*t*, 3-trans-hexadecenoic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid.

* Corresponding author. Tel.: +351 21 4544682; fax: +351 21 4544689.
E-mail addresses: cochichor@mail.telepac.pt, cochichor@iict.pt (J.C. Ramalho).

[1] Both authors contributed equally to this work.

0098-8472/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2011.06.001

thus reflecting diverse morphological, physiological and biochemical characteristics (Campos et al., 2003; Ramalho et al., 2003; Silva et al., 2004).

Cellular membranes are dynamic structures formed essentially by lipids and proteins, constituting permeable selective barriers that support many biophysical and biochemical reactions and the development of numerous biological responses. However, they are also a main target of environmental stresses, therefore playing a major role in the acclimation to environmental adverse conditions, including to low temperatures (Leshem, 1992; Routaboul et al., 2000). Lipid changes from the fluid-crystalline to a solid–gel stage are believed to occur under temperatures varying from 10 to 15 °C in some tropical species (Harwood, 1997), provoking flexibility loss in the membrane lipid bilayer. That will impair protein transport and favor the action of hydrolytic enzymes, resulting in membrane fissures and the consequent loss of solutes, constraining acclimation and threatening cell integrity, ultimately leading to cell death.

Temperature induced changes in membrane fluidity might be the first signal in the perception and/or damage (Siegenthaler and Trémolières, 1998). Membrane fluidity is strongly influenced by the lipid molecular species composition, unsaturation degree and environmental temperature (Harwood, 1998). Unsaturation of glycerolipid fatty acids (FAs) of photosynthetic organisms can be modified in response to temperature changes (Wada et al., 1994). Dienoic FAs are required for photosynthesis (McConn and Browse, 1998) while trienoic FAs are more specifically necessary for the biogenesis and maintenance of chloroplasts at low temperatures (Routaboul et al., 2000). A gradual reduction of temperature may promote a higher unsaturation of FA in order to maintain appropriate membrane fluidity, optimal photochemical and electron-transport reactions, ultrastructural integrity and thermal stability of the photosynthetic apparatus (Gombos and Murata, 1998; Kodama et al., 1995; Öquist, 1982; Routaboul et al., 2000; Siegenthaler and Trémolières, 1998; Vijayan et al., 1998). In fact, these changes in FAs, lipid class composition and unsaturation degree may play a decisive role in plant stress acclimation/survival to low temperatures (Campos et al., 2003; Kodama et al., 1995; Routaboul et al., 2000; Wang et al., 2006), granting cultivation viability in potentially less adequate regions.

Important crops, such as cotton, soybean, maize and many tropical and sub-tropical fruits are chilling sensitive plants. Functional definitions of chilling sensitivity vary to some extent but, in general, plants will be recognized as sensitive if they suffer irreversible damages in their structures, impaired growth or even dead after one or several days of exposure to temperatures in the range of 0–12 °C (Vijayan et al., 1998). On the other hand, chilling-resistant plants are able, *e.g.*, to preserve the integrity of their photosynthetic apparatus (Siegenthaler and Trémolières, 1998).

The genus *Coffea* includes at least 103 species, with particular relevance for *Coffea arabica* and *Coffea canephora*, that together are responsible for *ca*. 99% of world coffee production (Partelli et al., 2009). Low temperatures limit coffee geographical distribution, having strong negative effects on plant growth when month average temperatures are below 15–16 °C (Barros et al., 1997). Photosynthesis is affected below 18 °C (Ramalho et al., 2003) and chilling strongly depresses photosynthetic performance and yield (DaMatta et al., 1997; Partelli et al., 2009; Ramalho et al., 2003; Silva et al., 2004), with older leaves, root and shoot meristems being particularly affected (Alonso et al., 1997; Larcher, 1995). However, coffee plants display some ability to cold acclimate, that is related to a better protection against oxidative stress (Fortunato et al., 2010; Ramalho et al., 2003) and to the maintenance of higher cell membrane stability (Campos et al., 2003).

Previous work of our group granted the information that Icatu displayed a significant tolerance to low positive temperatures, while clone 02 and *Coffea dewevrei* showed higher cold sensitivity (Ramalho et al., 2003; Partelli et al., 2009), assuring an important sensitivity variation to relate with the characteristics and modifications of the chloroplast membranes matrix. In this context, this work presents a thorough characterization of the lipid components of chloroplast membranes in three *Coffea* spp. genotypes, covering three species (*C. arabica*, *C. canephora* and *C. dewevrei*), with agronomic or breeding value. We intend to provide new insights concerning the chloroplast lipid composition under adequate temperature conditions, as well as the qualitative and quantitative lipid changes (namely in fatty acid and lipid class composition) upon cold exposure, in order to establish the relevance of chloroplast membrane lipid dynamics to cold acclimation ability. Such evaluation will contribute to the management and selection of adequate genotypes to areas prone to low temperature incidence, constituting an important tool in coffee breeding programs and being, as far as we know, the first time such study is reported for *Coffea* genus.

2. Materials and methods

2.1. Plant material and growth conditions

The experiments were carried out as previously described (Ramalho et al., 2003) with minor modifications, using plants from the genotypes *Coffea arabica* cv. Icatu (IAC 2944), *C. canephora* cv. Conilon clone 02 (early ripening), which are important coffee producers in South America, and *C. dewevrei*, which is used in breeding programs as well as a production species in some areas of Africa. Note that Icatu is in fact an hybrid of *C. canephora* × *C. arabica,* but intensively backcrossed to *C. arabica*, which led coffee experts to consider it as a *C. arabica* cultivar.

Plants were grown in 10 L pots, under semi-controlled greenhouse conditions for *ca*. 1.5 years, after which they were transferred into walk-in growth chambers (10000 EHHF, ARALAB, Portugal) and placed under 25/20 °C (day/night) for 2 weeks. Plants were then submitted successively to (1) a gradual temperature decrease (0.5 °C per day) from 25/20 °C to 13/8 °C, over 24 days, to allow the expression of acclimation ability, (2) a 3-day chilling cycle (3 × 13/4 °C), where 4 °C were applied during the night and in the first 4 h of the morning (thus, with light), followed by a rise up to 13 °C, throughout the rest of the diurnal period, (3) a rewarming period of 15 days, with the first day after chilling at 20/15 °C and the rest at 25/20 °C, in order to allow recovery. Photoperiod was set to 12 h, RH to 65–70%, external CO_2 concentration to 380 μL L^{-1} and irradiance to *ca*. 750–850 μmol m^{-2} s^{-1} for the entire experiment. Determinations were performed in the two top pairs of recent mature leaves from each branch, collected from 8 to 10 plants *per* genotype, after 2–2:30 h of illumination.

2.2. Quantification of chloroplast membrane lipids

Chloroplast membranes were obtained from 3 to 4 g of leaf tissue as described earlier (Ramalho et al., 1998). Briefly, leaf material was collected and 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% (p/v) BSA and 2 mM Na-ascorbate, filtered (with eight layers of cheesecloth) and centrifuged (3000 × *g*, 5 min, 4 °C). The obtained pellet was used for FA and polar lipid analysis, as previously described (Pham Thi et al., 1985) with some modifications for *Coffea* spp. (Ramalho et al., 1998; Campos et al., 2003). For that, the pellet was resuspended in 9 mL of a chloroform/methanol/water (1/1/1, v/v/v) solution (Allen et al., 1966), centrifuged (4500 × *g*, 10 min, 4 °C), after which FAs were saponified and methylated with BF_3 using the method of Metcalfe et al. (1966) and the addition of heptadecanoic acid (C17:0) as an internal standard.