Biochemical Systematics and Ecology 46 (2013) 116–119

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

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

Foliar cuticular waxes of cultivated species and varieties of *Coffea*

Julia Toshie Kitagami [a], Antonio Salatino [a], Oliveiro Guerreiro-Filho [b], Maria Luiza Faria Salatino [a,*]

[a] Department of Botany, Institute of Biosciences, University of São Paulo, Rua do Matão 277, Cidade Universitária, 05508-09 São Paulo, SP, Brazil
[b] Agronomic Institute of Campinas, Rua Barão de Itapura 1481, CP 28, 13012-970 Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 18 April 2012
Accepted 8 September 2012
Available online 16 October 2012

Keywords:
Foliar cuticular wax
n-Alkanes
Fatty alcohols
Triterpenoids
Chemotaxonomy

ABSTRACT

The cuticular waxes of leaves of *Coffea arabica* cv. 'Catuaí Vermelho', *C. arabica* cv. 'Obatã', *Coffea canephora* cv. 'Apoatã', *Coffea racemosa* and two hybrids between *C. arabica* and *C. racemosa* were extracted by rapid washing of the surface with chloroform. The waxes were fractionated by thin layer chromatography over silicagel. The fractions of the constituent classes were characterized by infrared spectroscopy and the distribution of the homologs of the *n*-alkanes and *n*-primary alcohols was determined by GC/MS and GC/FID. Among the samples analyzed, leaves of *C. racemosa* have the highest content of foliar wax (22.9 μg cm^{-2}). Most samples contain either *n*-alkanes (*C. canephora* and *C. racemosa*) or *n*-primary alcohols (*C. arabica*) as predominant wax constituents. The distribution of *n*-alkanes allowed the distinction of *C. racemosa* from the other samples; the distribution of alcohols allowed the distinction of the three species. The two hybrids have waxes similar to the wax of *C. arabica*.

1. Introduction

A cuticle covering the leaf surface is a characteristic of vascular plants. The cuticle contains a biopolymer (cutin) and two types of waxes: the intracuticular and the epicuticular waxes (Stark and Tian, 2006). The former embeds the cutin matrix, while the latter is deposited on the cuticle surface (Guhling et al., 2005). Washing of the foliar surface with low polarity solvents removes both the intracuticular and epicuticular waxes. The product obtained is then referred to as cuticular wax (Jetter et al., 2006). The amount of cuticular wax varies widely among plant species. For example, leaves of varieties of soybean contain 8 μg cm^{-2} (Kim et al., 2007), while leaves of wild plants often contain thick deposits of cuticular wax; examples are leaves of *Tocoyena formosa* (82 μg cm^{-2}) and *Ziziphus joazeiro* (72 μg cm^{-2}), both species native to Brazil (Oliveira and Salatino, 2000).

The cuticular waxes are composed by long chain aliphatic substances, such as hydrocarbons (chiefly *n*-alkanes), esters of fatty acids and fatty alcohols, aldehydes, primary and secondary alcohols and free fatty acids (Bianchi, 1995). Other common wax constituents are triterpenes, while flavonoids occur more rarely in cuticular wax (Hamilton, 2004; Gao et al., 2012). Waxes play several roles in the plant biology, such as maintenance of an impermeable foliar surface (and thus contributing to avoid the growth of pathogens), restriction of the loss of water and protection against the attack of herbivore insects and UV irradiation (Baker, 1982; Hamilton, 2004; Gao et al., 2012).

* Corresponding author. Tel./fax: +55 11 3091 7547.
E-mail address: mlfsalat@usp.br (M.L. Faria Salatino).

0305-1978/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bse.2012.09.012

The composition of the cuticular wax is a characteristic of the plant species or variety. Hence it may be used for taxonomic purposes and to distinguish among taxa (Medina et al., 2006). The distribution of wax alkanes in particular has been often used with taxonomic purposes (Kolattukudy, 1976; Salatino et al., 1989; Mimura et al., 1998; Sonibare et al., 2005; Rodrigues and Salatino, 2006; Motta et al., 2009; de Souza et al., 2010; Huang et al., 2011) and to determine hybrid parentage (Chadwick et al., 2000).

Coffee is among the most consumed drinks in the world (FAO, 2006). Several species of *Coffea* are cultivated, such as *Coffea arabica* and *Coffea canephora*. The former contributes with 70% of the coffee consumed worldwide. Other species are cultivated to meet specific purposes. For example, *Coffea racemosa* is cultivated because it is more resistant to water deficiency than other species of *Coffea* (Guerreiro-Filho, 1992).

Stocker and Wanner (1977) reported that the chemistry of the foliar wax is useful for characterization of species of *Coffea*. The present work aims to compare the composition of the foliar cuticular wax of species and varieties of *Coffea*.

2. Material and methods

2.1. Plant material

Fully expanded and undamaged leaves were collected from specimens cultivated at the Agronomic Institute of Campinas, in the state of São Paulo (Brazil, Southeast). The accessions collected were: 1) *C. arabica* L. cv. 'Catuaí Vermelho'; 2) *C. arabica* L. cv. 'Obatã'; 3) *C. canephora* Pierre cv. 'Apoatã'; 4) *C. racemosa* Lour.; 5) hybrid H 13685-1, obtained from crosses between *C. arabica* and *C. racemosa*, followed by backcrosses with *C. arabica*; 6) hybrid H 13685-1-27, obtained from endocrosses among individuals of H 13685-1. The leaves were dried in ventilated oven at 35 °C.

2.2. Chemical analyses

The waxes were extracted by three consecutive immersions in chloroform, the first for 30 s, the second for 20 s and the last one for 15 s. The pooled extracts were evaporated to dryness in a rotatory evaporator. The waxes were dissolved in a small volume of chloroform; the solvent was evaporated on a steam bath and the flasks maintained in dessicator until constant mass. The total leaf areas were determined using the software "Leaf Measurement System", version 2.0 (Skye Instruments Ltd., Llandrindod Wells, UK.). The wax contents are expressed by the ratio between wax mass and leaf area ($\mu g\ cm^{-2}$), taking into account both adaxial and abaxial surfaces.

The classes of constituents of the waxes were isolated by thin layer chromatography over silicagel 60, impregnated with sodium fluorescein, using the mobile phase *n*-hexane: chloroform (7:3) (Salatino and Silva, 1988). For characterization of the respective organic functions, the isolated fractions were analyzed by infrared (IR) spectroscopy, using a spectrophotometer Bomem MB 100 FTIR. Ketones and diketones were distinguished by the lower Rf of the diketones and the absorption of the carbonyl bands at 1732 and 1725 cm^{-1}, respectively.

The primary alcohols were converted to the corresponding acetyl esters (Kanya et al., 2007) prior to analysis by GC/MS. Quantitative analyses of the homolog *n*-alkanes and acetyl esters of *n*-primary alcohols were carried out in a chromatograph HP 5890 series II coupled to a mass spectrometer HP 5989 B Engine Chem Station System, using a capillary column HP-5MS (Crosslinked 5% MePh Siloxane, 30 m × 0.25 mm) and He as carrier gas at a flux of 1 $cm^3.s^{-1}$. A column temperature program was used, starting at 150 °C in the beginning of a ramp rate of 5 °C.s^{-1} until 300 °C and holding this temperature for 10 min. The temperatures of the injector and detector were 250 °C and 300 °C, respectively. The mass spectra were obtained at 70 eV and the temperatures of the quadrupole and ion source were 100 °C and 150 °C, respectively. The alkane and alcohol homologs were identified by comparison of retention times and respective mass spectra with standards and data from the NIST library. Analysis of the *n*-alkanes and acetyl esters of *n*-primary alcohols were also performed by gas chromatography and detection by flame ionization (GC/FID), using the chromatographic conditions described above.

3. Results

The contents of wax and classes of constituents of the samples of *Coffea* analyzed are given in Table 1. *C. racemosa* stands out due to a wax content that is almost twofold the contents of the other genotypes. *C. canephora* and the two varieties of *C. arabica* have practically the same wax content. The contents of the two hybrids are very similar (Table 1) and practically the same as *C. arabica*.

The predominant classes of wax constituents of most *Coffea* samples analyzed were either *n*-alkanes or *n*-primary alcohols (Table 1). Other constituents may be abundant in waxes of some samples and appear as minor components in the waxes of other genotypes. Fatty acids and ketones, for example, are relatively abundant in the foliar wax of both hybrids, but the former are minor constituents in the foliar wax of the other samples. Diketones and esters are relevant constituents in the foliar wax of *C. arabica* cv. 'Obatã.' Esters are also present as major constituents in the foliar wax of the hybrid H 13686-1. *C. canephora* stands out for the nearly complete absence of diketones (Table 1). The content of *n*-alkanes is a characteristic that seemingly distinguishes the *Coffea* accessions analyzed: it is highest in *C. canephora*, intermediate in *C. racemosa* and lower in *C. arabica* and in both hybrids (Table 1).