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Original Article

Methylxanthines and phenolic compounds in mate (*Ilex paraguariensis* St. Hil.) progenies grown in Brazil

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Abstract

Methylxanthines and phenolic compounds contents of 16 mate (*Ilex paraguariensis* St. Hil.) progenies from four Brazilian regions (Ivaí, Quedas do Iguaçu, Cascavel and Barão de Cotegipe) and those grown in three places within in the state of Paraná (Ivaí, Rio Azul and Guarapuava) were evaluated. Results revealed significant changes in total methylxanthines, caffeine and theobromine contents in progenies, according to their origin. Total methylxanthines (0.560–0.734%) and caffeine (0.490–0.611%) contents followed the order Ivaí < Quedas do Iguaçu < Barão de Cotegipe < Cascavel. Theobromine (0.132–0.068%) contents were inversely related to caffeine contents. Chlorogenic acid in the Cascavel progenie was lower (0.786%) than that in the others (0.861–0.915%). No change occurred in total phenol and caffeic acid contents between progenies with regard to their origin. In the case of planting site, Rio Azul progenies contained low methylxanthines (0.574%) when compared to progenies from Ivaí (0.678%) and Guarapuava (0.739%). Caffeine (0.426–0.695%) contents were in the following order: Rio Azul < Ivaí < Guarapuava. Total phenol (7.910–9.591%) contents were Ivaí < Rio Azul < Guarapuava whereas high cholorogenic acid contents were detected in Rio Azul (0.953%) and Guarapuava (0.911%) progenies, and high caffeic acid occurred in Ivaí (0.018%) and Guarapuava (0.020%) progenies.

Keywords: *Ilex paraguariensis*; Mate; Methylxanthines; Caffeine; Theobromine; Total phenols; Chlorogenic acid; Caffeic acid

1. Introduction

Mate has long been part of the culture of the Latin American southern cone, where it is traditionally consumed as a tonic and as a stimulant to reduce fatigue and to suppress appetite. Nowadays, mate is exploited commercially and exported to several parts of the world, mainly to the USA and Europe. Antioxidant, diuretic, eupeptic and choleretic properties have been associated to mate (Gugliucci, 1996; Schinella et al., 2000; Gorzalczany et al., 2001). Mate is brewed from the dried leaves and stemlets of the perennial tree *Ilex paraguarensis* St. Hil. This plant belongs to the family Aquifoliaceae and grows between parallels 10° and 30° in the Paraná and Paraguay river basins. It is a plant typical of the Alto Paraná region, Alto Uruguay region and northeastern Argentina (Vazquez and Moyna, 1986). The chemical composition of the *Ilex* genus includes phenols and phenolic acids, amino acids and other nitrogenated compounds, fatty acids, anthocyanins, flavonoids, terpenic compounds, alcohols, carbohydrates, vitamins, carotenoids and methylxanthines. The latter compounds are the best known (Alikaridis, 1987).

The main methylxanthine found in mate is caffeine (0.89–1.73%), followed by theobromine (0.26–0.88%) and small amounts of theophylline (Clifford and Ramirez-Martinez, 1990). Methylxanthines, with several pharmacological properties which comprise stimulation of the central nervous system, peripheral vasoconstriction, relaxation of the smooth muscle and myocardial stimulation (Kikatani

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et al., 1993; Lorist and Tops, 2003), accumulate intracellularly in the epicuticular wax of mate leaves. Their contents have been associated to origin, genetic and environmental variability, harvest time and processing manner (Athayde et al., 2000). Contents change according to season, decrease from September to December (Da Croce, 2002), and differences in caffeine and theobromine contents may occur between varieties and progenies (Reginatto et al., 1999; Scherer et al., 2002).

Phenolic compounds constitute another important chemical group in mate, among which chlorogenic acids (CGAs) are the most known (Alikaridis, 1987). CGAs comprise a family of mono- and di-acyl esters of quinic acid, and the most common acyl residues are caffeic (CQA and di-CQA), ferulic (FQA) and *p*-coumaric (*p*-CoQA) acids. Mate has high concentration of caffeoyl derivatives, including caffeic, chlorogenic, 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic (3,5-di-CQA) and 4,5-dicaffeoylquinic acids (Filip et al., 2001). Commercial preparations of green mate contain high amount of CGAs, especially 3-CQA and 3,5-di-CQA (Clifford and Ramirez-Martinez, 1990). In general, CGAs have been associated to choleretic, antioxidant and hypocholesterolemic properties (Ohnishi et al., 1994; Meyer et al., 1998; De Maria and Moreira, 2004).

Taking into consideration the economic importance and the biological properties of mate, this research evaluates the contents of methylxanthines (caffeine and theobromine) and phenolic compounds (chlorogenic and caffeic acids) in leaves of 16 progenies of *Ilex paraguariensis*. These progenies are included in a genetic improvement program carried out in Brazil.

2. Materials and methods

2.1. Experimental design and sample collection

Progenies from four Brazilian regions (Ivaí—PR: 25 progenies; Quedas do Iguaçu—PR: 25 progenies; Cascavel—PR: 25 progenies; Barão de Cotegipe—RS: 21 progenies) were grown in different places in the state of Paraná (Ivaí, March 1997; Rio Azul, July 1997 and Guarapuava, August 1997). Experiments were arranged in a randomized block design, in eight (Guarapuava and Rio Azul) and ten replications (Ivaí), with six plants each one, linearly planted on a 3×2-m spacing between them. Progenies selection was randomized without hierarchic arrangement within each origin. Plants were first hand harvested in three blocks, from June to August 2001. The second harvest was carried out 2 years later. Sixteen progenies with high production of leaf mass were selected. Samples (3 kg) of leaves and branches (<3 mm in diameter) of each replication were collected, homogenized and immediately brought to the laboratory. Samples were boiled in water for 10 s and the green and clean leaves were separated. Leaves were dried in a forced draft oven (45 °C, 48 h). Next, the leaves were ground, placed in paper, wrapped in plastic packing and refrigerated until analysis.

2.2. Quantification of methylxanthines and phenolic compounds

Methylxanthines were extracted from samples (1.0 g) with sulfuric acid, followed by aqueous extraction at 75 °C and then with chloroform partition. Spectrophotometric quantification of total methylxanthines was made at 275 nm, using caffeine as standard (Instituto Adolfo Lutz, 1985). Phenolic compounds were extracted from samples (1.0 g) by water infusion, followed by reduction with phosphotungstic acid. Total phenol was quantified at 715 nm, using pyrogallic acid as a calibration standard (Costa, 1982).

Caffeine, theobromine, and caffeic and chlorogenic acids were extracted by maceration of sample (1.5 g) in 100 mL ethanol:water (70:30 v/v) for chromatography (Clifford and Ramirez-Martinez, 1990). Extracts were filtered with 0.45 μm nylon filters, and put into a chromatograph. Chromatography was performed on a Shimadzu (Mod. SCL-10A) high performance liquid chromatography (HPLC) system, consisting of SIL-10AF injector, LC-10AT pump, FCV-10AL mixer, DGU-14A degasser and injector valve, a 20-μl sample loop and an interphase module Shimadzu CLASS VP6.14SP2. A 5 μm Supelcosil LC-18, 4.6×250 mm analytical column was used. The column was maintained at 30 °C using the CTO-10AS oven integrated to the HPLC machine. The solvent system consisted of (A) acidulated water with a 0.3% acetic acid, and (B) methanol. Solvents were run at a flow rate of 1.0 mL min^{-1} using the following linear gradient: 15–20% B in 20 min; 20–85% B in 5 min; 85% B in 5 min (Filip et al., 2001). Detection was monitored at 265 nm for caffeine and theobromine, and at 325 nm for caffeic and chlorogenic acids using a SPD-10A UV–vis detector integrated in the chromatograph. All samples were run in triplicate. Chromatographic peaks were identified by comparing retention times with those of caffeine, theobromine, chlorogenic and caffeic acids standards (Sigma Chemical Co., USA), recorded in the same conditions. All reagents used were of the purest grade available or chromatographic grade. Calibration curves were obtained with standards diluted in the mobile phase. Linearity was determined by regression, while precision and accuracy were determined by variation coefficient (<3%). Correlation coefficients were as follows $r^2 = 0.9999$ for caffeine, $r^2 = 0.9976$ for theobromine, $r^2 = 0.9997$ for chlorogenic acid, and $r^2 = 0.9995$ for caffeic acid.

2.3. Statistical analysis

Significant differences were observed undertaken by one-way variance analysis with Sisvar software package (Version 4.3, UFLA, Brazil). Difference between parameters was evaluated by Scott–Knott test, taking $P < 0.05$ as statistically significant. Results are given as percentage of dry weight (mean of three independent experiments ± S.E.).