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Natural infraspecific variation in fatty acid composition of *Cuphea* (Lythraceae) seed oils

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ABSTRACT

Fatty acid analyses of seed lipids were performed for 28 populations representing three widely distributed *Cuphea* species. Locality and climatic data for all samples were also compiled. The objectives of this study were to examine the extent of variation in seed oil composition among the wild populations, and to discover any patterns of relationship between fatty acid composition of seed oils and environmental factors. While we found the dominant fatty acid in the oil of each species remained consistent over the environmental and geographical ranges of the samples in the present study, variation as high as 30.6% in the amount of the dominant fatty acid produced was observed. Correlation analysis between fatty acid composition and the individual environmental factors of latitude, elevation or temperature showed no consistent pattern of influence. However, when considered together, the interaction of all three, and especially latitude and elevation, contributed significantly to the variation among populations. Environmental data at microhabitat level and through controlled environmental experiments will be needed for more precise understanding of factors affecting *Cuphea* seed oil composition at the population level.

1. Introduction

Medium chain fatty acids (MCFAs) – those with carbon chain lengths ranging from 8 to 14 – have been recognized for decades for their economic importance. They are used as feed-stocks and in the manufacture of food products, household cleaners, personal care products, industrial lubricants, coatings and plastics (Thompson, 1984; Forcella et al., 2005). There is great interest in MCFAs as a potential alternative to motor oils and diesel fuel derived from petroleum (Geller et al., 1999; Cermak and Isbell, 2004). There is also a growing interest in developing plant oils as a source of biodiesel fuel. Biodiesels possess performance characteristics identical to those of petroleum-derived diesel, but without the attendant pollutants (Buchanan et al., 2000). At present, tropical coconut (*Cocos nucifera* L.) and palm kernel (*Elaeis guineensis* Jacq.) oils are the major sources of the world's supply of plant-derived MCFAs, and the demand for these oils as industrial ingredients has been growing in developed countries in recent years (FAOSTAT, 2006). Under tropical environmental conditions, however, plants are often subject to drought and diseases that result in dramatic variations in yield. As a result, efforts have been made to find temperate sources of MCFAs as a supplement or replacement for the tropical oils (Hirsinger, 1985; Knapp, 1993; Gesch et al., 2006).

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Cuphea P. Browne is one of the few temperate flowering plant genera that have the potential to produce oil MCFAs, especially through development of high-yielding capric–lauric acid plants (Knapp, 1993). It is the largest genus in the Lythraceae, comprising more than 260 species that are distributed from the eastern United States to Argentina (Cavalcanti and Graham, 2005). *Cuphea* species produce highly diversified seed oils that are rich in a variety of MCFAs, including caprylic (C8:0), capric (C10:0), lauric (C12:0) and myristic (C14:0) acids (Graham and Kleiman, 1992; Forcella et al., 2005). This contrasts with most other angiosperms whose oily seeds generally produce primarily linoleic acid (C18:2).

Numerous projects have been undertaken in recent years to develop *Cuphea* plants suitable for large-scale agriculture. Genetic and agronomic studies have been undertaken aiming at evaluating species for fatty acid content, determining the feasibility of domesticating *Cuphea*, and understanding the barriers to domestication (Hirsinger and Roebbelen, 1980; Hirsinger, 1985; Roebbelen and von Witzke, 1989; Knapp, 1993; Knapp and Crane, 2000; Gesch et al., 2005, 2006; Forcella et al., 2005). Although such efforts have resulted in a few germplasm lines with favorable agronomic traits such as partial non-shattering and non-dormancy (e.g. *Cuphea* PSR23; Knapp and Crane, 2000), many issues related to fatty acid production and commercialization of *Cuphea* seed oils remain to be resolved.

Studies of fatty acid composition of *Cuphea* seeds obtained from wild species have previously been reported (Hirsinger, 1980; Hirsinger and Roebbelen, 1980; Graham et al., 1981; Graham, 1989; Graham and Kleiman, 1992). However, these studies were limited and sampled within restricted geographical and environmental ranges. For instance, Graham et al.'s (1981) study involved two *Cuphea* species of Mexican distribution. Based on these and many other studies, seed oil composition in flowering plants is considered to be a genetically controlled and conserved trait (Levin, 1974; Ohlrogge and Jaworski, 1997; Hobbs et al., 2004). However, fatty acid composition also is likely subject to adaptation that results in natural infraspecific variation (O'Neill et al., 2003). This means that certain environmental conditions could result in selection for seed oil composition with enhanced amounts of specific fatty acids.

In the present study, we conducted a survey of infraspecific variation in MCFAs from three widely distributed wild species of *Cuphea*: *C. strigulosa* Kunth, *C. carthagenensis* (Jacq.) Macbr., and *C. wrightii* A. Gray. Our objectives were to: (1) test the extent of variation in seed oil composition among wild populations of species throughout their geographical range and across natural environmental and ecological differences; and (2) discover any patterns of relationship between fatty acid composition of the seed oils and selected environmental factors to provide information on the influence of the environment on fatty acid composition.

2. Materials and methods

2.1. Plant material

Mature seeds from 28 distinct localities ranging from South Carolina, USA to Paraguay, and representing three wild *Cuphea* species were sampled for this study, including 7 *C. strigulosa*, 12 *C. carthagenensis* and 9 *C. wrightii* samples (Table 1). Only fully matured seeds were utilized because immature seeds, either still within the capsule or exposed but not yet with mature brown coloration, have not reached the final fatty acid composition. Most populations were visited and seeds collected during field studies in Mexico, Central America and Brazil. Five seeds constituted a sample and the samples were selected across the geographic range of each species. Samples of *C. strigulosa* were collected from Everglades, Florida to Peru and Southeastern Brazil, across a range of 45.0° latitude, 2349 m elevation and 8.1 °C temperature. The 12 samples of *C. carthagenensis* were collected from South Carolina, USA through Mexico and Central America to Argentina, a range of 60.4° latitude, 1749 m elevation and 10.2 °C temperature. *C. wrightii* samples were collected from Nayarit to Oaxaca, Mexico, a range of 43.0° latitude, 984 m elevation and 5.4 °C temperature. Herbarium vouchers are deposited at the Missouri Botanical Garden (St. Louis, MO, USA).

2.2. Fatty acid content in Cuphea seeds

2.2.1. Extraction and derivatization procedure

Fatty acids were extracted and derivatized into fatty acid methyl esters following a stepwise hydrolysis and methylation procedure. Five debris-free, mature seeds were first soaked in 1 ml hexane in a 10 ml conical centrifuge tube for 5 min and were crushed with Teflon glass rod for 35 s. The mixture was then incubated at 50 °C for 15 min in a dry heat block and allowed to settle down for 2 min. The solvent was evaporated and residue was refluxed with 0.1 ml of ethyl ether followed by a 0.1 ml of KOH in methanol. Next, 0.1 ml of 0.15 M HCl was added followed immediately by 1 ml of hexane by a pipette through a condenser. The mixture was swirled in the tube and allowed to settle down for 30 min until separation into two layers was complete. Approximately 0.5 ml of the hexane (top) layer was transferred into an autosampler vial using a Pasteur pipette and the sample was placed in the automated carousel of the GC instrument. Following Gören et al. (2003), the fatty acid methyl esters were recovered after solvent evaporation under vacuum.

2.2.2. Gas chromatography analysis

The fatty acid methyl esters were analyzed using Agilent Technologies 6890 N network gas chromatography with a flame ionization detector (FID) and an Agilent 122–2332 DB-23 column. The carrier gas was helium at a rate of 17 ml/min. Oven temperature was kept at 50 °C for 1 min and programmed to 185 °C at a rate of 30 °C/min with a 4 min hold at the final temperature. The injector and detector temperatures were 220 and 230 °C, respectively. The injection volume was 1 μl for all samples with a split ratio of 1:20. Data were collected using Agilent Chemstation software. Retention times for eluted peaks included: methyl caproate (2.470 min), methyl caprylate (3.419 min), methyl caprate (4.220 min), methyl laurate (4.969 min), methyl myristate (5.563 min), methyl myristolate (6.019 min), methyl palmitate (6.240 min), methyl stearate (7.256 min), methyl oleate (7.472 min) and methyl lineolate (7.802 min). All samples were run in duplicate and the relative percentage of separated compounds was calculated