Fatty Acid and Oil Diversity of *Cuphea viscosissima*: A Source of Medium-Chain Fatty Acids

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***Cuphea viscosissima* Jacq. is being developed as a commercial source of caprylic, capric, lauric, and myristic acids. Germplasm resources for characterizing the genetic diversity of this species became available following explorations by the United States Department of Agriculture (USDA) in 1986 and 1987. In this paper, we describe the fatty acid and oil percentage diversity of forty-two populations of *C. viscosissima* collected from seven states within the United States. Caprylic (18.0%) and capric acid (69.9%) were the major fatty acids of these populations. The fatty acid percentage ranges were narrow for every fatty acid, *e.g.*, 16.4 to 20.4% for caprylic acid and 66.6 to 71.3% for capric acid. The maximum lauric acid percentage was 3.4%. Oil ranged from 27.3 to 33.4%. Although the populations surveyed cover a fairly wide geographic range, they display limited fatty acid diversity. Surveys of germplasm from other parts of the range are needed to further characterize the fatty acid diversity of this species.**

KEY WORDS: Capric acid, caproic acid, caprylic acid, lauric acid, medium-chain triacylglycerols, myristic acid, oilseed.

Nearly three decades ago, several *Cuphea* species were discovered by Miller *et al.* (1) to be excellent sources of medium-chain fatty acids (MCFA). Surveys of fatty acid variation between *Cuphea* species have since been done (2,3), and efforts to domesticate *Cuphea* have been started in Europe and the United States (U.S.). *Cuphea* is a large and diverse genus found over a wide range of climates in North, Central, and South America (4,5). *Cuphea viscosissima* Jacq., the sole species of *Cuphea* indigenous to middle and northern latitudes of the U.S. (4,5), is the focus of the U.S. effort to domesticate *Cuphea*.

C. viscosissima is an excellent source of caprylic and capric acid (3). The caprylic and capric acid percentages previously reported for this species are 9.1% and 75.5%, respectively (3). These percentages were estimated from limited seed samples collected from a wild population (3). The fatty acid and oil diversity of this species has not been characterized.

Prior to 1986, the germplasm resources of *C. viscosissima* were limited to an accession from West Virginia (Balough SN 412). Two explorations have since been made, yielding seed of fifty-six populations from several midwestern and southeastern states and important information about the distribution and habitat of wild *C. viscosissima* populations. This germplasm has become a vital resource for breeding *C. viscosissima*. In this paper, we describe the fatty acid and oil diversity of 42 populations of *C. viscosissima*.

MATERIALS AND METHODS

Forty-two populations of *C. viscosissima* were grown at Corvallis, Oregon, in 1988 and 1989. These populations were derived from bulk seed samples of PI 534734 through PI 534771, collected by W.W. Roath and M.D. Widrlechner (USDA-ARS, Ames, Iowa) in Kansas, Arkansas, Missouri, Iowa, and Illinois, PI 534772 collected by M.D. Widrlechner in Indiana, and Balough SN 412 collected by S.A. Graham (Kent State University, Kent, Ohio) in West Virginia.

To overcome dormancy, seed was germinated by excising embryos from their seed coats. Ten plants of each population were grown in the greenhouse in April of 1988 and 1989 and transplanted to field nurseries at Corvallis, Oregon, In June of 1988 and 1989. A completely randomized experiment design was used. There were two replications of each line and five transplants in each replication. Seed was repeatedly harvested and bulked during September and October of both years.

Oil percentages were measured by wide line nuclear magnetic resonance (NMR) (Oxford, Inc., Villanova, PA). A *C. viscosissima* oil percentage standard was used to calibrate the NMR. The oil percentage of the standard was determined from a petroleum ether extract of Balough SN 412 seed. Seeds were dried at 50°C for 24 hr and stored in a desiccator to ensure uniform moisture among samples. Bulk 10-g seed samples from each replication were assayed.

Fatty acid percentages were measured in a Varian 3400 gas chromatograph fitted with a J&W (Folsom, CA) DB-225 (15.0 m × 0.533 mm) fused silica column. Fatty acid methyl esters were prepared from 30-mg samples of mature seed from each replication, essentially using previously described methods (6). Samples were ground in 10 mL of hexane and centrifuged for 4 min at 200 × *g*. The supernatant was transferred to a clean tube and evaporated to dryness at 50°C under a stream of N_2. Seed triglycerides were brought into solution with 0.5 mL of ethyl ether and transesterified in 0.5 mL of 0.1 N methanolic KOH. Fatty acid methyl esters were extracted by adding 0.5 mL of pentane and 0.5 mL of 0.15 N HCl followed by vigorous shaking. A sample from the top layer was injected into the gas chromatograph. The helium gas flow rate was set at 18 mL min^{-1}. The column oven temperature was programmd to increase from 85.0 to 180.0°C at 20.0°C min^{-1} and 180.0 to 195.0°C at 4.0°C min^{-1}. Temperatures of the injector (split/splitless) and flame ionization detector were set at 220°C. Nu Chek Prep (Elysian, MN) standards were used to identify peaks and monitor analyses. Peak counts were expressed as percentages of total fatty acids.

Analysis of variance was used to estimate and test hypotheses about population, year, and population by year means for caprylic and capric acid and oil percentage.

RESULTS AND DISCUSSION

The fatty acids percentage ranges of the *C. viscosissima* populations surveyed were narrow (Table 1). The ranges of 8:0 and 10:0 across years were 4.0 and 4.7%, respectively (Table 1). The caprylic acid percentages of these popula-

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TABLE 1

Minimum, Maximum, and Mean Fatty Acid and Oil Percentages of Forty-Two *C. viscosissima* Populations Grown at Corvallis, Oregon, in 1988 and 1989

	1988			1989			Across years		
Trait[a]	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
					(%)				
Oil	26.6	33.4	30.7	27.9	33.3	31.3	28.7	33.1	31.0
6:0	0.7	1.1	0.8	0.7	1.0	0.9	0.7	1.0	0.9
8:0	15.9	21.2	17.8	16.9	20.2	18.3	16.4	20.4	18.1
10:0	65.9	71.5	69.8	67.4	71.4	70.0	66.6	71.3	69.9
12:0	2.7	3.6	3.0	2.5	3.3	2.8	2.7	3.4	2.9
14:0	0.8	1.3	0.9	0.8	1.2	0.9	0.8	1.2	0.9
16:0	1.4	1.9	1.6	1.4	1.9	1.6	1.4	1.9	1.6
18:0	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.1
18:1	1.4	2.6	2.0	1.6	2.4	1.9	1.5	2.5	2.0
18:2	3.2	4.0	3.6	3.0	3.6	3.3	3.2	3.8	3.5
18:3	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.3	0.2

[a]6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 18:1, 18:2, and 18:3 are caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, linoleic, and linolenic acid, respectively.

TABLE 2

Analysis of Variance of Caprylic (8:0) and Capric (10:0) Acid and Oil Percentages of Forty-Two *C. viscosissima* Populations Grown at Corvallis, Oregon, in 1988 and 1989

		Trait					
		8:0		10:0		Oil	
Source of variation	df[a]	Mean square	p-value	Mean square	p-value	Mean square	p-value
Population	41	2.88	⩽ 0.0001	3.84	⩽ 0.0001	5.07	⩽ 0.0001
Year	1	7.73	⩽ 0.0001	1.12	⩽ 0.0001	12.13	⩽ 0.0001
Population × year	41	0.25	0.03	0.19	0.15	1.58	⩽ 0.0001
Residual	84	0.15		0.15		0.38	

[a]df is degrees of freedom.

tions are greater than those previously reported, whereas the capric acid percentages are less (3). The difference may be attributed to sampling variation as opposed to genetic variation. Wolf *et al.* (3) estimated 75.5% capric acid from a small sample of seed harvestd from a wild population. The maximum 10:0 percentage we observed was 71.3 (Table 1). The difference might be attributed to seed quality and sample size.

The mean caprylic and capric acid percentages we observed were 18.1 and 69.9%, respectively (Table 1); thus, 8:0 and 10:0 comprise approximately 88.0% of the total fatty acids of the seeds of this species. Lauric acid comprises 2.9% (Table 1).

There were significant caprylic and capric acid percentage differences among populations and years (Table 2). There was a significant line-by-year interaction for caprylic acid percentage, but not for capric acid percentage (Table 2). Oil percentages for these populations ranged from 28.7 to 33.1% (Table 1). The mean oil percentage across populations and years was 31.0%. Population, year, and population-by-year interaction effects for oil percentage were significant (Table 2).

The fatty acid diversity of the 42 *C. viscosissima* populations we characterized was limited (Table 1). There is, for example, limited 12:0 variation among them and, as a consequence, no natural genetic variation for significantly increasing the percentage of lauric acid (Table 1). There was, however, significant natural genetic variation for oil percentage.

Although natural genetic variation for fatty acids is limited within *C. viscosissima*, several mutants have been developed that transgress the fatty acid percentage ranges of natural populations (7). Lauric acid and caprylic acid, for example, have been increased from 3.0 to 14.3% and 17.9 to 28.4%, respectively, by individual point mutations (7). Although fatty acid diversity seems to be limited in wild populations of *C. viscosissima*, induced genetic variation can be exploited to greatly expand the range of fatty acid phenotypes within this species.

Graham *et al.* (2) found somewhat limited fatty acid variation between *C. wrightii* and *C. aequipetala* populations. The lauric acid percentages of nine wild populations of *C. wrightii*, for example, ranged from 48.2 to 60.5% (2). The myristic acid percentage range of wild populations of *C. aequipetala* was found to be 20% (2). Although these ranges exceed those observed for *V. viscosissima*, they show there are archetypal fatty acid profiles within *Cuphea* species (2).

Additional populations of *C. viscosissima* have been collected from Tennessee, Kentucky, North Carolina, and South Carolina and additional explorations are expected over the next several years. The fatty acid and oil percentages of these populations need to be estimated to further characterize the diversity of *C. viscosissima.*

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