Evaluation of Gamma-linolenic Acid Composition of Evening Primrose (*Oenothera*) Species Native to Texas

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Abstract. The oil of evening primrose (*Oenothera* sp.) is an important source of gamma-linolenic acid (GLA). GLA [C18:3Δ6,9,12] is an unsaturated fatty acid in demand for its nutritional and pharmaceutical application. *Oenothera biennis* L. is the primary commercial source of evening primrose oil. A study was conducted to determine if species of *Oenothera*, adapted to Texas, produce GLA levels comparable to *O. biennis*. This project identified and evaluated the fatty acid composition of eight species of evening primrose native to Texas. GLA levels of 54 accessions evaluated from collected seed ranged from 0.0% to 11.0%. Field experiments were then conducted to determine oil content, fatty acid composition, seed yield, and potential adaptation to commercial production of selected accessions. Mean GLA levels of cultivated seed from these accessions ranged from 0.0% to 10.1%. Mean seed oil content ranged from 7.3% to 21.7%. Of the species examined, *O. elata* subsp. *hirsutissima* (A. Gray ex S. Watson) W. Dietrich and *O. jamesii* (Torrey & Gray) demonstrated GLA levels and seed yields adequate for commercial production. Based on these results, *O. elata* subsp. *hirsutissima* and *O. jamesii* demonstrated sufficiently high GLA levels, oil content, and seed yields to be considered for commercial production.

In recent years, there has been increased interest in plants that produce gamma-linolenic acid (GLA). In humans and other mammals, GLA is an important intermediate in the conversion of linoleic acid to prostaglandins, thromboxanes, and other long-chain polyunsaturated fatty acids (Horrobin, 1992). This compound is considered an essential fatty acid, since, like vitamins, it cannot be synthesized by animals and must be consumed in the diet from plant sources (Lehninger, 1982). Prostaglandins and thromboxanes are hormone regulators which affect many cellular functions and regulate some blood platelet activities. The rate-determining step in the bioconversion of linoleic acid to arachidonic acid, the precursor of prostaglandins and thromboxanes, is believed to be the formation of the double bond at the C-6 carbon by the enzyme Δ6-desaturase (Horrobin, 1992). This enzyme catalyzes the conversion of linoleic acid to gamma-linolenic acid. Although most humans consume sufficient quantities of linoleic acid, it has been shown that certain factors such as stress, aging, diabetes, and alcohol consumption can inhibit or reduce the activity of the Δ6-desaturase enzyme (Horrobin, 1992; Phillips and Huang, 1996). In recent medical research, it has been found that dietary supplementation of GLA can be effective in the treatment of these conditions (Carter, 1988).

Evening primrose (*Oenothera biennis* L.) was the first plant used for the isolation of GLA (Gunstone, 1992), and it is the primary source of GLA used in clinical and pharmaceutical applications (Phillips and Huang, 1996). GLA was extracted from the oil in the seeds of this plant. Hudson (1984) reported that evening primrose seed contains 24% oil with gamma-linolenic acid comprising 7% to 14% of the fatty acid component. Evening primrose oil is available as a nutritional supplement in over 30 countries and *Oenothera biennis* is grown commercially in at least 15 countries (Carter, 1988). In 1988, Canada and the United States produced 300–400 tons of evening primrose seed. Most of the commercial production of evening primrose oil in North America is located in Canada and in the eastern United States (Brandle et al., 1993; Hall et al., 1988). These are areas of high rainfall and moderate temperatures. Little work has been done to evaluate the fatty acid composition of *Oenothera* species native to semiarid regions of the United States. A native evening primrose with commercial levels of GLA would have potential as a high-value oil crop in areas of high temperatures and low annual rainfall. The objectives of this project were to: 1) identify and collect species of evening primrose native to the western regions of Texas, 2) analyze the fatty acid composition of the seed oil of these species, and 3) evaluate their adaptability to commercial field production.

Methods and Materials

The collection area included the Northern High Plains and Southern High Plains, the Texas Rolling Plains, and the Edwards Plateau

regions of Texas. In the spring and early summer, tagging trips were taken to locate evening primrose species while they were in bloom so that identification could be made. Voucher specimens were collected for each species of the study. Each collection site was recorded using a hand-held GeoExplorer global positioning unit. In the late summer or fall, plant populations were relocated and ripened seed capsules were collected. A threshing machine was used to break open capsules and separate seed from other plant debris. Seed was further cleaned with a seed blower (General Mfg.). Cleaned seed was stored in air-tight plastic containers in a refrigeration unit held at 4 °C.

Seeds from each collection site were analyzed for fatty acid composition (Speed, 1995). A 300-mg sample of seed from each accession was used. Oil was extracted from the seed by grinding whole seeds in 20 mL of hexane using a Polytron grinder. The oil/hexane mixture was poured through a Whatman filter into 20-mL scintillation vials. Vials were placed into a heating block at 50 °C and the hexane was evaporated with a continuous flow of nitrogen gas. Methyl esters of the oil were made by adding 1 mL of methylation mixture to each sample and inverting the capped sample for mixing. The methylation mixture was composed of 29.1 mL of 14% borontrifluoride in methanol, 20 mL of toluene, and 50.9 mL of methanol. Samples were placed in a heating block at 100 °C for 20 min. Samples were then removed from the heating block and allowed to cool for 10 min. Each sample was rinsed with 2 mL of deionized water and 1 mL of hexane and shaken to mix well. The mixture was allowed to separate into two layers and the hexane layer was pipetted into vials for gas chromatograph analysis. Nitrogen gas was blown into each sample vial before it was capped to prevent oxidation of the sample.

Samples were analyzed using a Hewlett Packard 5890 Series II Gas Chromatograph equipped with a Supelco 30 m × 0.53 mm × 0.5 μm fused silica capillary column and flame ionization detector (Speed, 1995). The initial oven temperature of 210 °C was increased at the rate of 4 °C per minute until a final temperature of 240 °C was reached. The injector port was set at 200 °C and the flame ionization detector was set at 250 °C. Output of the flame ionization detector was recorded on an HP 3396 Series II Integrator. Fatty acid composition was calculated by summing the total area of all fatty acids detected and dividing each fatty acid by the total area. Peaks were identified by comparing retention times to reference standard retention peaks of known composition (Sigma AOCs No. 3, Sigma AOCs No. 5, Alltech Methyl Ester Standards).

Seeds harvested from cultivated plots were analyzed for percent oil at the Univ. of Idaho, using a Newport MKIII nuclear magnetic resonance instrument (Newport of North America, Villanova, Pa.). Analyses were made on oven-dried seed, using a canola standard of 36.2% oil.

Species with high levels GLA were evaluated in field studies in 1996, 1997, 1998, and 1999. Seeds were sown in a soilless mix in

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the greenhouse in January of each year and transplanted to the field in May. Plants were grown at the Erskine Research Farm in Lubbock, Tex., under drip irrigation. The soil at this site was an Amarillo fine sandy loam. Each species was planted in a completely randomized block design with four replicates. Seeds were harvested at maturity and threshed and analyzed for fatty acid composition using the above procedure.

Data were subjected to analysis of variance procedures (SAS Institute, 1998). Mean separations were performed using Fisher's Protected Least Significant Difference procedures at the $P \leq 0.05$ level.

Results and Discussion

From 1995 to 1997, seed from 83 accessions of nine species of evening primrose were collected. The area surveyed included portions of the High Plains, the Rolling Plains, the Edwards Plateau, and the Crosstimbers and Prairies regions of Texas. *Manual of the Vascular Plants of Texas* (Correll and Johnston, 1970) and *Systematic Botany Monographs Vol. 50* (Dietrich et al., 1997) were used to identify species.

Fatty acid composition of oil from native collected seed showed minimal differences among species in levels of palmitic, stearic, oleic, and linoleic acids (Table 1). Linoleic acid was the dominant fatty acid found in all species tested. This is in agreement with fatty acid composition of *Oenothera* reported by Gunstone (1992). Levels of GLA varied widely among species but were consistent within accessions of a species with the exception of several accessions of *O. rhombipetalata*, which showed significant variation in GLA content, ranging from 1.5% to 6.8%.

GLA levels of 11.0% were found in one accession (R50) of *O. jamesii* collected in 1997. The accession of *O. elata* collected in 1996 had a GLA level of 6.1%. GLA levels of *O. rhombipetalata* ranged from 1.2% in 1997 to 6.8% in 1996 and averaged 4.3% (four accessions) in 1996 and averaged 4.1% (five accessions) in 1998. GLA levels of *O. speciosa* ranged from 0.0% to 2.2% but averaged 1.7%

in 1996 and 0.0% in 1997. All accessions of *O. grandis*, *O. missouriensis*, *O. brachycarpa*, and *O. laciniata* had GLA levels of <0.5%. Statistical analyses were not conducted on fatty acid composition of native collected seed due to the low numbers of accessions collected of some species. Accessions with high levels of GLA were included in subsequent field evaluations.

Each collected species was grown in the field for a minimum of 2 years. Species exhibiting <2.0% GLA levels were not evaluated in subsequent years. *Oenothera biennis* was used as the control in 1996 and 1997 in field trials, but insect infestations limited its continued use as a control in 1998 and 1999. *Oenothera elata* was used as the control in 1998 and 1999 because it represented a better adapted check.

Selected accessions of *Oenothera* species were grown under cultivated conditions at Lubbock, Tex. In 1996, *O. rhombipetalata*, *O. grandis*, and *O. engelmannii* were grown along with a commercial source of *O. biennis*. GLA levels of *O. biennis* were significantly higher than those of *O. rhombipetalata* (Table 2). Levels of GLA in *O. rhombipetalata* were significantly higher than those of *O. grandis* and *O. engelmannii*. In 1997, oil of *O. elata* with 10.1% GLA was significantly higher than *O. biennis* with 8.0%. *Oenothera biennis* had significantly higher levels than *O. rhombipetalata* with 6.3%. *Oenothera speciosa*, *O. grandis*, and *O. missouriensis* had levels <2.2%, which severely limited their commercial potential. In 1998, *O. jamesii* with 9.1% and *O. elata* with 8.0% had significantly higher GLA levels than *O. rhombipetalata*, which had 4.1%. *Oenothera speciosa* contained the lowest amount of GLA (1.5%). Again in 1999, *O. jamesii* and *O. elata* had GLA levels of over 8%, which was significantly higher than *O. rhombipetalata* at 3.6%.

Oenothera elata, *O. rhombipetalata*, and *O. biennis* had significantly higher GLA levels in 1997 than in other years (Table 2). *Oenothera speciosa* and *O. jamesii* showed no differences among years of this study.

Comparisons of fatty acid composition between native collected seed and cultivated seed of the same accessions of eight species

showed a modest correlation in concentration of GLA levels ($r = 0.73^{**}$, $df = 12$). Seeds from cultivated plants of *O. jamesii* had GLA levels ranging from 8.3% to 10.5% (data not shown) in 1998 and 6.8% to 10.7% in 1999 compared to the 1997 native collection with 11% and the 1998 native collection with 6.8%. *Oenothera jamesii* was collected in a mesic habitat that may have greater water availability comparable to field conditions. In all three years of cultivation, *Oenothera elata* demonstrated greater GLA levels (8.0% to 10.1%) than the original collected seed (6.1%).

In 1999, seeds harvested from the upper (terminal) half of a branch, which matured in October, were compared to seeds harvested from the lower half of the branch in two species *O. jamesii* and *O. elata*. Comparisons of fatty acid composition showed that although there was a tendency toward higher GLA levels in the upper half of the branches, differences were not statistically significant (data not shown). Average GLA levels of seed in the upper halves of *O. elata* and *O. jamesii* were 8.3% and 8.9%, respectively, while those of the lower halves were 6.6% and 7.3%.

In 1996, mean oil percentages of cultivated plants ranged from 10.8% to 17.3%, with oil content of *O. rhombipetalata* and *O. engelmannii* being significantly higher than *O. biennis* and *O. grandis* (Table 2). In 1997, oil content ranged from 11.1% to 19.3%, with *O. speciosa* and *O. rhombipetalata* having significantly higher oil content than *O. biennis* and *O. grandis*. In 1998, oil content ranged from 7.3% in *O. elata* to 20.7% in *O. speciosa*, which had significantly higher oil content than all others in that year. In 1999, oil content ranged from 13.5% in *O. rhombipetalata* to 18.6% in *O. elata*, which was significantly higher than *O. jamesii* (17.3%) and *O. rhombipetalata*. It was interesting to note that over the 4-year period the two species with the highest average oil content (*O. speciosa* and *O. engelmannii*) were two species that had low GLA percentages. *Oenothera elata*, a species with consistently high GLA levels, had the lowest average oil content in 1998.

It has been reported that seeds maturing under cooler conditions generally produce more unsaturated fatty acids than under

Table 1. Average fatty acid composition of seed collected from nine species of evening primrose (*Oenothera* sp.) native to Texas in 1996, 1997, and 1998.

Year	Species	Access. (no.)	Fatty acid composition					
			C16:0	C18:0	C18:1	C18:2	γC18:3	αC18:3
			-----% methyl esters (std error)-----					
1996	<i>O. engelmannii</i>	7	9.4 (±0.4)	0.8 (±0.2)	10.1 (±0.8)	71.0 (±4.6)	0.0	0.0
	<i>O. grandis</i>	4	9.3 (±0.4)	1.0 (±0.2)	9.8 (±0.9)	74.7 (±2.3)	0.0	0.0
	<i>O. missouriensis</i>	3	7.5 (±0.5)	1.1 (±0.3)	13.2 (±0.4)	75.3 (±0.7)	0.0	0.0
	<i>O. rhombipetalata</i>	4	7.8 (±0.3)	2.0 (±0.0)	9.2 (±0.9)	72.2 (±2.1)	4.3 (±1.1)	0.1 (±0.1)
	<i>O. speciosa</i>	3	10.1 (±0.5)	2.3 (±0.1)	12.2 (±0.3)	66.8 (±4.7)	1.7 (±0.3)	0.1 (±0.0)
	<i>O. elata</i>	1	6.3	1.6	10.1	72.4	6.1	0.0
1997	<i>O. engelmannii</i>	3	9.4 (±0.6)	1.0 (±0.3)	7.8 (±0.4)	74.3 (±2.1)	0.0	0.0
	<i>O. missouriensis</i>	8	8.5 (±0.3)	1.4 (±0.2)	12.2 (±0.9)	68.3 (±1.5)	0.0	0.0
	<i>O. rhombipetalata</i>	1	7.8	2.1	8.6	73.4	1.2	0.1
	<i>O. speciosa</i>	4	12.6 (±0.2)	2.3 (±0.2)	10.5 (±0.8)	64.5 (±3.0)	0.0	0.0
	<i>O. brachycarpa</i>	2	8.6 (±0.2)	2.2 (±0.1)	12.9 (±1.3)	68.6 (±0.1)	0.0	0.0
	<i>O. jamesii</i>	1	8.2	1.4	11.7	58.6	11.0	0.0
	<i>O. laciniata</i>	2	10.3 (±0.1)	1.7 (±0.2)	7.2 (±0.6)	70.0 (±0.5)	0.0	0.0
	<i>O. rhombipetalata</i>	5	8.6 (±0.2)	1.6 (±0.3)	10.0 (±0.4)	65.1 (±0.9)	4.1 (±1.4)	0.0
1998	<i>O. jamesii</i>	1	7.6	1.5	13.5	60.5	6.8	0.0

Table 2. Average fatty acid composition and percent oil content of 10 accessions of *Oenothera* species grown at Lubbock, Tex., in 1996, 1997, 1998, and 1999.

Year	Species	Access.	Fatty acid composition ^z						Oil
			C16:0	C18:0	C18:1	C18:2	γC18:3	αC18:3	content
			---% methyl ester-----						---%---
1996	<i>O. biennis</i>	P118	6.9 a ^y	1.5 b ^y	21.5 a ^y	63.2 b ^y	6.2 a ^y	0.1 a ^y	10.8 b ^y
	<i>O. rhombipetala</i>	P113	8.1 a	2.0 a	8.5 b	74.1 a	4.0 b	0.1 a	17.3 a
	<i>O. grandis</i>	P7	7.7 a	2.0 a	10.4 b	76.7 a	0.1 c	0.1 a	13.4 b
	<i>O. engelmannii</i>	P95	7.5 a	1.5 b	8.2 b	76.4 a	0.0 c	0.1 a	18.0 a
1997	<i>O. elata</i>	R50	7.2 bcd ^y	1.5 c ^y	7.3 d ^y	67.0 b ^y	10.1 a ^y	0.3 a ^y	14.6 bc ^y
	<i>O. biennis</i>	P118	6.8 cd	1.5 c	17.5 a	60.0 c	8.0 b	0.2 a	12.5 c
	<i>O. rhombipetala</i>	R46	7.6 bc	1.9 b	7.9 cd	69.3 b	6.3 c	0.1 a	16.5 ab
	<i>O. speciosa</i>	R36	9.6 a	2.6 a	12.1 b	66.6 b	2.2 d	0.3 a	19.3 a
	<i>O. grandis</i>	R1	7.3 bcd	1.9 b	9.7 c	70.7 ab	1.8 d	0.0 a	11.1 c
	<i>O. missouriensis</i>	R21	6.7 d	1.5 c	14.1 b	68.1 b	0.0 e	0.3 a	13.6 bc
	<i>O. jamesii</i>	T60	9.6 b ^y	1.5 b ^y	6.8 b ^y	62.5 b ^y	9.1 a ^y	0.3 b ^y	12.8 b ^y
1998	<i>O. elata</i>	R50	11.1 a	2.2 a	7.0 b	52.5 c	8.0 a	0.5 a	7.3 c
	<i>O. rhombipetala</i>	R46	7.1 c	2.1 a	6.9 b	73.9 a	4.4 b	0.2 bc	14.2 b
	<i>O. speciosa</i>	R36	10.5 ab	2.4 a	11.1 a	65.4 b	1.5 c	0.1 c	20.7 a
	<i>O. jamesii</i>	T60	7.2 a ^y	1.6 b ^y	9.1 a ^y	68.6 a ^y	8.9 a ^y	0.1 b ^y	17.3 b ^y
1999	<i>O. elata</i>	R50	7.5 a	1.8 b	7.4 a	63.8 a	8.3 a	0.1 b	18.6 a
	<i>O. rhombipetala</i>	R46	7.6 a	2.2 a	8.5 a	63.5 a	3.6 b	2.3 a	13.5 c

²Composition reported for six major fatty acids: C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; γC18:3, gamma-linolenic acid; αC18:3, alpha-linolenic acid.

³Means within a year and a column not followed by the same letter differ at the 0.05 level by Fisher's Protected Least Significant Difference Test.

warmer conditions (Bewley and Black, 1994). Canvin (1965) reported that oil of sunflower had high linoleic acid and low oleic acid under low temperatures, and oil of flax had decreased linoleic and linolenic acid percentages under higher temperatures. He found that fatty acid composition was not affected by temperature in the oil of castor and safflower. Yaniv and Perl (1987) demonstrated that the sum of linoleic acid and linolenic acid fractions decreased with increasing temperature in *O. biennis* and *O. lamarkiana*. Of all the species evaluated in these trials, the two producing oil containing the highest GLA levels (*O. elata* and *O. jamesii*) bloomed in the fall and had seed maturing in October and November when night temperatures were cooler. This could be an important factor determining which species of *Oenothera* produce oils containing high levels of GLA.

Seed yields in 1996, calculated on a per plant basis, showed *O. biennis* with an average 22.6 g per plant (363 kg·ha⁻¹) and *O. rhombipetala* with 22.9 g per plant (369 kg·ha⁻¹) had significantly higher yields than *O. grandis*, *O. engelmannii*, and *O. missouriensis*, which produced less than 3.0 g per plant (48 kg·ha⁻¹) (Table 3). In 1997, *O. elata* produced an average of 47.6 g of seed per plant (766 kg·ha⁻¹), which was significantly higher than *O. biennis* with 27.5 g per plant (443 kg·ha⁻¹). *Oenothera rhombipetala* seed yields of 9.1 g per plant (147 kg·ha⁻¹) were less than half of 1996 seed yields and were significantly less than *O. biennis*. In 1999, *O. elata* had the highest seed yields of any species in any year with an average of 78.5 g per plant (1264 kg·ha⁻¹). Seed yields of *O. jamesii*, 31.6 g per plant (508 kg·ha⁻¹), were significantly less than *O. elata* but significantly more than *O. rhombipetala*, whose seed yields were less than half of what they had been in 1997. Seed yield data for 1998 was not included because of problems with threshing equipment. All plantings were at 60 cm spacing. In a field study comparing seed yield of three different plant spacings in three species of *Oenothera* (*O. elata*, *O. jamesii*,

Table 3. Seed yield of eight species of cultivated *Oenothera* grown at Lubbock, Tex., in 1996, 1997, and 1999.

Year	Species	No. of accessions	Seed yield per plant	
			Avg	Range
-----g-----				
1996	<i>O. biennis</i>	1	22.6 a ^z	10.2–53.2
	<i>O. rhombipetala</i>	1	22.9 a	16.3–31.4
	<i>O. grandis</i>	3	2.7 b	0.3–4.3
	<i>O. englemannii</i>	4	1.3 b	0.1–2.6
	<i>O. missouriensis</i>	1	0.2 b	0.1–0.3
1997	<i>O. elata</i>	1	47.6 a ^z	26.6–71.3
	<i>O. biennis</i>	1	27.5 b	16.3–35.9
	<i>O. rhombipetala</i>	9	9.1 c	0.6–16.1
	<i>O. missouriensis</i>	4	5.7 cd	1.0–14.9
	<i>O. grandis</i>	3	4.2 cd	2.1–6.4
	<i>O. englemannii</i>	4	3.6 de	1.1–13.0
	<i>O. speciosa</i>	8	1.6 e	0.3–3.8
1999	<i>O. elata</i>	1	78.5 a ^z	51.4–130.2
	<i>O. jamesii</i>	1	31.6 b	15.5–56.3
	<i>O. rhombipetala</i>	1	3.8 c	1.2–7.7

²Means within a year and column not followed by the same letter differ at the 0.05 level by Fisher's Protected Least Significant Difference Test.

and *O. rhombipetala*), Murphy (2000) found that a 30 cm plant spacing gave the highest yield for all species evaluated, but only in *O. jamesii* was the 30 cm spacing significantly higher than 60 cm plant spacing.

Plants of *O. elata*, *O. jamesii*, and *O. rhombipetala* had an upright growth habit with cylindrical seed capsules held erect. As the seed capsules matured, shattering became an issue, but the erect seed capsules prevented excessive seed loss. These characteristics would be beneficial to mechanical harvesting with commercial combines. Plants of *O. grandis* and *O. missouriensis* had a prostrate growth habit, which would be nonconductive to mechanical harvesting. *Oenothera engelmannii* had stout seed capsules that were difficult to thresh. The accessions of *O. grandis*, *O. missouriensis*, *O. engelmannii*, and *O. speciosa* did not produce adequate seed yields to be commercially acceptable for an oilseed crop.

This study identified and evaluated nine

species of *Oenothera* native to Texas for fatty acid composition, oil content, and seed yields. Of these nine species, *O. elata* and *O. jamesii* had high GLA levels, sufficient oil content, and acceptable seed yields to be considered as potential candidates for commercial production. Native species showed little insect damage, whereas the non-native *O. biennis* was susceptible to insect damage. It has been reported that seed yields, oil content, and GLA levels are affected by precipitation and temperature during flowering and seed set (Brandle et al., 1993). Since *O. elata* and *O. jamesii* are found growing in mesic sites along riverbanks, these species may have potential as crops to be grown in Canada and the northeastern U.S., although the late-maturing seed may be limited by the length of the growing season. These two high GLA producing species demonstrated good seed production and showed promise as a potentially valuable crop for the nutritional and pharmaceutical industry.

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