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Fatty Acids in Vernonia Produced in the Mid-Atlantic Region of the United States

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Abstract *Vernonia galamensis* [(Cass.) Less.] is a native of Ethiopia and Eritrea. Seed of vernonia contain substantial quantities of naturally epoxidized oil, which is used in the paint industry to reduce emissions of volatile organic compounds that produce smog resulting from the use of petroleum-based (alkyd-resin) paint. Epoxidized oil is also used in the manufacture of plasticizers, additives to polyvinyl chloride, polymer blends and coatings, and cosmetic and pharmaceutical applications. Previous research has indicated that vernonia has potential for commercialization in the mid-Atlantic region of the United States. This study characterized fatty acids in oil from vernonia grown in this latter region. Vernonia oil, from 14 vernonia lines grown during 1995 and 1996 under field conditions in Virginia, contained 3.3, 3.0, 5.0, 15.0, 0.2, 0.5, 0.4, and 72.7%, respectively, of C16:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, and vernolic (C18:1 epoxy) fatty acids. Effects of genotypes on vernonia oil quality were generally not significant whereas the effects of years were significant. The concentration of vernolic acid was positively correlated with oil concentration but negatively correlated with concentrations of all individual fatty acids, except for C18:3.

Keywords *Vernonia galamensis* · Vernolic acid · Oil content · Fatty acids · Epoxy acids

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Introduction

Vernonia galamensis [(Cass.) Less.], a native of Ethiopia and Eritrea, is receiving increasing attention due to the high concentration of naturally epoxidized oil in its seed [1–3]. Oils rich in epoxy fatty acids are useful in the paint industry to reduce emissions of volatile organic compounds that produce smog as a result of using petroleum-based (alkyd-resin) paints [4]. Epoxy fatty acid oils are also useful in the manufacture of plasticizers, as additives to polyvinyl chloride, in polymer blends and coatings, and in cosmetic and pharmaceutical applications. Vernonia is one of only a few plants containing naturally occurring epoxy oils in its seeds. Currently, the requirement for epoxidized oils is met with petrochemicals or by chemical epoxidation of fats and vegetable oils such as soybean [*Glycine max* (L.) Merr.]. Preliminary work in the United States and elsewhere has indicated that vernonia has potential for commercialization.

Interest in vernonia started when *Vernonia anthelmintica* [(L.) Willd.] was identified by the United States Department of Agriculture in the 1950s as having a high concentration of epoxy oil [5]. Extensive research focused on domestication of *V. anthelmintica* but lack of seed retention and other agronomic limitations resulted in discontinuation of this research [6]. Interest was revived in the 1960s when *V. galamensis* with increased seed retention was identified [3]. *V. galamensis* seed contained substantially more oil and vernolic acid than the best *V. anthelmintica* selections [7]. However, the short-day flowering response limited the usefulness of this species in the United

States. The discovery of a day-neutral germplasm in one accession of *V. galamensis* ssp. *galamensis* var. *petitiana* [8] greatly improved its potential use.

Vernonia galamensis germplasm was evaluated for seed yield, plant height, maturity of seedheads, oil, and vernolic acid concentration in the mid-Atlantic region of the United States during 1994, 1995, and 1996 [1]. However, information regarding other fatty acids in vernonia produced in this region is unknown. Our objective was to characterize the variation in fatty acids in seed oil of vernonia grown in this region.

Materials and Methods

Field experiments were conducted at the Randolph Farm of the Virginia State University, Petersburg, VA (latitude 37°–15′N, longitude 77°–30.8′W). The soil type was an Abell sandy loam (fine-loamy, mixed, semiactive, thermic aquic hapridult). This region is characterized by short days when vernonia starts to flower.

During 1995 and 1996, 12 day-neutral flowering selections from the United States Water Conservation Laboratory (USDA-ARS), Phoenix, AZ, and two selections from Virginia were evaluated under field conditions. It was assumed that, since the lines included in this study were developed under different environments from different populations, the 14 lines would differ genetically. These 14 lines were planted in a randomized complete block design with four replications on 16 May 1995 and 21 May 1996. Each plot consisted of four rows 4.2 m long spaced 75 cm apart. One gram of acid-delinted seed was direct-seeded in each individual row. The middle two rows were harvested by hand for data recording, in the middle of November of each year.

The oil was extracted from 1 g ground mature seed at room temperature by homogenization for 2 min in 10 ml hexane/isopropanol (3:2 v/v) with a Biospec Model 985–370 Tissue Homogenizer (Biospec Products, Racine, WI) and centrifuged at 4,000*g* for 5 min [9]. The oil extraction was repeated for each sample for three times to ensure full oil recovery and the three extractions were combined. The hexane-lipid layer was washed and separated from the combined extract by shaking and centrifugation with 10 ml 1% $CaCl_2$ and 1% NaCl in 50% methanol. The washing procedure was repeated and the purified lipid layer was removed by aspiration and dried over anhydrous Na_2SO_4 [9]. The oil percentage (g/100 g dry basis) was determined gravimetrically after drying under vacuum at 40°C and stored under N at –10°C until analysis.

Fatty acid methyl esters (FAME) were prepared by an acid-catalyzed transestrification method [9, 10]. The oil samples (5 mg) were vortexed with 2 ml sulfuric acid/methanol (1:99 v/v) in 10 ml glass vials containing a Teflon boiling chip. The open vials were placed in a heating block at 90°C until the sample volume was reduced to 0.5 ml. After cooling to room temperature, 1 ml hexane, followed by 1 ml distilled water was added. The mixture was vortexed and the upper hexane layer containing the FAME was taken and dried over anhydrous Na_2SO_4. The hexane phase containing FAME was transferred to a suitable vial and kept under N_2 at 0°C for gas chromatographic analysis.

Analyses of FAME were carried out [11] using a SupelcoWax 10 capillary column (25 m × 0.25 mm i.d. and 0.25 μm film thickness, SupelcoWax, Bellefonte, PA) in a Varian model Vista 6000 GC equipped with a flame ionization detector (FID) (Varian, Sugar Land, TX). An SP-4290 Integrator (Spectra Physics, San Jose, CA) was used to determine relative concentrations of the detected fatty acids.

Peaks were identified by reference to the retention of FAME standards and quantified with the aid of heptadecanoic acid (C17:0) as an internal standard. The concentration of each fatty acid was calculated as a percentage (w/w) of the total fatty acids.

All data were analyzed using the analysis of variance procedures in version 8 of SAS [12]. The means were compared using Fisher's protected least significant difference at a 5% level of significance. Pearson correlation coefficients were calculated to determine the relationships between various pairs of traits.

Results and Discussion

The effect of genotype on the concentration of oil and most fatty acids was not significant (Table 1). Although analysis of variance indicated the genotypic effect on the concentration of C20:1 fatty acid to be significant, the concentration of this fatty acid was very low (0.35% of oil, Table 2) indicating that this effect may be due to random error. However, the growing environment, as indicated by different years, significantly affected the concentrations of oil, and C16:0, C18:0, C18:1, and C20:0 fatty acids (Table 1). The effect of the year on the concentration of vernolic (C18:1 epoxy) acid was also significant (Table 1). The interaction between genotype and year was not significant, indicating that means of main effects i.e., genotypes and years, could be combined across years for genotypes and genotypes for years.

Based on individual plot results, the oil concentration among the 14 genotypes varied from 29.3 to 40.8%, with a mean of 36.9%. The mean values varied from 32.4 to 36.9% with a mean of 35.0% (Table 2). Vernolic acid (C18:1 epoxy) was the predominant fatty acid in our

Table 1 Analysis of variance (ANOVA) for oil and fatty acid concentrations in vernonia grown during 1995 and 1996 at Petersburg, Virginia

Mean squares						
Variable	Genotypes	Years	Genotype* years	Residual	R^2	CV (%)
Oil (%)	9.6	89.8[b]	8.1	6.0	67.3	7.0
Fatty acids[c]						
C16:0	0.1	1.2[b]	0.2[a]	0.1	75.5	9.1
C18:0	0.1	3.4[b]	0.2	0.2	65.9	13.7
C18:1	0.2	6.3[b]	0.4	0.2	77.1	9.1
C18:2	1.8	0.4	2.6	1.3	74.5	7.6
C18:3	0.0	0.0	0.0	0.0	60.8	30.0
C20:0	0.0	0.0[a]	0.0	0.0	53.4	18.9
C20:1	0.0[a]	0.0	0.0	0.0	71.3	14.5
Vernolic[d]	3.5	40.6[b]	9.2	4.7	73.6	3.0

[a, b] Mean squares significantly different from the residual error mean squares at 5 and 1% levels, respectively. In this table, the mean squares are presented as one decimal point whereas the significance is based on two decimal points

[c] Fatty acids as percentage of oil

[d] C18:1 epoxy fatty acid

Table 2 Concentrations of oil and fatty acids, averaged over 2 years and two replications per year, in 14 vernonia genotypes grown during 1995 and 1996 at Petersburg, Virginia

Genotype	Oil	C16:0[a]	C18:0[a]	C18:1[a]	C18:2[a]	C18:3[a]	C20:0[a]	C20:1[a]	Vernolic[a,b]
AZ95-1	36.9	3.3	3.1	4.7	14.2	0.1	0.5	0.3	73.8
AZ95-2	35.7	3.4	3.0	4.8	15.0	0.1	0.6	0.4	72.6
AZ95-3	34.2	3.3	3.2	4.9	15.1	0.1	0.6	0.4	72.4
AZ95-4	36.2	3.4	3.2	5.1	15.4	0.1	0.5	0.4	71.8
AZ95-5	35.4	3.2	2.8	4.8	14.0	0.1	0.5	0.4	74.3
AZ95-6	35.8	3.5	3.0	5.1	14.4	0.1	0.5	0.3	72.9
AZ95-7	35.9	3.6	3.1	5.1	16.5	0.2	0.6	0.4	70.5
AZ95-8	36.7	3.0	3.0	4.7	15.0	0.2	0.5	0.3	73.2
AZ95-9	32.9	3.6	2.9	5.3	15.0	0.2	0.6	0.4	72.1
AZ95-10	33.5	3.4	3.1	5.2	14.3	0.2	0.5	0.3	72.8
AZ95-11	32.4	3.4	2.8	4.7	14.7	0.2	0.5	0.3	73.3
AZ95-12	32.7	3.1	3.0	5.0	14.5	0.2	0.5	0.3	73.4
VX95-1	36.2	3.2	2.8	5.1	15.8	0.1	0.5	0.3	72.2
VX95-2	34.7	3.2	2.9	4.8	15.1	0.1	0.5	0.3	73.0
Mean	35.0	3.3	3.0	5.0	14.9	0.1	0.5	0.3	72.7
LSD (5%)	NS	NS	NS	NS	NS	NS	NS	0.1	NS

[a] Fatty acids as percentage of oil

[b] C18:1 epoxy fatty acid

material, comprising 73% of total fatty acids, followed by linoleic acid (15% of total fatty acids). The remaining fatty acids comprised less than 12% of total fatty acids. Vernonia fatty acids can be arranged in the following descending order: vernolic > linoleic > olieic > palmitic > stearic > arachidic > eicosenoic > linolenic.

The vernolic acid content in our studies varied from 71 to 74% as compared to the concentration of 54–74% reported by Baye et al. [13] from 41 accessions. The range of 71–74% in our studies is based on an average over 2 years and two replications. If plot values are used, then the range in our studies was from 65 to 78%, which is more similar to that reported by Baye et al. [13]. Another report [14] indicated that the mean vernolic acid content from 480 accessions differing in maturity, plant height, flower color, and branching pattern varied from 34 to 87%, with a mean of 74%. The lower values in this latter report could result from the use of immature seeds in the analysis. Moreover, we limited our studies to day-neutral flowering lines, which may have resulted in lower variation. These observations indicate that a potential exists for increasing the vernolic acid in vernonia produced in Virginia and the mid-Atlantic region of the United States.

In our study, the mean contents of fatty acids in vernonia seed oil are different from those obtained from greenhouse-grown accessions [13] and 114 accessions collected from Ethiopia and grown in the field in Ethiopia [2]. In all cases, the minimum values of fatty acid concentrations were of higher magnitude, whereas the maximum concentration values were of lower magnitude in seed produced in Virginia compared to previously characterized accessions. These differences may be attributable to environmental and

genotypic differences between the Virginia- and Ethiopian-grown accessions. These studies establish upper and lower values for the contents of various fatty acids in vernonia seed oil as of now but evaluations with wider germplasm over more environments may produce different limits.

The growing environment, as reflected by different years, significantly affected vernonia oil (Table 3). The contents of C16:0, C18:0, C18:1, and C20:0 fatty acids were significantly higher, whereas the concentrations of oil and vernolic acid were significant lower, in seed produced in 1995 as compared to that produced in 1996. We speculate that these differences might be due to differing environments during 1995 and 1996. The average monthly temperature (°C) from May to November during 1995 and 1996 was 21.7 and 20.7, respectively. However, average monthly precipitation (cm) from May to November during 1995 and 1996 was 10.5 and 12.7, respectively, the 1995 growing season being drier than that in 1996. The observation that yields of oil and vernolic acid were higher in 1996 was in contrast to our expectations. We had expected that vernonia, having originated in the drier climate of eastern Africa, might be more acclimatized to drier years and produce more oil and vernolic acid in the drier year of 1995. Apparently, a better understanding of vernonia stress responses is required.

Our studies were not replicated over locations; therefore, the data are not suitable for a study of genotype × environment interactions for fatty acids. This issue of genotype × environment (genotype × location, genotype × year, and genotype × year × location) needs to be evaluated further with a larger germplasm replicated over time and space to identify either optimal location and or a genotype with stable performance over environments.

The vernonia oil in our studies contained 5.5–9.4% saturated fatty acids, which indicates a potential for its use in manufacture of biodiesel. There is increasing interest in the manufacture of biodiesel from vegetable oils. Canola is currently the most promising vegetable oil for biodiesel production because of its low saturated fat concentration (varying from 6.4 to 7.5% [15]), which reduces the gel point, thereby reducing cold flow issues. The saturated fatty acid content in the vernonia oil used in our studies was comparable to that in canola. Therefore, although vernonia has specialized uses and may be more profitable if used for its naturally epoxidized nature, the potential still exists for its use in the manufacture of biodiesel.

Significant positive and negative correlations exist between the concentrations of oil and various fatty acids, and between fatty acids (Table 4). The concentration of vernolic acid, the desired fatty acid, was positively correlated with oil content but negatively correlated with contents of all fatty acids, except that of C18:3. The positive correlation ($r = 0.34$) between oil and vernolic acid concentrations suggests the possibility of improving both these traits simultaneously. However, this conclusion is constrained by lack of variation among 14 vernonia lines, indicating a lack

Table 3 Effect of production year on concentrations of oil and fatty acids in 14 vernonia genotypes grown at Petersburg, Virginia

Year	Oil	C16:0[a]	C18:0[a]	C18:1[a]	C18:2[a]	C18:3[a]	C20:0[a]	C20:1[a]	Vernolic[a, b]
1995	33.74	3.47	3.27	5.30	15.03	0.14	0.56	0.36	71.87
1996	36.19	3.19	2.76	4.63	14.87	0.15	0.50	0.34	73.56
LSD (5%)	1.36	0.17	0.23	0.25	NS	NS	0.06	NS	1.21

[a] Fatty acids as percentage of oil

[b] C18:1 epoxy fatty acid

Table 4 Correlation coefficients between various oil traits among 14 vernonia genotypes grown during 1995 and 1996 at Petersburg, Virginia

	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	Vernolic[a]
Oil	–0.37**	–0.31*	–0.46**	0.23	–0.31*	0.12	0.04	0.34*
C16:0		0.78**	0.83**	0.70**	0.19	0.74**	0.50**	–0.87**
C18:0			0.85**	0.64**	0.04	0.69**	0.58**	–0.85**
C18:1				0.71**	0.14	0.61**	0.49**	–0.89**
C18:2					0.15	0.54**	0.51**	–0.93**
C18:3						0.02	0.07	0.16
C20:0							0.70**	–0.69**
C20:1								–0.60**

*, ** Correlation coefficient significantly different from zero at 5 and 1% probability, respectively

[a] C18:1 epoxy fatty acid

of genetic effects and the predominance of environmental effects.

The lack of variation between various fatty acids among the 14 lines evaluated in our studies was interesting. We speculate that selection based on adaptability in North America has, perhaps, reduced the genetic diversity of *V. galamensis*. This issue is of critical importance for future production and utilization of *V. galamensis* as a natural source of epoxidized oil and may indicate a need for expansion of genetic diversity in vernonia. However, an evaluation of fatty acids in a larger germplasm collection, under field conditions conducted in North America over several years, would be needed to address this issue. Evaluations over years would be needed given the significant effect of year of growth on the fatty acid content. In addition, breeding of vernonia for development of day-neutral or short-day genotypes would also be needed.

References

1. Bhardwaj HL, Hamama AA, Rangappa M, Dierig DA (2000) Vernonia oilseed production in the mid-Atlantic region of the United States. Ind Crop Prod 12:119–124
2. Baye T, Becker HC (2004) Analyzing seed weight, fatty acid composition, oil, and protein contents in *Vernonia galamensis* germplasm by near-infrared reflectance spectroscopy. J Am Oil Chem Soc 81:641–645
3. Perdue RE Jr, Jones E, Nyati CT (1987) *Vernonia galamensis*: a promising new industrial crop for the semi-arid tropics and sub-tropics. In: Wickens GE, Haq N, Day P (eds) New crops for food and industry. Chapman & Hall, New York, pp 197–207
4. Anonymous (1989) Vernonia, new industrial oil crop. Agric Res 37:10–11
5. Gunstone FD (1954) Fatty acids, part II: the nature of oxygenated acids present in *Vernonia anthelmintica* (Willd.) seed oil. J Chem Soc Lond:1611–1616
6. Massey JH (1971) Harvesting *Vernonia anthelmintica* [(L.) Willd.] to reduce seed shattering losses. Agron J 63:812
7. Thompson AE, Dierig DA, Kleiman R (1994a) Variation in *Vernonia galamensis* flowering characteristics, seed oil and vernolic acid contents. Ind Crop Prod 3:175–183
8. Thompson AE, Dierig DA, Johnson ER, Dahlquist GH, Kleiman R (1994b) Germplasm development of *Vernonia galamensis* as a new industrial oilseed crop. Ind Crop Prod 3:185–200
9. Bhardwaj HL, Hamama AA (2000) Oil, erucic acid, and glucosinolate contents in winter hardy rapeseed germplasms. Ind Crop Prod 12:33–38
10. Dahmer ML, Fleming PD, Collins GB, Hildebrand DF (1989) A rapid screening technique or determining the lipid composition of soybean seeds. J Am Oil Chem Soc 66:543–549
11. Bhardwaj HL, Hamama AA (2003) Accumulation of glucosinolate, oil, and erucic acid contents in developing *Brassica* seeds. Ind Crop Prod 17:47–51
12. SAS (1999) SAS system for Windows, version 8. SAS Institute, Cary, NC
13. Baye T, Becker HC, Witzke-Ehbrecht W (2005) *Vernonia galamensis*, a natural source of epoxy oil: variation in fatty acid composition of seed and leaf lipids. Ind Crop Prod 21:257–261
14. Baye T, Becker HC (2005) Exploration of *Vernonia galamensis* in Ethiopia and variation in fatty acid composition of seed oil. Genet Resour Crop Evol 52:805–811
15. Hamama AA, Bhardwaj HL, Starner DE (2003) Genotype and growing location effects on phytosterols in canola oil. J Am Oil Chem Soc 80:1121–1126