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Characterization of *Vernonia galamensis* germplasm for seed oil content, fatty acid composition, seed weight, and chromosome number

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Abstract

Vernonia galamensis (Cass.) Less. has potential for domestication as a new industrial oilseed source of natural epoxy fatty acids. Thirty-four accessions from the six subspecies of the *Vernonia galamensis* complex were characterized for seed weight, seed oil content, and fatty acid composition. The mean seed weight of the six subspecies was 3.42 g/1000 seeds, with a range of 2.46 g to 5.43 g for ssp. *mutomensis* and ssp. *afromontana*, respectively. The mean seed oil content for the six subspecies was 30.8%, ranging from 25.1% to 39.2% for ssp. *mutomoensis* and ssp. *lushotoensis*, respectively. The 18:1 epoxy fatty acid (vernolic acid) was predominant. The mean vernolic acid content for the six subspecies was 72.5%, and ranged from 66.9% to 76.6% for ssp. *mutomoensis* and ssp. *lushotoensis*, respectively. Within the four varieties of ssp. *galamensis*, in which most of the current domestication effort is centered, oil contents ranged from 31.8% to 38.4%, and 18:1 epoxy fatty acid ranged from 68.0% to 77.0%. Mean levels of other fatty acids within the species were about 14% for linoleic acid (18:2), 7% for oleic acid (18:1), and from 2 to 3% for both palmitic acid (16:0) and stearic acid (18:0). The basic chromosome number for *Vernonia galamensis* was found to be $n = 9$. It was concluded that selection for improved levels of both oil and vernolic acid contents should be possible within a germplasm enhancement and plant breeding program.

Keywords: Epoxy fatty acid; Natural epoxidized oil; Industrial oilseed; Domestication; New crops; Commercialization

1. Introduction

Currently, no oilseed crop has been commercialized as a source of natural epoxidized oils. Epoxy fatty acids are useful raw materials for manufacturing paints and coatings with low or no volatile organic compounds (VOC), thermoset resins and coatings, polymer blends, dibasic acids, adhesives, and epoxy composite materials. Vernolic acid (*cis*-12,13-epoxy-*cis*-octadecenoic acid), an epoxidized fatty acid, was first discovered by Gunstone (1954) in the seed oil of *Vernonia anthelmintica*, a plant native to India and Pakistan. This species was also identified in the USDA-Agricultural Research Service (ARS), National Center for Agricultural Utilization Research (NCAUR) in its plant chemical screening program. The oil and vernolic acid (18:1 epoxy) contents were initially characterized over 30 years ago (Smith et al., 1959; Earle et al., 1960). Sub-

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stantial agronomic and utilization research studies conducted in the 1960's on this species were reviewed by Perdue et al. (1986).

The discovery of *Vernonia galamensis* ssp. *galamensis* var. *ethiopica* M. Gilbert (Perdue et al., 1986; Perdue, 1988) led to revived interest in the domestication of vernonia germplasm. Earle (1970) first reported that seeds of var. *ethiopica* (formerly *V. pauciflora* (Willd.) Less.) contained about 42% oil of which 73% was vernolic acid, and was higher than the best selections developed from *V. anthelmintica*. Extensive plant exploration efforts were undertaken in Africa to identify and collect potentially useful germplasm of various *Vernonia* species. Several accessions of the *Vernonia galamensis* complex were collected, and are now maintained in the USDA-ARS Working Vernonia Germplasm Collection at the U.S. Water Conservation Laboratory (USWCL), Phoenix, Arizona, and in the North Central Regional Plant Introduction Station, Ames, Iowa.

In 1989, USDA-ARS initiated a major research effort at the USWCL to evaluate the potential for commercialization of *Vernonia galamensis* as a new industrial oilseed crop. Research studies on germplasm evaluation, photoperiodicity, identification of day-neutral germplasm in *V. galamensis* ssp. *galamensis* var. *petitiana*, and initial breeding efforts have been reported (Kaplan, 1989; Thompson et al., 1992; Carlson et al., 1992; Dierig and Thompson, 1993). Increasing levels of research activities are in progress to characterize the chemical properties and utilize *V galamensis* seed oil and vernolic acid in industrial applications (Carlson et al., 1981; Carlson and Chang, 1985; Ayorinde et al., 1988, 1989, 1990a, b, 1993; Afolabi et al., 1989; Dirlikov et al., 1990, 1991; Ologunde et al., 1990).

A taxonomic description of the *Vernonia galamensis* complex was made by Gilbert (1986). This information was utilized to define potential differences in the morphological, ecological, and geographic adaptations of the germplasm pool (Perdue et al., 1986; Perdue, 1988).

The objective of this paper was to characterize all available accessions of *Vernonia galamensis* in regard to seed oil content, fatty acid composition, seed weights, and chromosome numbers. This information will aid in the domestication and development of improved germplasm necessary for the ultimate commercialization of *Vernonia* as a new industrial oilseed crop.

2. Materials and methods

Thirty-four accessions of the *Vernonia galamensis* complex have been assembled in the working germplasm collection at the USWCL, Phoenix, Arizona, and were evaluated in this study. Most of these accessions were collected through the efforts of Dr. Robert E. Perdue, Jr. The collection has been previously characterized as to subspecies, variety, and country of origin (Table 1). All accessions are identified as to PI or North Central Regional Plant Introduction Station (Ames, Iowa) numbers. For additional reference, most of the accessions are identified by Dr. Perdue's V (Vernonia) number.

To date, 21 accessions of *V. galamensis* have been cytologically examined for chromosome number at the USWCL (Table 1). Flower buds were collected from greenhouse-grown plants for meiotic analysis. Buds were placed in Farmer's solution (3 parts ethanol, 1 part glacial acetic acid) for one hour, and then transferred and stored in 70% ethanol. Young anthers were extracted from the buds, and chromosome smears were prepared using the standard acetocarmine squash technique.

Sufficient seeds from the original collections of 30 accessions were available for chemical analysis. Most of the accessions were grown in Arizona in 1989 for evaluation and seed increase. Enough seeds were produced for the four accessions that lacked sufficient original seeds for chemical analysis. Furthermore, seed production for six additional accessions was sufficient to compare the chemical composition of the original and 1989 produced seeds. Since the variations in oil and fatty acid composition among the two samples were minimal, the means of the two samples were reported herein for the six accessions. Seed weight determinations (g/1000 seeds) were made on all analyzed samples.

Oil content and fatty acid composition were determined at the NCAUR, Peoria, Illinois. Seed oil contents were measured using a Bruker PC120

Table 1
Identification, origin, and chromosome numbers of accessions of the *Vernonia galamensis* (Cass.) Less. complex in the USDA germplasm collection

Subspecies	Variety	Accession numbers [a]		Origin	Chromosome number (n)
		PI or Ames	V		
1. ssp. *galamensis*	(a) var. *galamensis*	PI 508535	V 003	Ghana	9
		A 20276	V 004	Nigeria	9
		A 20277	V 034	Nigeria	–
		–	V 036	Ghana	9
	(b) var. *petitiana*	A 20294	V 027	Kenya	–
		A 20295	V 029	Kenya	9
		A 20296	V 032	Kenya	9
		A 20297	V 014	Tanzania	9
		A 20298	V 015	Tanzania	9
	(c) var. *australis*	PI 503141	V 009	Malawi	9
		PI 503142	V 010	Malawi	–
		–	V 008	Malawi	9
	(d) var. *ethiopica*	PI 312852	V 001	Ethiopia	9
	(e) var. undetermined	A 20278	V 013	Tanzania	9
2. ssp. *nairobensis*		PI 321654	–	Kenya	–
		A 20288	V 026	Kenya	9
		A 20289	V 028	Kenya	9
		A 20290	V 031	Kenya	–
		A 20291	V 033	Kenya	9
		A 20292	V 011	Kenya	–
		A 20293	V 012	Kenya	9
3. ssp. *lushotoensis*		A 20284	V 017	Tanzania	–
		A 20285	V 016	Tanzania	–
4. ssp. *mutomoensis*		A 20286	V 018	Kenya	9
		A 20287	V 025	Kenya	9
		–	V 019	Kenya	9
5. ssp. *afromontana*		PI 321664	–	Uganda	–
		PI 321684	–	Kenya	–
		PI 500003	V 002	Kenya	–
		A 20279	V 020	Kenya	9
		A 20280	V 030	Kenya	9
6. ssp. *gibbosa*		A 20281	V 024	Kenya	–
		A 20282	V 022	Kenya	–
		A 20283	V 021	Kenya	9

[a] PI = USDA Plant Inventory number; A = North Central Regional Plant Introduction Station, Ames, Iowa, accession number; V = Dr. Robert E. Perdue's Vernonia accession number.

Pulsed NMR analyzer. Fatty acid compositions were determined by trans-esterification of the oil and GC analysis of the resulting esters. The method consisted of first placing 25 seeds in a 1.5-dram screw-capped glass vial, wetted with hexane, then shattering them with a stainless steel rod. Approximately 1 ml of 1.5% sodium in methanol was added to the shattered seeds, the vial capped, and shaken vigorously. After standing for a minimum of 10 min, about 0.5 ml of 1% NaCl solution was added. 1 ml of hexane was then added and the contents shaken vigorously. After allowing the layers to separate, the hexane layer was withdrawn from the top and transferred into a separate, clean vial. Hexane extraction was done three times. The combined hexane extracts were

Table 2
Seed weight, seed oil content, and fatty acid composition of *Vernonia galamensis* ssp. *galamensis* accessions

Variety	Accession number	Seed weight (g/1000)	Oil (%)	Fatty acid composition (%)				
				16:0	18:0	18:1	18:2	18:1 epoxy
galamensis	PI 508535	2.20	31.1	3.0	3.4	5.7	13.0	74.4
	A 20276	2.46	28.6	3.2	4.2	7.8	16.4	67.6
	A 20277	2.90	33.7	2.9	3.8	6.1	13.8	72.9
	V 036	2.11	33.9	3.1	3.6	9.2	18.4	65.1
	Mean ($\bar{x}$)	2.42	31.8	3.0	3.8	7.2	15.4	70.0
petitiana	A 20294	2.22	32.4	2.6	3.5	7.5	16.7	68.6
	A 20295	2.28	34.9	4.3	4.4	9.4	21.4	59.9
	A 20296	1.74	31.4	3.4	4.4	9.6	19.4	62.6
	A 20297	2.07	38.3	2.3	3.0	5.7	13.9	74.2
	A 20298	2.82	36.8	2.4	3.0	6.0	13.0	74.5
	Mean ($\bar{x}$)	2.23	34.8	3.0	3.7	7.6	16.9	68.0
australis	PI 503141	2.79	29.1	1.9	3.6	8.2	14.1	71.2
	PI 503142	3.50	38.2	2.6	2.7	4.2	13.2	77.0
	V 008	3.55	33.7	2.1	3.7	6.6	13.1	73.4
	Mean ($\bar{x}$)	3.28	33.7	2.4	3.3	6.3	13.5	73.9
ethiopica	PI 312852	3.33	38.4	2.5	2.2	3.6	14.4	77.0
Undetermined	A 20278	3.37	33.2	2.4	3.1	6.6	12.1	75.3
Subspecies total ($\bar{x}$)		2.93	34.4	2.7	3.2	6.3	14.5	72.8

used for fatty acid analysis by GC. A 25-m 25QC3/BPX70–0.25 column (SGE, Victoria, Australia [1]), in a Hewlett-Packard 5890 [1] gas chromatograph, programmed from 125°C to 245°C at 3°C/min was used for analysis.

3. Results and discussion

In all instances, the chromosome numbers of all accessions, regardless of subspecies or varieties within the *V. galamensis* complex were $n = 9$ (Table 1). Additionally, cytological examination of the F_1's of the five intraspecific hybrids within the complex had $n = 9$ chromosomes with no observable meiotic abnormalities. It is reasonable to conclude that nine is the basic chromosome number for this species.

Seed weights, seed oil contents, and fatty acid compositions for the fourteen accessions of the four varieties of ssp. *galamensis*, and the twenty accessions of the other five subspecies are summarized in Tables 2 and 3, respectively. Considerable variation in seed weight exists within the species (Tables 2 and 3). Even though environment undoubtedly modifies seed weight, the data strongly suggest that genetic variation exists among the various subspecies and varieties. The overall mean for the six subspecies was 3.42 g/1000, with a range from 2.46 g to 5.43 g for ssp. *mutomoensis* and ssp. *afromontana*, respectively (Table 3). The smallest seed size, 2.23 g/1000, was exhibited by ssp. *galamensis* var. *petitiana* (Table 2). Accession 20295 (V 029) of this variety with a 1000-seed weight of 2.28 g, essentially the same as the varietal mean, is being extensively utilized as a day-neutral flowering parental line in our current germplasm enhancement effort.

Environmental factors also undoubtedly play an important role in the biosynthesis of both oil and fatty acid contents. However, it is likely that significant genetic variation exists for both oil content and the fatty acid composition. The overall mean

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

Table 3
Seed weight, seed oil content, and fatty acid composition of five *Vernonia galamensis* subspecies

Subspecies	Accession number	Seed weight (g/1000)	Oil (%)	Fatty acid composition (%)				
				16:0	18:0	18:1	18:2	18:1 epoxy
nairobensis	PI 321654	3.26	29.4	2.0	4.1	10.3	14.4	68.6
	A 20288	3.12	26.2	2.6	3.4	7.6	15.4	70.4
	A 20289	3.42	33.0	2.1	3.5	6.9	13.1	74.4
	A 20290	2.75	28.2	2.3	3.0	5.5	15.6	72.6
	A 20291	2.96	31.7	2.3	2.9	4.5	14.1	76.1
	A 20292	3.92	35.4	2.2	2.6	5.5	11.9	77.7
	A 20293	3.47	31.3	2.1	3.7	7.5	14.7	71.0
	Mean ($\bar{x}$)	3.25	30.4	2.2	3.3	6.8	14.2	73.0
lushotoensis	A 20284	2.82	38.6	2.2	3.2	5.6	12.1	76.6
	A 20285	3.09	39.7	2.2	3.0	6.0	11.7	76.6
	Mean ($\bar{x}$)	2.96	39.2	2.2	3.1	5.8	11.9	76.6
mutomoensis	A 20286	2.76	28.3	2.6	4.0	8.7	15.1	69.0
	A 29287	2.83	26.5	2.6	4.2	8.6	17.2	66.5
	V 019	1.80	20.4	2.5	2.0	9.7	18.9	65.2
	Mean ($\bar{x}$)	2.46	25.1	2.6	3.4	9.0	17.1	66.9
afromontana	PI 321664	4.76	26.1	2.7	2.7	3.9	14.5	75.7
	PI 321684	4.04	17.5	3.6	3.6	9.7	23.9	57.5
	PI 500003	4.59	32.2	2.7	3.9	9.9	14.2	68.2
	A 20279	5.20	36.1	1.9	2.4	5.1	10.8	79.3
	A 20280	8.57	35.0	2.0	2.6	4.7	13.5	76.8
	Mean ($\bar{x}$)	5.43	29.4	2.6	3.0	6.7	15.4	71.5
gibbosa	A 20281	3.07	28.4	2.9	3.0	4.6	13.9	74.7
	A 20282	3.20	22.7	2.7	3.4	4.6	15.0	73.3
	A 20283	4.22	29.6	2.5	3.4	5.8	12.0	75.1
	Mean ($\bar{x}$)	3.50	26.4	2.7	3.3	5.0	13.6	74.4
Mean ($\bar{x}$)[a]		3.42	30.8	2.5	3.2	6.6	14.4	72.5

[a] Mean of six subspecies, including ssp. *galamensis*.

oil content of the six subspecies was 30.8%, with a range of 25.1% to 39.2% for ssp. *mutomoensis* and ssp. *lushotoensis*, respectively (Tables 2 and 3). The mean oil content of ssp. *galamensis*, within which most of our current domestication efforts are centered, was relatively high at 34.4% (Table 2). Oil contents within the four varieties of ssp. *galamensis* appears to vary significantly, ranging from 31.8% to 38.4%.

The 18:1 epoxy fatty acid, vernolic acid, is clearly the predominant constituent of vernonia seed oil with the mean of the six subspecies being 72.5% (Tables 2 and 3). Again there appears to be significant genetic variation, with the means for the six subspecies ranging from 66.9% to 76.6% for ssp. *mutomoensis* and ssp. *lushotoensis*, respectively. Variation is also observed among the varieties within ssp. *galamensis*, which ranged from 68.0% to 77.0% (Table 2).

Linoleic acid (18:2) is the second most prevalent fatty acid in *V. galamensis* seed oil at about 14% (Tables 2 and 3). Oleic acid (18:1) makes up about 7% of the content, with palmitic (16:0) and stearic (18:0) acids each contributing from 2 to 3%. Variation within these minor fatty acid constituents among the various accessions of the species most likely has a genetic basis.

These data indicate that selection for improved

levels of both oil and vernolic acid contents should be possible within a germplasm enhancement and plant breeding program. The current status and objectives of the USDA-ARS germplasm development program were recently summarized by Dierig and Thompson (1993). The development effort is primarily based upon inter-varietal and inter-subspecific hybridization utilizing A 20295 (V 029) of var. *petitiana*, which is day-neutral in flowering and self incompatible, as the female parent. Completely fertile hybrids were made utilizing four varieties of ssp. *galamensis* [PI 312852 (V 001), var. *ethiopica*; PI 508535 (V 003) and A 20276 (V 004), var. *galamensis*; and A 20278 (V 013), an unclassified accession that appears to be a distinct variety of the subspecies] and one accession [A 20286 (V 018)] of ssp. *mutomoensis* as the male parents.

4. Conclusions

The basic chromosome number of the *Vernonia galamensis* complex has been determined to be $n = 9$. Relatively large variation was measured for seed weight, seed oil content, and vernolic acid composition among the 34 accessions of *V. galamensis* within the USDA-ARS working germplasm collection. There appears to be as much variation within as between subspecies for these characters. It is highly unlikely that this collection encompasses the full range of genetic variability to be found within the species. However, the variability appears to be adequate to support an intensive germplasm enhancement and plant breeding program. It is concluded that these data will provide a useful base for planned genetic improvement as well as for future plant exploration and collection efforts.

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References

Afolabi, O.A., Aluko, M.E., Wang, G.C., Anderson, W.A. and Ayorinde, F.O., 1989. Synthesis of a toughened elastomer from *Vernonia galamensis* seed oil. J. Am. Oil Chem. Soc., 66: 983–985.

Ayorinde, F.O., Osman, G., Shepard, R.L. and Powers, F.T., 1988. Synthesis of azelaic acid and suberic acid from *Vernonia galamensis* oil. J. Am. Oil Chem Soc., 65: 1774–1777.

Ayorinde, F.O., Powers, F.T., Streete, L.D., Shepard, R.L. and Tabir, D.N., 1989. Synthesis of dodecanedioic acid from *Vernonia galamensis* oil. J. Am. Oil Chem Soc., 66: 690–692.

Ayorinde, F.O., Butler, B.D. and Clayton, M.T., 1990a. *Vernonia galamensis*: a rich source of epoxy acid. J. Am. Oil Chem. Soc., 67: 844–845.

Ayorinde, F.O., Carlson, K.D., Pavlik, R.P. and McVety, J., 1990b. Pilot plant extraction of oil from *Vernonia galamensis* seed. J. Am. Oil Chem. Soc., 67: 512–518.

Ayorinde, F.O., Nwaonicha, C.P., Parchment, V.N., Bryant, K.A., Hassan, M. and Clayton, M.T., 1993. Enzymatic synthesis and spectroscopic characterization of 1,3-divernoloyl glycerol from *Vernonia galamensis* seed oil. J. Am. Oil Chem. Soc., 70: 129–132.

Carlson, K.D. and Chang, S.P., 1985. Chemical epoxidation of a natural unsaturated epoxy seed oil from *Vernonia galamensis* and a look at epoxy oil markets. J. Am. Oil Chem. Soc., 62: 934–939.

Carlson, K.D., Schneider, W.J., Chang, S.P. and Princen, L.H., 1981. *Vernonia galamensis* seed oil: a new source for epoxy coatings. Am. Oil Chem. Soc. Monogr., 9: 297–318.

Carlson, K.D., Knapp, S.A., Thompson, A.E., Brown, J.H. and Jolliff, G.D., 1992. Nature's abundant variety: new oilseed crops on the horizon. In: New Crops, New Uses, New Markets, 1992 Yearbook of Agriculture. USDA, Washington, D.C., pp. 124–133.

Dierig, D.A. and Thompson, A.E., 1993. Vernonia and lesquerella potential for commercialization. In: J. Janick and J.E. Simon (Editors), New Crops. John Wiley, New York, N.Y., pp. 362–367.

Dirlikov, S.K., Frischinger, I., Islam, M.S. and Graham, J., 1990. Vernonia oil: a new reactive monomer. Polymer Mater. Sci. Eng., 62: 217–221.

Dirlikov, S., Islam, M.S., Frischinger, I., Lepkowski, T. and Muturi, P., 1991. Vernonia oil: a new reactive diluent for high-solids alkyd and epoxy resins and coatings. Proc. Water-Borne, Higher-Solids, Powder Coatings Symp., 18: 129–144.

Earle, F.R., 1970. Epoxy oils from plant seeds. J. Am. Oil Chem. Soc., 47: 510–513.

Earle, F.R., Wolff, I.A. and Jones, Q., 1960. Search for new industrial oils, III. Oils from Compositae. J. Am. Oil Chem. Soc., 37: 254–256.

Gilbert, M.G., 1986. Notes on East African *Vernonieae* (*Compositae*). A revision of the *Vernonia galamensis* complex. Kew Bull., 41: 19–35.

Gunstone, F.D., 1954. Fatty acids, Part II. The nature of the oxygenated acids present in *Vernonia anthelmintica* (Willd.) seed oil. J. Chem. Soc. (London), May: 1611–1616.

Kaplan, K., 1989. Vernonia, new industrial oil crop. Agric. Res., 37(4): 10–11.

Ologunde, M.O., Ayorinde, F.O. and Shepard, R.L., 1990. Chemical evaluation of defatted *Vernonia galamensis* meal. J. Am. Oil Chem. Soc., 67: 92–95.

Perdue, R.E., Jr., 1988. Systematic botany in the development of *Vernonia galamensis* as a new industrial oilseed crop for the semi-arid tropics and subtropics. Symb. Bot. Ups., 28(3): 125–135.

Perdue, R.E., Jr., Carlson, K.D. and Gilbert, M.G., 1986. *Vernonia galamensis*, a potential new crop source of epoxy fatty acid. Econ. Bot., 40: 54–68.

Smith, C.R., Jr., Koch, K.F. and Wolff, I.A., 1959. Isolation of vernolic acid from *Vernonia anthelmintica* oil. J. Am. Oil Chem. Soc., 36: 219–220.

Thompson, A.E., Dierig, D.A. and White, G.A., 1992. Use of plant introductions to develop new industrial crop cultivars. In: H.L. Shands and L.E. Weisner (Editors), Use of Plant Introductions in Cultivar Development, Part 2. Crop Sci. Soc. Am. Spec. Publ. No. 20, Madison, Wisc., pp. 9–48.