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Oil and Squalene in *Amaranthus* Grain and Leaf

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Amaranthus grain of 104 genotypes from 30 species was investigated for oil and squalene contents and fatty acid profiles. The overall average oil content in *Amaranthus* grain was 5.0%, ranging from 1.9 to 8.7%. Squalene concentration in extracted oils ranged from trace to 7.3%, with an average concentration of 4.2%. The average contents of three major fatty acids in *Amaranthus* grain were 22.2, 29.1, and 44.6% for palmitic, oleic, and linoleic, respectively. The average fat content in dried mature leaves of 45 *Amaranthus* genotypes was 1.63%, ranging from 1.08 to 2.18%. The squalene concentration in leaf lipid extracts averaged 0.26%, ranging from trace to 0.77%, which is much lower than that from seeds. The major fatty acids of leaf extracts were linolenic, linoleic, and palmitic. Linolenic ranged from 56.5 to 62.0% of total fatty acids; linoleic, from 15.5 to 24.7%; and palmitic acid, from 13.5 to 15.5%. As for the fatty acid compositions at different growth stages, fatty acid content in leaf lipid was lower in mature leaves than in young leaves. The saturated/unsaturated ratio decreased when the leaf grew to maturity. Principal component analysis (PCA) was carried out on compositional characteristics of grain. The first two components accounted for 70% of the total variance (38.3 and 21.7%, respectively). There was a positive correlation between oil content and squalene yield, and a negative correlations were found between linoleic and either of the other two major fatty acids, palmitic and oleic. The taxonomic relationship among the species was also elucidated by PCA.

KEYWORDS: Amaranthus grain; leaves; squalene; oil contents; fatty acid; principal component analysis; taxonomy

INTRODUCTION

Squalene is a type of unsaponifiable lipid and acts as a biosynthetic precursor to all steroids in plants and animals. This compound is now widely used as an important ingredient in skin cosmetics and as a lubricant for precision instruments, such as computer disks (*1*, *2*). Squalene has also been reported to have important beneficial effects on health, such as decreasing the risk for various cancers (*3*) and reducing serum cholesterol levels (*4*, *5*). Application of squalene in nutraceutical and pharmaceutical fields has been increasing progressively, so the demand for this substance is expected to increase continuously. The traditional source for squalene is primarily from shark (*Centrophorus squamosus*) and whale (*Physeter macrocephalus*) liver oil (*6*−*8*), which contain ∼30−45% of squalene. Use of squalene in cosmetic applications is limited by the uncertainty of its availability as a result of international concern for the protection of marine animals. In addition, the presence of similar compounds, such as cholesterol, which are not recommended for human consumption (*9*), in the oils from marine animal liver can make squalene purification difficult.

Plant resources were considered as another potential source of squalene and have been widely prospected. Squalene contents in some commercially important oils, such as olive, rice bran, corn, peanut, rapeseed, sunflower, and cottonseed oils, are in the 0.01−0.4% range (*10*), which is not high enough for them to be considered as viable resources. It was reported that squalene was present up to 0.46% of the leaf dry weight of Macaronesian *Echium* plants (*9*). In addition, the extraction yield and squalene content in extracts from *Terminalia catappa* leaves by supercritical carbon dioxide extraction were reported to be 12.2 mg/g and 12.29%, respectively (*11*). Recently, more attention has been focused on squalene from *Amaranthus* grain. Oils from *Amaranthus* grain have been reported to contain larger amounts of squalene (2.4−8.0%) than other common vegetable oils (*12*−*16*). Squalene content in *Amaranthus cruentus* has been reported to be 0.43% of the total seed weight (*10*). In addition, squalene was also present at 0.73% in the seed of *Amaranthus hypochondriacus* and at 1.32% in the seed of *Amaranthus pumilus* (*14*).

In this study, we investigated the oil content, squalene concentration, and fatty acid profile in a seeds of a much wider range of *Amaranthus* genotypes cultivated in China. In addition, the *Amaranthus* plant can offer high production of leaf and stem, amounting to 30000−60000 kg/ha or even higher (*17*), so the occurrence of squalene in vegetative parts is also of interest. The squalene content in *Amaranthus* leaves of 45 genotypes

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was also investigated. Analysis of the taxonomic relationship among species in *Amaranthus* was carried out by multivariate statistical methods using the oil data.

MATERIALS AND METHODS

Materials. The seeds of 104 genotypes from 30 species originating from 44 countries and regions, obtained from the USDA National Plant Germplasm collection held at Iowa State University, Ames, IA, were planted in May at the experimental farm of Hubei Academy of Agricultural Science, Wuhan, China. The seeds of 45 genotypes representing 20 *Amaranthus* species, having good leaf production, were chosen for planting in the next year for leaf sampling. The mature leaves were collected for oil and squalene testing, and some genotypes were sampled at different growth stages before maturity. The first time for collecting leaf sample was on June 12, 2002, and a total of four samplings at intervals of ~15 days were carried out. The leaves collected at the last stage were mature. The fresh leaves were prepared by cutting the petiole and were immediately dried in a microwave oven (at 100 W for 60 s) to inhibit uncontrolled enzymatic reactions. Then the leaves were freeze-dried and ground to powder (to pass an 850 μm sieve) with a laboratory mill (Kenwood). The ground samples were collected in polyethylene zipper bags and kept in a cool room (4 °C) until needed for analysis. Standards of squalene (99% purity) and fatty acid methyl esters were from Sigma Chemical Co. (St. Louis, MO).

Oil Extraction. Ground samples were dried in an oven at 50 °C overnight to reduce the moisture to below 5% before use for oil extraction. The powdered samples were extracted for oils by an extraction/desolventizing unit, Soxtec System HT6 (Tecator, Sweden), with petroleum ether (boiling range 40−60 °C) containing 0.01% butylated hydroxytoluene as an antioxidant to avoid the possible deterioration of unsaturated fatty acids (*18*). Each sample was analyzed in duplicate, and average values are shown in the tables.

Fatty Acid Analysis. The American Oil Chemists' Society (AOCS) official method (*19*) with minor changes was used to prepare the fatty acid methyl esters (FAMEs) for fatty acid analysis. The FAMEs were detected by an HP-6890 gas chromatograph (GC) (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and a Supelco (Bellefonte, PA) Omegawax 250 capillary column (30 m × 0.25 mm). The GC conditions were as follows: initial oven temperature, 170 °C; initial time, 15 min; heating rate, 2 °C/min; final temperature, 220 °C; final holding time, 10 min; injection port temperature, 280 °C; detector temperature, 260 °C. Peaks were identified by comparison with FAME standard from Sigma. Nonadecanoic acid (19:0) methyl ester was added as an internal standard. The absence of 19:0 in the samples was previously checked before sample analysis.

Squalene Analysis. Squalene contents in extracted oil samples were determined using a high-performance liquid chromatography system (Hewlett-Packard 1100) under the conditions described before (*18*).

Statistical Analysis. Statistical analyses were conducted by software Statistica 6.0 (StatSoft). Principal component analysis (PCA) was used to discover the possible correlations among measured parameters and the relationships among different species. The basic idea of PCA is to describe the variation of a set of multivariate data by a set of orthogonal variables, each of which is a combination of the original variables (*20*). An advantage of PCA is that in addition to portraying the multidimensional scatter of objects via efficient variance extraction, the relationships among variables can also be evaluated thoroughly.

RESULTS AND DISCUSSION

Compositional Characteristics of Grain. A total of 104 genotypes of *Amaranthus* grain of 30 species were investigated for oil content, squalene concentration, and fatty acid profiles, as listed in **Tables 1** and **2**. A crude seed production value (no replicated experiment was carried out) was also listed as a reference value to evaluate the squalene yield per plant. The average oil content in *Amaranthus* grain based on a dry matter is 5.0%, ranging from 1.9% in PI 511714 of *A. cruentus* to 8.7% in PI 607462 of *A. rudis* (**Table 1**). Amaranth grain has a higher lipid content than most cereal grains (*9*), although its content is much lower than the major oilseeds, which are usually between 24 and 48%. These results for oil content are consistent with previous reports (*1*, *12*, *21*) except that a wider range of oil content was obtained because of the larger number of genotypes of *Amaranthus* seeds examined in this study. The oil contents of four *Amaranthus* genotypes, grown in Nigeria, were reported to be much higher than those in other studies, ranging from 9.75% in *A. arthropurpureus* to 16.95% in the common weed *A. spinosus* (*22*). Although we have investigated more than 100 *Amaranthus* genotypes, the maximum oil content found (8.7%) was still much lower than their results. We planted several genotypes of those species also originating from Nigeria, but not enough seeds were obtained to carry out the relevant testing. Other genotypes originating from Nigeria, Ames 25135 (*A. dubius*), Ames 5647 (*A. hybrid*), and Ames 25136 (*A. viridis*) (**Table 1**), did not give obviously high lipid contents. The average oil contents among species varied greatly, and there is also a high variation among genotypes within most species. The variations among species and within species were probably mostly due to genetic compositions, but environmental factors and cultural practices would also have some impact.

Although *Amaranthus* grain was low in oil content as compared to many other oil-containing grains, *Amaranthus* oil contained a considerably higher concentration of squalene. Squalene contents in some important commercial oils are quite low, such as 0.002% in coconut, 0.01% in sunflower and cotton, 0.03% in maize, peanut, and rapeseed, 0.3% in rice bran, and 0.4% in olive oil (*9*). Extracted oils from genotypes PI 604574 (*A. hybridus*) and Ames 2178 (*A. hypochondriacus*) contained as high as 7.0% squalene (**Table 1**). Squalene was found in trace amounts in *Amaranthus* oils in several genotypes, Ames 21715 (*A. crispus*), Ames 13779 (*A. deflexus*), and PI 605739 (*A. standleyanus*). The average squalene concentration was 4.2% in extracted *Amaranthus* oil, and the average squalene content in seeds was 2.13 mg of squalene/g of dry seed. The two genotypes having the highest squalene contents in seed were PI 607462 (*A. rudis*) (5.6 mg of squalene/g of dry seed) and PI 604574 (*A. hybridus*) (5.11 mg of squalene/g of seed).

The fatty acid compositions and saturated/unsaturated (S/U) ratios are important indicators in evaluating nutritional and functional value. The major fatty acids of oils from seeds of *Amaranthus* genotypes were similar. The preponderant fatty acids were palmitic acid, oleic acid, and linoleic acid. The remaining fatty acids, stearic, linolenic, arachidic, and behenic, occurred in much lower amounts (trace−3%). The average fatty acid composition for each species is shown in **Table 2**, in which several minor fatty acids, linolenic, arachidic, and behenic, are not listed. A distinct diversity in the relative percentage of fatty acids in the oils existed within both inter- and intraspecies. Therefore, there is no typical *Amaranthus* seed oil. The overall average contents of three major fatty acids were 21.3, 28.2, and 46.5% for palmitic, oleic, and linoleic, respectively, possessing a fatty acid profile similar to oils from buckwheat, corn, and cotton (*1*, *6*, *23*). In addition, *Amaranthus* oils were highly unsaturated. The S/U fatty acid ratios ranged from 0.14 to 0.57, with an average ratio of 0.32.

Compositional Characteristics of Leaf. The average fat content in dried mature *Amaranthus* leaves was 1.63%, ranging from 1.08% in PI 482057 (*A. spinosus*) to 2.18% in Ames 23388 (*A. viridis*) (**Table 3**). The squalene concentration in leaf lipid extracts averaged 0.26%, which is much lower than that from seeds. Squalene was found in trace amounts in *Amaranthus* leaf extract in several genotypes, Ames13789 (*A. albus*), PI 604196

Table 1. Oil and Squalene Contents in Different *Amaranthus* Species Seeds

	species	genotype	origin	oil (%)	squalene (% in oil)	squalene (mg/g of seed)	1000 seed wt (g)
1	*A. aff. blitum*	PI 490298	Kenya	6.45	5.16	3.33	0.68
2		PI 606282	Bangladesh	2.69	2.97	0.80	0.46
3	*A. albus*	Ames 13788	Canada	4.95	4.11	2.03	0.28
4		Ames 13789	Spain	6.75	5.32	3.59	0.28
5		Ames 18499	United States	5.75	3.50	2.01	0.36
6		PI 608029	United States	5.25	4.05	2.13	0.32
7	*A. arenicola*	Ames 21664	Mexico	4.18	2.2	0.92	0.11
8	*A. asplundii*	PI 604196	Ecuador	5.4	1.36	0.73	0.44
9	*A. blitoides*	PI 608662	Hungary	3.3	6.46	2.13	1.10
10		PI 608663	Canada	3.3	4.95	1.63	1.17
11	*A. blitum*	Ames 5103	Hong Kong	8.5	4.07	3.46	0.35
12		Ames 23387	Brazil	6.5	4.24	2.76	0.33
13		PI 288277	India	4.7	2.61	1.23	0.57
14		PI 606751	Switzerland	7.3	4.47	3.26	0.30
15		PI 612860	United States	7.8	5.03	3.92	0.26
16	*A. californicus*	PI 595319	United States	3.6	1.04	0.37	0.28
17	*A. cannabinus*	PI 568124	United States	7.9	4.88	3.86	0.25
18	*A. crassipes*	Ames 10339	Czech Republic	7.5	4.64	3.48	0.29
19	*A. crispus*	Ames 21715	Hungary	2.7	Tr	Tr	0.18
20	*A. cruentus*	Ames 1978	Ghana	3.7	3.87	1.43	0.32
21		Ames 5604	Taiwan	5.0	3.72	1.86	0.34
22		PI 462371	Sudan	3.9	4.93	1.92	0.28
23		PI 511714	Peru	1.9	4.08	0.78	0.37
24		PI 511720	Guatemala	2.2	4.35	0.96	0.40
25		PI 566896	United States	2.2	3.32	0.73	0.43
26		PI 566897	India	3.6	4.21	1.52	0.30
27	*A. deflexus*	Ames 13779	Portugal	3.20	Tr	Tr	
28		Ames 13785	France	3.98	3.90	1.55	0.27
29		Ames 15314	Argentina	3.47	0.37	0.13	
30	*A. dubius*	Ames 10340	Czech Republic	5.81	5.63	3.27	0.25
31		Ames 25135	Nigeria	6.24	2.72	1.70	0.32
32		PI 482047	Zimbabwe	6.20	3.27	2.03	0.31
33		PI 536444	Maldives	3.32	3.59	1.19	0.22
34		PI 599681	India	5.24	3.55	1.86	0.29
35		PI 612850	United States	5.01	4.21	2.11	0.26
36	*A. fimbriatus*	PI 605738	Mexico	5.49	4.40	2.42	0.42
37		PI 612855	United States	4.80	6.27	3.01	0.42
38	*A. hybrid*	Ames 5647	Nigeria	3.05	5.66	1.73	0.45
39		Ames 23385	Brazil	5.69	4.45	2.53	0.28
40		Ames 24806	United States	5.18	5.46	2.83	0.36
41		PI 511752	Peru	4.26	5.07	2.16	0.31
42		PI 572255	United States	4.51	3.38	1.52	0.61
43		PI 612849	United States	4.68	6.20	2.90	0.25
44	*A. hybridus*	Ames 2028	China	4.05	5.89	2.39	0.27
45		Ames 5331	Argentina	2.83	4.14	1.17	0.26
46		Ames 5684	United States	5.03	6.00	3.02	0.29
47		Ames 23371	Brazil	6.69	4.94	3.30	0.26
48		PI 604574	Mexico	7.00	7.30	5.11	0.18
49		PI 607463	United States	2.36	2.26	0.53	0.30
50	*A. hypochondriacus*	Ames 2178	Nepal	5.23	6.98	3.65	0.63
51		Ames 5158	Puerto Rico	4.10	5.07	2.08	0.68
52		PI 538794	Russia	5.97	5.09	3.04	0.55
53		PI 612177	China	3.03	4.74	1.44	0.35
54	*A. muricatus*	Ames 21716	Spain	2.18	3.20	0.70	0.63
55	*A. palmeri*	Ames 5665	Mexico	5.20	2.20	1.14	0.18
56		Ames 15298	United States	4.42	2.76	1.22	0.21
57		PI 549158	Mali	4.56	2.47	1.13	0.25
58		PI 612856	United States	3.46	2.46	0.85	0.21
59	*A. powellii*	PI 538793	Russia	6.04	4.29	2.59	0.45
60		PI 572257	Rwanda	5.74	5.20	2.98	0.38
61		PI 572258	Hungary	6.80	3.86	2.62	0.46
62		PI 572259	Czech Republic	6.17	4.07	2.51	0.52
63		PI 572260	France	5.95	3.92	2.33	0.43
64		PI 572261	Germany	6.31	4.73	2.98	0.35
65		PI 604671	United States	6.07	4.35	2.64	0.49
66	*A. pumilus*	PI 553085	United States	3.19	5.05	1.61	0.31
67	*A. quitensis*	Ames 23384	Brazil	5.09	3.50	1.78	0.29
68		PI 511751	Peru	3.60	3.89	1.40	0.41
69	*A. retroflexus*	Ames 5328	Canada	5.04	3.82	1.93	0.40
70		Ames 21767	China	7.09	4.55	3.23	0.33
71		Ames 22592	Mongolia	4.94	4.23	2.09	0.44
72		Ames 23890	Italy	6.25	4.20	2.63	0.40
73		PI 177261	Turkey	5.86	3.78	2.22	0.35
74		PI 607465	United States	6.43	4.54	2.92	0.35
75		PI 612857	United States	4.89	4.56	2.23	0.36

Table 1 (Continued)

	species	genotype	origin	oil (%)	squalene (% in oil)	squalene (mg/g of seed)	1000 seed wt (g)
76	*A. rudis*	Ames 24593	United States	7.80	4.93	3.85	0.20
77		PI 607462	United States	8.69	6.49	5.64	0.18
78	*A.* sp.	Ames 14944	Canada	5.44	3.69	2.01	0.32
79		Ames 22374	United States	4.96	4.56	2.26	0.37
80		PI 274275	Pakistan	4.17	5.22	2.18	0.56
81		PI 481609	Bhutan	6.60	5.72	3.78	0.20
82	*A. spinosus*	Ames 2043	Indonesia	7.21	3.20	2.31	0.18
83		Ames 2053	Thailand	6.81	2.98	2.03	0.17
84		PI 482057	Zimbabwe	6.12	2.72	1.66	0.19
85		PI 500294	Zambia	5.67	2.71	1.54	0.17
86	*A. standleyanus*	PI 605739	Argentina	4.76	Tr	Tr	
87	*A. tricolor*	Ames 1980	Zaire	5.34	5.33	2.85	0.70
88		Ames 5163	Puerto Rico	4.24	5.22	2.21	0.35
89		Ames 5317	Hong Kong	4.31	4.73	2.04	0.52
90		Ames 5368	Bangladesh	3.34	5.00	1.67	0.58
91		Ames 15330	China	5.34	5.75	3.07	0.68
92		Ames 18049	Nepal	5.58	5.52	3.08	0.93
93		NSL 6100	United States	3.56	5.68	2.02	0.61
94		PI 607446	Thailand	4.31	4.99	2.15	0.72
95	*A. tuberculatus*	PI 603887	United States	3.09	4.75	1.47	0.12
96	*A. viridis*	Ames 5583	Philippines	5.27	5.74	3.02	0.33
97		Ames 10828	United States	5.13	3.28	1.68	0.37
98		Ames 15313	Argentina	5.08	3.71	1.88	0.28
99		Ames 23271	India	5.09	3.45	1.76	0.27
100		Ames 23388	Brazil	5.40	4.81	2.60	0.36
101		Ames 23806	Israel	4.93	3.71	1.83	0.26
102		Ames 25136	Nigeria	4.22	4.69	1.98	0.26
103		Ames 25413	South Africa	4.69	4.26	2.00	0.42
104		PI 540445	Indonesia	5.23	4.39	2.30	0.39
	overall mean and standard deviation			5.0 ± 1.5	4.2 ± 1.4	2.1 ± 1.0	0.38 ± 0.19

[a] Oil contents based on dry matter were the means of duplicate determination. [b] Squalene contents in *Amaranthus* oils were the means of duplicate determination. [c] Trace.

Table 2. Average Composition of Fatty Acids in *Amaranthus* Grain[a]

species	*N*[b]	FAs in oil (%)	palmitic 16:0	stearic 18:0	oleic 18:1	linoleic 18:2	S/U ratio[c]
A. aff. Blitum	2	58.1 ± 2.8	24.1	1.3	31.3	37.8	0.38
A. albus	4	58.7 ± 3.8	11.7 ± 1.7	1.3 ± 0.1	23.0 ± 1.2	61.5 ± 1.7	0.17 ± 0.02
A. arenicola	1	43.7	22.1	1.9	21.9	54.1	0.32
A. asplundii	1	61.1	24.1	0.1	33.6	39.3	0.35
A. blitoides	2	84.8 ± 19.3	17.2 ± 8.5	1.5 ± 0.3	22.2 ± 3.5	56.3 ± 11.8	0.26 ± 0.13
A. blitum	5	48.8 ± 11.9	25.0 ± 1.3	1.0 ± 0.1	28.9 ± 3.6	42.6 ± 4.7	0.38 ± 0.03
A. cannabinus	1	21.3	27.7	1.2	26.1	42.8	0.43
A. crassipes	1	44.6	24.9	0.6	30.6	41.7	0.36
A. cruentus	7	53.5 ± 13.2	27.0 ± 3.1	1.4 ± 0.8	27.9 ± 2.4	38.1 ± 3.1	0.44 ± 0.07
A. deflexus	3	62.6	22.7	0.9	29.0	44.9	0.33
A. dubius	6	55.8 ± 9.6	25.9 ± 1.5	0.7 ± 0.1	30.5 ± 4.8	40.6 ± 3.6	0.39 ± 0.03
A. fimbriatus	2	45.0 ± 15.5	19.4 ± 7.1	1.1 ± 0.8	28.8 ± 6.3	48.1 ± 14.2	0.29 ± 0.13
A. hybrid	6	61.8 ± 8.1	22.4 ± 4.5	0.7 ± 0.2	30.3 ± 5.1	43.6 ± 8.6	0.34 ± 0.08
A. hybridus	6	48.2 ± 12.9	22.0 ± 5.0	1.3 ± 1.0	26.3 ± 2.8	47.4 ± 7.2	0.34 ± 0.08
A. hypochondriacus	5	63.8 ± 6.4	24.0 ± 1.7	0.9 ± 0.4	33.7 ± 6.5	38.9 ± 5.1	0.37 ± 0.02
A. muricatus	1	49.2	16.3	1.5	33.1	40.7	0.25
A. palmeri	4	64.9 ± 9.3	22.3 ± 0.9	3.9 ± 6.8	21.0 ± 1.5	52.4 ± 1.7	0.37 ± 0.10
A. powellii	7	64.8 ± 11.2	16.5 ± 1.0	0.6 ± 0.1	28.0 ± 1.2	52.3 ± 1.0	0.23 ± 0.01
A. pumilus	1	76.2	19.7	0.6	26.0	50.9	0.28
A. quitensis	2	78.5 ± 3.1	22.8 ± 0.3	0.7 ± 0.0	31.1 ± 4.7	42.6 ± 4.7	0.34 ± 0.00
A. retroflexus	8	69.5 ± 5.3	14.3 ± 1.8	0.7 ± 0.2	27.1 ± 2.5	55.2 ± 4.3	0.20 ± 0.03
A. rudis	2	63.5 ± 4.5	13.2 ± 0.7	2.3 ± 0.5	23.1 ± 1.6	57.9 ± 1.2	0.21 ± 0.01
A. sp.	4	71.5 ± 7.6	18.1 ± 5.8	1.2 ± 0.9	27.6 ± 4.3	50.5 ± 9.1	0.27 ± 0.09
A. spinosus	4	67.0 ± 6.0	24.9 ± 0.4	0.9 ± 0.1	27.4 ± 0.5	44.6 ± 0.3	0.37 ± 0.01
A. standleyanus	1	21.9	31.1	0.6	49.4	16.3	0.49
A. tricolor	8	56.8 ± 16.2	24.3 ± 1.6	1.1 ± 0.4	25.9 ± 2.9	46.4 ± 2.7	0.36 ± 0.03
A. tuberculatus	1	70.6	12.1	1.8	24.6	53.9	0.18
A. viridis	9	60.1 ± 12.6	23.0 ± 1.3	1.3 ± 0.1	34.4 ± 2.8	38.7 ± 2.9	0.35 ± 0.03
overall mean and standard deviation		61.3 ± 10.1	21.3 ± 5.1	1.1 ± 1.4	28.2 ± 4.5	46.5 ± 7.9	0.32 ± 0.09

[a] Means of duplicate determinations. [b] Number of genotypes in relevant species. [c] S/U ratio = saturated/unsaturated = (14:0 +16:0 + 18:0 + 20:0 + 22:0)/(18:1 + 18:2).

(*A. asplundii*), and Ames 5103 (*A. blitum*). Although also a genotype of *A. albus*, Ames 13788 contained the highest squalene concentration in its leaf lipid extract. Therefore, the genetic differences within a species could be great. With respect

Table 3. Oil and Squalene Content in Mature *Amaranthus* Leaves

	species	genotype	oil % (DB)	squalene in oil (%)	squalene (mg/10 g of dry leaf)
1	*A. aff. blitum*	PI 490298	1.76	0.30	0.53
2	*A. albus*	Ames 13788	1.86	0.77	1.43
3		Ames 13789	2.11	Tr	0.00
4	*A. asplundii*	PI 604196	1.93	Tr	0.00
5	*A. blitoides*	PI 608663	1.54	0.33	0.51
6	*A. blitum*	Ames 5103	2.17	Tr	0.00
7		PI 288277	2.17	0.37	0.80
8		PI 606751	1.43	0.18	0.26
9		PI 612860	2.11	0.23	0.49
10	*A. crassipes*	Ames 10339	1.47	0.27	0.40
11	*A. cruentus*	Ames 5604	1.90	0.24	0.46
12	*A. dubius*	Ames 10340	1.72	0.25	0.43
13		PI 612850	1.47	0.31	0.46
14	*A. hybrid*	Ames 5647	2.07	0.22	0.46
15		Ames 23385	1.58	0.42	0.66
16		Ames 24806	1.66	0.37	0.61
17		PI 612849	1.52	0.25	0.38
18	*A. hybridus*	Ames 2028	1.68	0.26	0.44
19		Ames 5684	1.66	0.17	0.28
20		PI 604574	1.63	0.28	0.46
21	*A.hypochondriacus*	Ames 5158	1.75	0.28	0.49
22		PI 538794	1.34	0.26	0.35
23	*A. powellii*	PI 572258	1.5	0.23	0.35
24		PI 572259	2.07	0.18	0.37
25		PI 604671	1.63	0.22	0.36
26	*A. pumilus*	PI 553085	1.15	0.2	0.23
27	*A. quitensis*	Ames 23384	1.21	0.36	0.44
28	*A. retroflexus*	Ames 5328	1.38	0.14	0.19
29		Ames 21767	1.50	0.16	0.24
30		Ames 23890	1.75	0.26	0.46
31		PI 607465	1.77	0.14	0.25
32	*A. rudis*	Ames 24593	1.43	0.18	0.26
33		PI 607462	1.46	0.36	0.53
34	*A.* sp.	Ames 14944	1.32	0.46	0.61
35		Ames 22374	1.53	0.18	0.28
36		PI 481609	1.24	0.31	0.38
37	*A. spinosus*	Ames 2043	1.64	0.18	0.30
38		PI 482057	1.08	0.4	0.43
39		PI 500294	1.13	0.21	0.24
40	*A. tricolor*	Ames 1980	1.36	0.20	0.27
41		Ames 15330	1.28	0.37	0.47
42		Ames 18049	1.81	0.44	0.80
43		PI 607446	1.49	0.31	0.46
44	*A. viridis*	Ames 23388	2.18	0.104	0.23
45		Ames 25413	1.91	0.16	0.31
	overall mean		1.63	0.26	0.41
	standard deviation		0.30	0.13	0.23

to squalene yield in leaves of *Amaranthus* genotypes, Ames 13788 (*A. albus*) had the highest squalene yield of 1.43 mg/10 g of dry leaf, followed by 0.80 mg/10 g of dry leaf in PI 288277 (*A. blitum*) and Ames 18049 (*A. tricolor*). Although oil and squalene occurred in *Amaranthus* leaf at much lower levels than in *Amaranthus* grain, the leaves of these genotypes might be regarded as potential resources for squalene, because this plant can offer much cheaper leaf than grain. The average squalene yield was 0.41 mg/10 g of leaf. The variability among species and within species must probably be due to genetic composition, environmental factors, and cultural practices.

The oil content in *Amaranthus* leaves of 20 genotypes and squalene concentration in lipid extracts at different growth stages were studied (data not shown). Some genotypes and species show a certain trend in the changes, but when all of the genotypes were examined, no overall apparent trend of variation in oil content and squalene concentration at different growth stages was observed. In some genotypes, this variation was very slight, whereas in other genotypes this variation can be great.

Table 4. Fatty Acid Composition in Mature *Amaranthus* Leaves

species	genotype	palmitic 16:0	stearic 18:0	oleic 18:1	linoleic 18:2	linolenic 18:3	fatty acids in oil (%)	S/U ratio
A. crassipes	Ames 10339	14.33	1.21	5.61	21.71	56.81	28.2	0.18
A. cruentus	Ames 5604	15.50	1.37	6.96	18.10	56.46	24.0	0.21
A. dubius	Ames 10340	13.69	1.49	5.76	15.49	62.02	29.0	0.18
A. hypochondriacus	PI 538794	13.51	1.74	6.68	18.63	58.53	26.3	0.18
overall mean		14.26	1.45	6.25	18.48	58.46	26.88	0.19
standard deviation		0.90	0.22	0.67	2.55	2.54	2.23	0.01

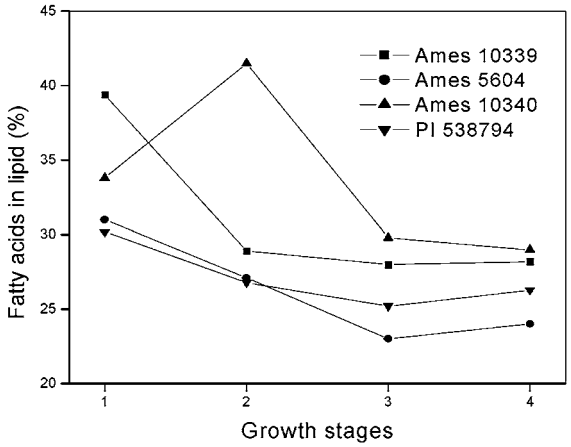

Figure 1. Total fatty acid content in leaf lipid at different growth stages (stage 1, seedling; stage 4, mature plant; stages 2 and 3, intermediate between seedling and mature plant).

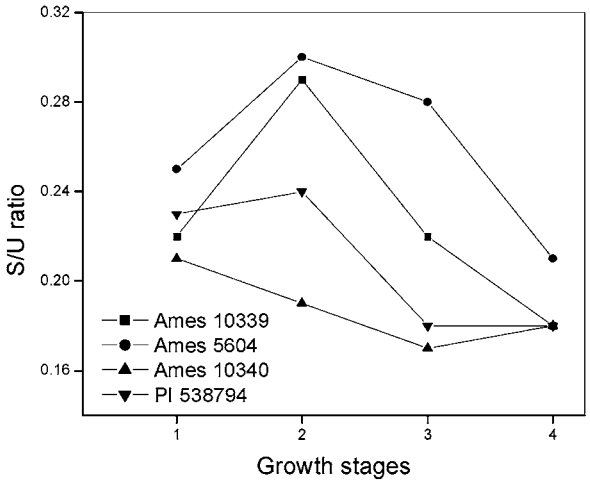

Figure 2. S/U ratio of leaf lipid at different growth stages (stage 1, seedling; stage 4, mature plant; stages 2 and 3, intermediate between seedling and mature plant).

As a result, to develop *Amaranthus* leaves as a resource of squalene, it is very important to choose both the right genotype and the right harvest time for optimum squalene yield.

Four genotypes of *Amaranthus* leaf lipid were tested for fatty acid composition. The average fatty acid content in mature leaf lipid was 26.9% (**Table 4**). The major fatty acids of leaf extracts were linolenic, linoleic, and palmitic (**Table 4**). The major fatty acids of the lipids from seeds of *Amaranthus* genotypes were similar. Linolenic ranged from 56.5 to 62.0% of total fatty acids; linoleic, from 15.5 to 24.7%; and palmitic, from 13.5 to 15.5% (**Table 4**). Unlike the fatty acid profile of seed extract, the leaf extract contained little linoleic acid, ranging from 5.6 to 7.0%,

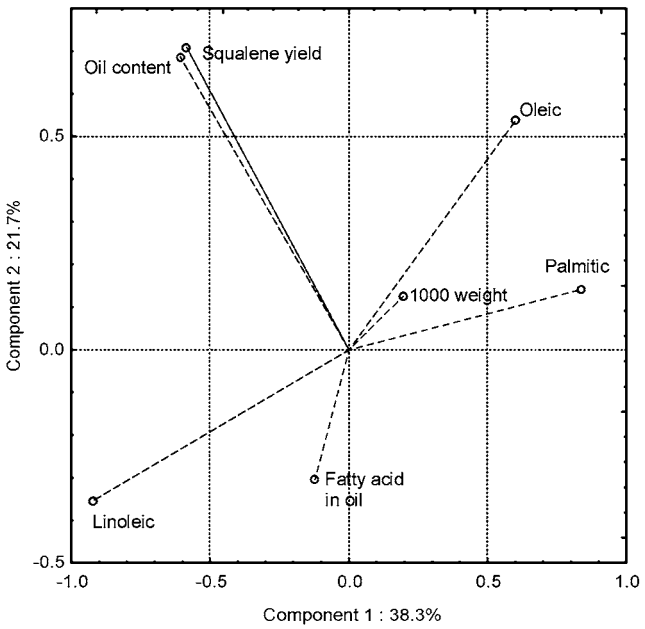

Figure 3. PCA ordination of variables based on component correlations.

which is the most abundant fatty acid in *Amaranthus* grain. Similar to *Amaranthus* grain, the leaf lipid also had little stearic acid, <2% of the total fatty acid. In addition, *Amaranthus* leaf lipids were highly unsaturated. The S/U fatty acid ratios of different genotypes were close, ranging from 0.18 to 0.21 (**Table 4**). This ratio was found to be superior to that of *Amaranthus* grain lipid. The lower S/U ratios, especially high in linolenic acid, make *Amaranthus* leaves potentially a better nutritional source of feeds and vegetables. As for the fatty acid compositions at different growth stages, it is obvious that the fatty acid content in leaf lipid was lower in mature leaves than young leaves (**Figure 1**). The S/U ratio decreased when the leaf grew to maturity (**Figure 2**). Additionally, *Amaranthus* leaf is a source of nutritionally important fatty acids, especially linolenic acid, found abundantly in all investigated genotypes.

Multivariate Statistical Analysis. To select suitable data that can well represent the species, only species containing data for four or more genotypes were selected for the following statistical analysis. A total of 81 genotypes belonging to 14 species were used for PCA on seven independent variables, oil content (%), squalene yield (mg/g seed), fatty acid in oil (%), compositions of three major fatty acids (palmitic, oleic, linoleic), and 1000 seed weight (g).

The PCA of the present data showed that the first two components (**Figure 3**) accounted for 70% of the total variance (38.3 and 21.7%, respectively) in the seven variables, leaving only 30% for the remaining five components. The lengths of the dashes reflect the variance of the corresponding variables, and the angles between them indicate the degree of their correlations, small angles corresponding to high correlations, opposite sides of the plot corresponding to negative correlations. Component 1 is positively correlated with palmitic and oleic contents and negatively correlated with linoleic content; component 2 is positively correlated with oil content and squalene yield (**Figure 3**). The remaining two variables (fatty acid in oil and 1000 seed weight), falling close to the origin, seem to be independent of the other variables, and their role is negligible in determining data structure. Therefore, the compositional characteristics of *Amaranthus* species in this study are not correlated to seed size, reflected by 1000 seed weight. We previously observed no correlation between compositional characteristics and seed color by analysis of a relatively small sample set (*18*). In addition, a positive correlation between oil content and squalene yield was observed (**Figure 3**). This might be due to the presence of squalene as one component in the oil. Negative correlations between linoleic and either of the other two major fatty acids, palmitic and oleic, were also observed (**Figure 3**). This might be due to the enzymatic system

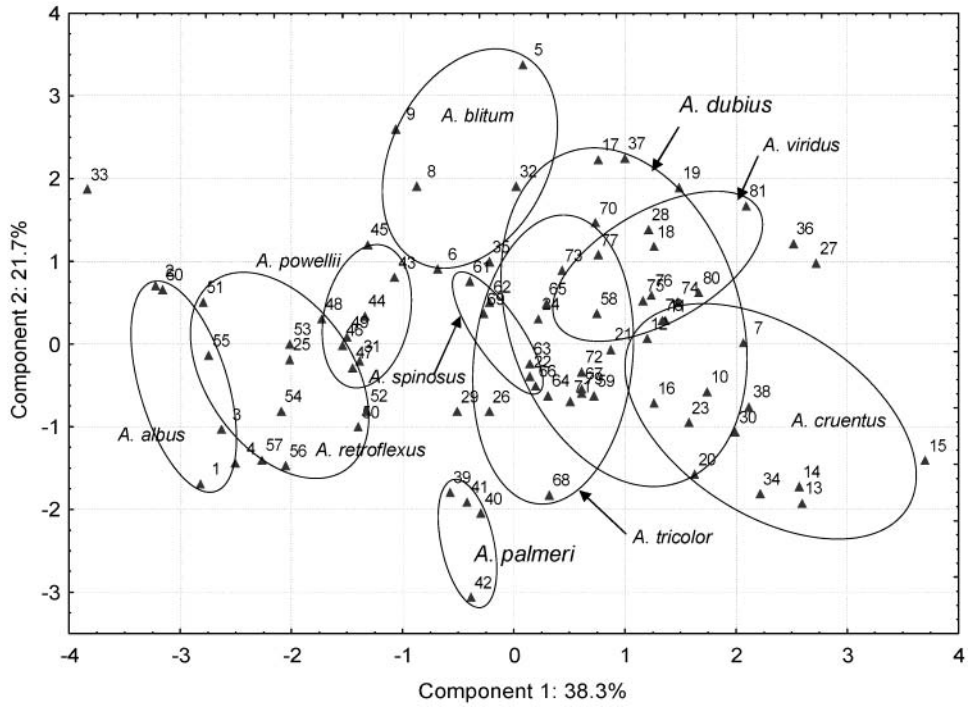

Figure 4. Scatter diagram of the first two principal components for *Amaranthus* genotypes: 1–4, *A. albus*; 5–9, *A. blitum*; 10–16, *A. cruentus*; 17–22, *A. dubius*; 23–28, *A. hybrid*; 29–34, *A. hybridus*; 35–38, *A. hypochondriacus*; 39–42, *A. palmeri*; 43–49, *A. powellii*; 50–56, *A. retroflexus*; 57–60, *A.* sp.; 61–64, *A. spinosus*; 65–72, *A. tricolor*; 73–81, *A. viridis*.

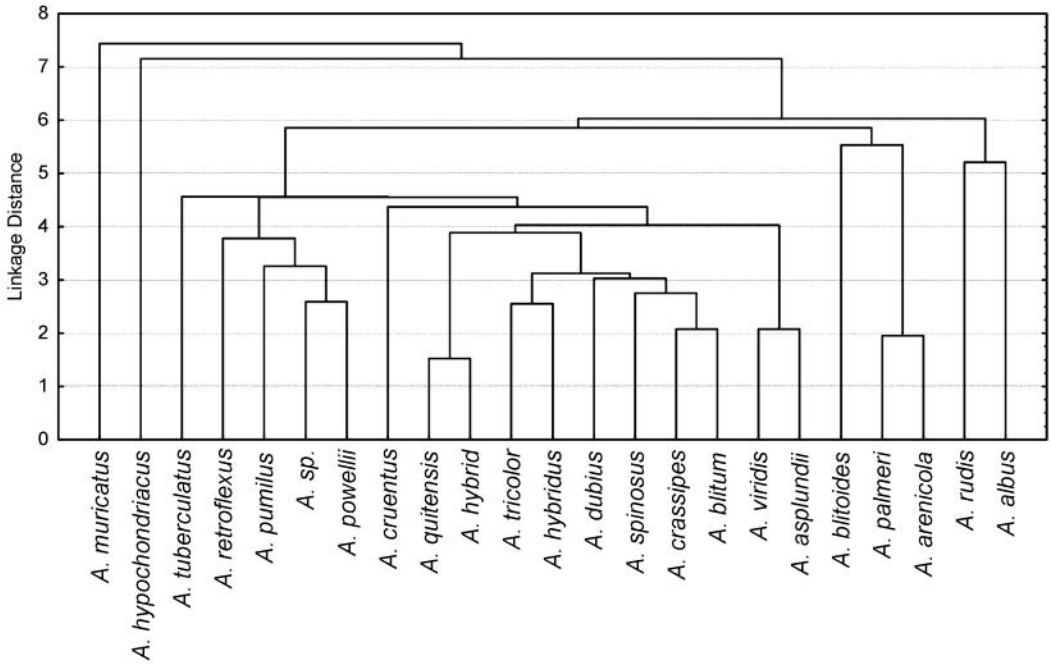

Figure 5. Single-linkage dendrogram for *Amaranthus* species on some seed composition data.

responsible for the metabolism of palmitic, oleic, and linoleic acids. No significant correlation was observed between other variables.

The scatter diagram of investigated genotypes on the first two principal components (**Figure 4**) indicates that some *Amaranthus* species can be grouped according to several lipid characteristics (contents of oil, squalene, and major fatty acids). The relative positions of the points corresponding to genotypes indicate the similarities and differences of their compositional characteristics. Genotypes within one species are relatively close to one another in the scatter diagram, overlaps indicate those species might have close relationships. The close relationships between *A. dubius* and *A. spinosus*, *A. powellii*, and *A. retroflexus*, proposed by Sauer (*24*), were observed in this scatter diagram (**Figure 4**). The separation of *A. palmeri* from other species might be due to its low squalene yield. Profiles of genotypes within several species, *A. blitum*, *A. hybrid*, *A. hybridus*, *A. hypochondriacus*, and *A.* sp., were spread widely in the scatter diagram. The possible reason might be their wide genetic variation for these traits.

There are relatively few taxonomic reports on *Amaranthus*, and this group has a perhaps undeserved reputation for taxonomic difficulty, mainly because of hopeless attempts to recognize taxa by pigmentation (which segregates within populations) and by growth form, which is extremely plastic under different day lengths and other environmental variables (*24*). Sauer attempted to elucidate the taxonomic relationships among *Amaranthus* species (*24*, *25*). The genotypes and their parameters used for PCA were also used for cluster analysis to discover the relationships among *Amaranthus* species. The analysis results on relationships of *Amaranthus* species (**Figure 5**) were close to those determined by Sauer (*24*).

Conclusion. Amaranth is a widely adapted plant, tolerant of adverse conditions. This work studied the concentration of squalene present in the *Amaranthus* grain and leaf. The oil fraction of the grain is considerably higher in squalene compared to many other cereal or pseudocereal grain sources. As the clinical and nutraceutical functions of squalene are becoming better known, the demand for this substance will likely increase continuously. This will help support the large-scale production of *Amaranthus* grain as a source of squalene and for other value-added uses.

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