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BREEDING AND GENETICS

Fatty Acid Profiles of Cottonseed Genotypes from the National Cotton Variety Trials

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ABSTRACT

Cottonseed oil fatty acid composition was determined for several cotton genotypes included in the 2006 and 2007 years of the National Cotton Variety Trials. Seed was collected from a total of nine environments that included six locations, and 35 genotypes were included in the analysis. Oil was extracted from seed with hexane, and the glycerides were converted to fatty acid methyl esters and analyzed by gas chromatography. Results indicated that commercially acceptable cotton genotypes vary modestly in their distribution of fatty acids, covering a range slightly greater than the range specified in the Codex trading standard for cottonseed oil. Analysis of variance, based on a random effects model, indicated the relative level of most fatty acids was influenced by environment and genetics, but the interaction of these effects was relatively small. Correlations were found between the levels of several major and minor fatty acids. Many of the observed associations appeared to have some foundation with known and proposed biosynthesis pathways. Although the results indicate that breeding cotton for modified oil composition should be feasible, the range of variation observed within the genotypes studied was insufficient to provide useful traits for breeding. A more extensive survey of cottonseed genotypes will be needed for this purpose.

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Cottonseed oil (*Gossypium hirsutum* L.) has long been considered to be a good vegetable oil for frying, in part because it tends to impart a toasted aroma to fried products. Cottonseed oil also has disadvantages that have resulted in some food companies limiting their use of the oil. Specifically, the oxidative stability of cottonseed oil can be lower than for other vegetable oils because of its high concentration of linoleic acid (18:2). When used for frying, this instability accelerates the formation of off-flavors (rancidity) and shortens oil life. To compensate, cottonseed oil can be partially hydrogenated, which reduces the level of 18:2 and improves the oil's stability, but the process also forms undesirable trans-fatty acids that raise serum low-density lipoprotein cholesterol levels (Sacks and Katan, 2002). In addition, the concentration of palmitic acid (16:0), a saturated fatty acid, is higher in cottonseed oil (~24%) than in many other vegetable oils. Although higher levels of saturated fatty acids contribute functionality in food systems, they also contribute negatively to serum cholesterol profiles (Zock et al., 1994) and consumers have expressed a desire for reduced levels of these acids in processed foods. Cottonseed oil also contains modest levels of cyclopropenoid fatty acids, which are considered anti-nutritional. Although the level of these acids is significantly reduced by oil deodorization, they would be fully present in whole seed or kernel feeding of cottonseed to animals, a practice that would increase if current efforts to reduce seed gossypol levels prove successful (Sunilkumar et al., 2006). Consequently, although cottonseed oil is considered a premium vegetable oil, tailoring its composition to overcome some of these issues would likely expand its marketability and increase its value.

Because most of the value in the cotton plant resides in the fiber, most cottonseed development efforts have focused on improving fiber yield and quality. Nevertheless, DNA techniques have been used to modify cottonseed oil traits (Chapman et al., 2001; Liu et al., 2002; Sunilkumar et al., 2005). Classical breeding to improve cottonseed oil qual-

ity has not been reported, although techniques that avoid intellectual property concerns, regulatory hurdles, and potential public non-acceptance should be considered advantageous. To follow this approach, however, considerable background information is needed about the degree of compositional variation that exists in the seed oil of cotton genotypes.

Consequently, a series of studies is underway to determine the variation that exists in fatty acid composition of cotton germplasm, the effect that growing environment has on oil composition, and the potential of developing cotton plants with modified oil properties. As a first step in this process, our objective was to evaluate the fatty acid variation existing in seeds of commercially acceptable cotton genotypes. Toward this end, the fatty acid profiles were determined for a subset of genotypes[1] from the 2006 and 2007 "Regional High-Quality" section of the National Cotton Variety Trials. The study included 35 genotypes, six locations, and two growing seasons.

MATERIALS AND METHODS

Field Trials and Seed Samples. In 2006, 20 genotypes were grown in Bella Mina, AL; Keiser, AR; Las Cruces, NM; Lubbock, TX; and Stoneville, MS. In 2007, 23 genotypes were grown in Florence, SC; Keiser, AR; Lubbock, TX; and Stoneville, MS. Each genotype was grown in duplicate row plots. The experiment was designed as a randomized complete block. A 50-boll sample was harvested from each row and was ginned to recover fiber and fuzzy seed. Fifteen grams of fuzzy seed was then subsampled for determination of fatty acid composition.

Seed Preparation. Seed was dehulled in a 1-L stainless-steel Waring blender operated at variable speed. Typically, a handful of seed was pulsed in the blender then poured over stacked #4, #12, and base plate sieves. Hull pieces (with linters) and uncracked seeds were retained on the surface of the #4 sieve; whole kernels and large pieces were retained on the surface of the #12 sieve; and fines (both hull and kernel pieces) passed through the #12 sieve. The top fraction was then re-milled increasing the duration and intensity of the milling and re-sieved. The process was repeated until all of the kernels were cracked, and most of the larger kernel pieces were recovered on the #12 sieve. Recovered kernels were then ground for approximately 30 sec with a Braun hand-chopper to pass a #20 sieve, freeze-dried, and stored at -20 °C until used for oil recovery.

Oil Extraction and Trans-methylation Chemistry. Approximately 100 mg of the ground seed material was weighed into a 2-mL microcentrifuge vial. Two 2.3-mm diameter chrome-steel balls and 1 mL of hexane were then added. The tube was sealed and the contents pulverized on a Beadbeater-8 microcentrifuge mill (Bio-spec Products, Bartlesville, OK) operated at 90% maximum speed for 2 min. Tubes were then shaken on a platform shaker overnight at room temperature to extract the oil. After extraction, each tube was centrifuged (~10,000*g*) for 2 min to pelletize seed debris, and the miscella (solvent plus crude oil) was transferred to a test tube.

To form fatty acid methyl esters, 200 μL of 0.5 N methanolic base (Supelco, Inc., Bellefonte, PA) was added to the miscella, and the tube was capped and heated to 70 °C for 10 min with periodic vortex mixing. Upon cooling, 1 mL of brine and 1 mL of hexane were added, and the contents were vortex mixed again. After allowing the phases to separate, 1 mL of the organic phase containing the methyl esters was transferred to a gas chromatography autoinjector vial.

Gas Chromatography. For the 2006 samples, the analysis was conducted with a Hewlett-Packard (Palo Alto, CA) model 5890 Series 2 plus gas chromatograph. For the 2007 samples, the gas chromatograph was replaced with an Agilent (Santa Clara, CA) model 7890A gas chromatograph. Each instrument was fitted with a split/splitless injector, a flame-ionization detector (FID), and a Supelco SP-2380 capillary column (0.25 mm i.d. x 30 m x 0.2mm film thickness). Other than the instrument, the methods used to detect and measure fatty acid profiles were identical. Injectors were operated in split mode with a split ratio of 1:100. Injector and detector temperatures were set at 240 °C. Helium was used as carrier gas and was controlled in constant flow mode at a linear velocity of 20 cm/sec. The oven was programmed to start at 170 °C, which was held for 3 min; then the temperature was ramped at 1 °C/min

[1] Genotypes are referenced in this report by the names used for the 2006 and 2007 National Cotton Variety Trials, which allows for comparisons with trial data that are available online (USDA, National Cotton Variety Trials). It should be noted that some of these genotypes have progressed into commercial varieties with different names. For example, AR 9704-13-08 is now known as Arkot 9704, ARK 9610 is known as Arkot 9610, ARK JJ46 is known as Arkot JJ46, etc.

to 180 °C; then the temperature was ramped at 4 °C/min to 240 °C, which was held for an additional 5 min. Injection volumes were 1 μL.

Peaks were identified by comparison of elution times with known standards. All expected major components were separated on the polar column and were easily identified. Several minor and trace components were also observed. Some of these components were identified from prior reports and expected retention times; others will require mass spectroscopy to confirm their identities (a future effort). Fatty acid distributions were based on a common set of acids that included myristic (14:0), 16:0, palmitoleic (16:1), stearic (18:0), oleic (18:1(n-9)), *cis*-vaccenic (18:1(n-7)), 18:2, α-linolenic (18:3), arachidic (20:0), behenic (22:0), lignoceric (24:0), malvalic (cpe18:1), and sterculic (cpe19:1) acids. Together, these acids accounted for >99.5% of the fatty acid peak areas observed in the chromatograms. Although additional peaks were apparent, their small contributions to the profiles should not significantly impact the relative proportions of the more common components. In calculating distributions, individual FID peak areas were corrected for response factor differences as recommended in AOCS Official Method Ce 1e-91 (1998). Each sample was extracted, derivatized, and analyzed in duplicate.

Calculations and Statistics. From measured distributions, a number of characteristic fatty acid parameters were also calculated. Total saturated acids were determined from the sum of 14:0, 16:0, 18:0, 20:0, 22:0, and 24:0; total unsaturated acids were determined as the sum of 16:1, 18:1(n-7), 18:1(n-9), 18:2, and 18:3; and total cyclopropenoid fatty acids were determined from the sum of cpe18:1 and cpe19:1. Ratio of 16- to 18-carbon fatty acids and acids with >18-carbon atoms (i.e., 20:0 to 24:0) were also calculated.

Variability in fatty acid composition was measured by analysis of variance (ANOVA) based on a random effects model. The model divided variability into the following components: environment (σ_e^2), genotype (σ_v^2), environment x genotype (σ_{vxe}^2), replication within environment (σ_r^2), and residual error (σ_{err}^2). Thirty-five genotypes were used to estimate the effect of genotype. To estimate the effect of environment, combinations of location and years were considered as individual environments. Because only two growing seasons were included in the study, no attempt was made to partition environmental variance among locations and years. SAS Proc Mixed was used to perform ANOVA and estimate variance components (Littell et al., 2006). Means were estimated from this analysis as best linear unbiased predictors (BLUP) (Littell et al., 2006).

RESULTS AND DISCUSSION

Variation of fatty acid parameters was first divided into two groups: σ_1^2 ($=\sigma_r^2+\sigma_{err}^2$) representing replication within an environment and σ_2^2 ($=\sigma_e^2+\sigma_v^2+\sigma_{vxe}^2$) representing phenotypic variance or the additional variation due to environment and genotype (Table 1). Replicate variance was small, indicating fatty acid profiles of duplicate field rows of the same genotype produced in the same environment were consistent. The duplicate gas chromatographic measurements were also consistent indicating measurement error contributed only a minor part of the total variation.

Most of the observed variation was modeled by genotype, environment, and their interaction (σ_2^2). This variance component was partitioned into its three sources (σ_e^2, σ_v^2, and σ_{vxe}^2) and was expressed as a percent of σ_2^2 (Table 1). Most of the phenotypic variance was due to genotype and environment main effects, which together generally accounted for greater than 90% of σ_2^2. For example, 16:0, 18:1(n-9), and 18:2, which combined account for >92% of the oil's fatty acids, had genotypic variances of 62.4, 50.3, and 44.0%, respectively; environmental variances of 33.7, 39.7, and 50.5%, respectively; and interaction variances of 3.9, 9.9, and 5.4%, respectively. For most fatty acids, environment and genotype each accounted for between one-third and two-thirds of the component variation (Table 1).

Variation in components that fell toward the end of biosynthesis pathways, e.g., 18:3, 18:1(n-7), and 24:0 (Fig. 1), appeared to be more sensitive to environment (63-67%) than genetics (20-31%). Variation in cyclopropenoid fatty acids, both individually and collectively, also appeared to be affected more by environment (52-61%) than genetics (31-41%). 18:0 and longer chain 22:0 and 24:0 components exhibited the greatest interaction effects. 18:0 is a major branch point in the biosynthetic pathway (Fig. 1) leading primarily to unsaturated fatty acids but also to longer chain fatty acids. Its relative concentration might be more affected by a larger array of biochemical influences, which might account for the greater potential for interaction between environment and genotype.

Table 1.Variance components from cottonseed oil composition of genotypes from the 2006 and 2007 National Cotton Variety Trials

Fatty acid	Mean[z]	$\sigma_1^{2\,y}$	$\sigma_2^{2\,x}$	Partitioning of phenotypic variance, %(Z-value)		
				Environment (σ_e^2/σ_2^2 x 100)	Genotype (σ_v^2/σ_2^2 x 100)	Genotype x Environment ($\sigma_{vxe}^2/\sigma_2^2$ x 100)
14:0	0.866	0.00111	0.02693	40.8 (1.9)	52.5 (4.0)	6.8 (7.0)
16:0	23.9	0.08281	2.91104	33.7 (2.0)	62.4 (4.1)	3.9 (6.5)
16:1	0.598	0.00034	0.00431	33.4 (1.9)	59.4 (4.0)	7.2 (5.6)
18:0	2.54	0.00536	0.04309	53.5 (1.9)	29.6 (3.6)	16.9 (6.3)
18:1(n-9)	17.2	0.34159	3.33879	39.7 (1.9)	50.3 (4.0)	9.9 (5.8)
18:1(n-7)	0.865	0.00057	0.00582	66.9 (1.9)	26.1 (3.9)	7.0 (5.5)
18:2	52.5	0.53483	9.83958	50.5 (2.0)	44.0 (4.0)	5.4 (6.0)
18:3	0.201	0.00005	0.00023	65.8 (1.9)	31.4 (3.8)	2.8 (1.5)
20:0	0.294	0.00018	0.00158	54.9 (1.9)	36.1 (3.9)	9.1 (5.4)
22:0	0.139	0.00005	0.00036	38.2 (1.9)	49.7 (3.9)	12.1 (5.6)
24:0	0.120	0.00010	0.00034	62.6 (1.9)	20.3 (3.1)	17.1 (4.6)
cpe18:1	0.424	0.00169	0.00530	57.6 (1.9)	35.4 (3.7)	7.0 (2.4)
cpe19:1	0.328	0.00047	0.00206	51.7 (1.9)	40.8 (3.8)	7.5 (3.0)
Sat[w]	27.9	0.11064	3.92024	38.7 (2.0)	57.0 (4.1)	4.3 (6.7)
Unsat[w]	71.3	0.10584	3.71690	36.3 (2.0)	59.4 (4.1)	4.2 (6.6)
Cpe acids[w]	0.752	0.00363	0.01223	61.9 (1.9)	30.8 (3.6)	7.4 (2.6)
20:0-24:0	0.554	0.00062	0.00531	54.2 (1.9)	34.8 (3.8)	11.0 (5.9)
16/18 ratio	0.336	0.00003	0.00101	34.4 (2.0)	61.4 (4.1)	4.3 (6.5)

[z] Values represent best linear unbiased predictor (BLUP) of random effects.

[y] Residual error and replicate variance, i.e., $\sigma_1^2 = \sigma_r^2 + \sigma_{err}^2$

[x] Variance caused by variety, environment, and their interaction, i.e, $\sigma_2^2 = \sigma_v^2 + \sigma_e^2 + \sigma_{vxe}^2$

[w] Sat = Total saturated fatty acids, Unsat = Total unstaturated fatty acids, Cpe = Total cycloproprenoid fatty acids.

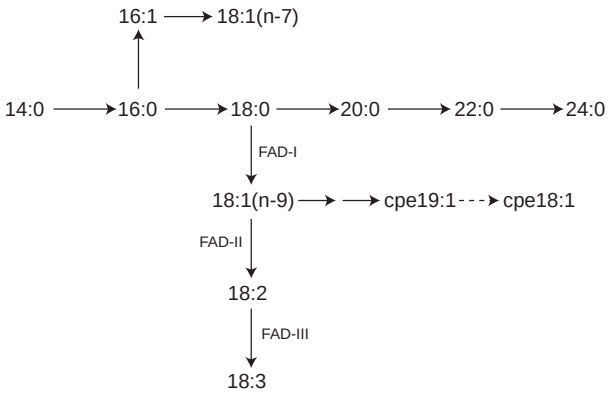

Figure 1. Simplified synthesis pathway of oilseed fatty acids.

Among the population of genotype means (Table 2), JAJO 3077 and JAJO 3007 had the lowest levels of 16:0 (21.3 and 21.1%, respectively) followed closely by several Deltapine genotypes (e.g., DP 445BR with 21.5% and DP 161BG2RF with 21.6%). The highest level of 16:0 was obtained from ARK 9610 (25.9%), which also had the highest levels of 14:0 (1.08%) and close to the highest level of longer-chain 20:0 to 24:0 (0.604%). FiberMax genotypes exhibited fatty acid profiles similar to ARK 9610, with FM 9063B2F having the lowest levels of 18:2 (48.4%) and 18:3 (0.185%) and a relatively high level of 16:0 (25.1%). In general, there appeared to be an inverse relationship between the levels of 16:0 and 18:2. The highest levels of 18:1 were found in PHY 745WRF (19.9%).

Eight genotypes were grown in all nine environments. The variation exhibited by this subset of genotypes was almost equal to the variation present in the full dataset. Graphical analysis of the components from this subset of genotypes illustrates a number of points regarding differences in the effects of genotype and environment observed in the full dataset (Fig. 2). The width of the component bands in these

plots reflects a measure of the variation due to genetics. A vertical shift in the bands reflects the influence of environment. That genotype x environment effects are generally small is reflected in the roughly parallel lines that connect individual genotypes as they run through the environments. Among these eight genotypes, DP 555BR exhibited fairly high levels of 18:2 and 18:3 and the lowest levels of saturated fatty acids, 18:1(n-9), and the individual and total cyclopropenoid fatty acids. This trend was generally maintained regardless of environment (Fig. 2). At the other end of the range, FM 9063B2F exhibited the lowest levels of 18:2 and 18:3 and close to the highest levels of saturated fatty acids. FM 960B2R had slightly greater levels of 16:0 and longer-chain saturated fatty acids than did FM 9063B2F.

Table 2. Genotype means for cottonseed oil fatty acid components[z]

Variety	14:0	16:0	16:1	18:0	18:1 (n-9)	18:1 (n-7)	18:2	18:3	20:0	22:0	24:0	cpe18:1	cpe19:1	Sat[y]	Unsat[y]	Cpe[y] acids	20:0-24:0	16/18 ratio
AR 9704-13-05	0.921	24.3	0.635	2.49	18.6	0.908	50.6	0.200	0.301	0.148	0.132	0.432	0.316	28.2	71.0	0.749	0.583	0.342
AR 9704-13-08	0.925	24.4	0.636	2.43	18.1	0.895	51.1	0.196	0.291	0.142	0.131	0.426	0.336	28.3	70.9	0.762	0.566	0.344
AR 9803-23-08	0.798	22.3	0.687	2.49	17.7	0.926	53.4	0.204	0.284	0.142	0.122	0.469	0.389	26.2	73.0	0.855	0.549	0.309
ARK 9610	1.08	25.9	0.595	2.60	17.0	0.899	50.6	0.199	0.314	0.152	0.136	0.378	0.284	30.1	69.2	0.664	0.604	0.372
ARK JJ46	0.734	22.2	0.651	2.43	17.4	0.905	54.1	0.204	0.268	0.127	0.116	0.438	0.327	25.9	73.4	0.765	0.512	0.305
CS 37	0.800	23.8	0.533	2.55	17.6	0.833	52.5	0.202	0.297	0.136	0.117	0.399	0.319	27.7	71.6	0.718	0.550	0.330
CS 38	0.900	24.7	0.553	2.59	17.1	0.836	51.9	0.206	0.304	0.145	0.125	0.357	0.295	28.8	70.6	0.654	0.575	0.348
CS 44	0.926	25.3	0.616	2.42	15.8	0.892	52.6	0.201	0.299	0.135	0.120	0.408	0.302	29.1	70.2	0.711	0.554	0.360
CS 45	0.791	23.7	0.528	2.54	17.3	0.831	52.8	0.203	0.295	0.137	0.115	0.410	0.318	27.6	71.7	0.728	0.546	0.329
CS 48	0.927	25.4	0.564	2.60	15.5	0.842	52.7	0.207	0.302	0.141	0.123	0.415	0.309	29.5	69.8	0.726	0.567	0.361
CS 53	0.937	25.0	0.608	2.49	17.1	0.869	51.6	0.196	0.294	0.137	0.122	0.419	0.313	28.9	70.3	0.733	0.554	0.354
DP 141BG2RF	0.840	23.3	0.515	2.61	14.5	0.796	56.1	0.209	0.260	0.114	0.104	0.486	0.319	27.2	72.0	0.805	0.475	0.321
DP 143BG2RF	0.977	24.3	0.564	2.58	15.5	0.841	53.8	0.198	0.271	0.126	0.109	0.454	0.305	28.4	70.9	0.758	0.505	0.342
DP 147RF	0.807	24.1	0.531	2.51	16.3	0.815	53.4	0.211	0.283	0.130	0.117	0.497	0.324	27.9	71.2	0.820	0.530	0.337
DP 161BG2RF	0.689	21.6	0.618	2.71	16.2	0.875	55.9	0.196	0.261	0.117	0.108	0.399	0.291	25.5	73.8	0.693	0.483	0.292
DP 164BG2RF	0.763	22.8	0.646	2.77	16.7	0.891	54.1	0.189	0.286	0.126	0.111	0.344	0.274	26.9	72.5	0.622	0.520	0.314
DP 445BR	0.686	21.5	0.602	2.59	17.5	0.865	54.6	0.202	0.262	0.120	0.117	0.497	0.332	25.3	73.9	0.828	0.499	0.293
DP 455BR	0.778	23.5	0.527	2.43	16.0	0.831	54.2	0.227	0.281	0.141	0.115	0.523	0.376	27.2	71.8	0.896	0.536	0.327
DP 555BR	0.726	23.1	0.494	2.65	15.0	0.772	55.9	0.209	0.265	0.121	0.115	0.384	0.297	26.9	72.4	0.682	0.501	0.316
FM 9063B2F	1.06	25.1	0.666	2.77	19.5	0.865	48.4	0.185	0.334	0.152	0.118	0.426	0.337	29.6	69.7	0.763	0.604	0.360
FM 960B2R	1.03	25.3	0.611	2.76	17.7	0.832	50.3	0.186	0.336	0.156	0.121	0.424	0.331	29.7	69.6	0.755	0.612	0.361
FM 960BR	0.850	25.3	0.576	2.64	17.6	0.808	50.6	0.190	0.325	0.158	0.119	0.443	0.351	29.4	69.8	0.793	0.602	0.360
JAJO 3007	0.624	21.3	0.589	2.63	17.5	0.873	54.9	0.201	0.281	0.135	0.119	0.457	0.351	25.1	74.1	0.807	0.534	0.286
JAJO 3077	0.629	21.1	0.598	2.33	15.9	0.869	57.3	0.200	0.226	0.110	0.114	0.424	0.303	24.5	74.8	0.728	0.450	0.283
MD 391	0.952	25.1	0.585	2.46	16.3	0.860	52.2	0.201	0.297	0.139	0.122	0.467	0.320	29.1	70.1	0.787	0.559	0.357
NM 03012	0.938	24.8	0.623	2.49	17.8	0.888	50.9	0.195	0.311	0.146	0.119	0.430	0.352	28.8	70.4	0.781	0.574	0.353
NM 03K1001	0.978	25.0	0.657	2.38	17.6	0.894	51.0	0.197	0.308	0.153	0.124	0.431	0.366	28.9	70.3	0.796	0.585	0.356
NM 03N1168	0.892	24.3	0.575	2.48	18.3	0.846	51.1	0.197	0.310	0.145	0.119	0.397	0.358	28.2	71.0	0.755	0.573	0.341
NM 03S1023	0.932	24.5	0.673	2.46	19.1	0.912	50.0	0.205	0.317	0.155	0.125	0.378	0.322	28.4	70.9	0.701	0.598	0.346
NM 1155	0.858	24.1	0.584	2.45	18.6	0.860	51.0	0.196	0.304	0.145	0.121	0.417	0.364	27.9	71.3	0.779	0.570	0.338
PHY 485WRF	0.931	24.2	0.640	2.51	18.3	0.903	51.0	0.210	0.301	0.147	0.134	0.421	0.339	28.3	71.0	0.759	0.584	0.342
PHY 72	0.901	24.5	0.542	2.52	16.8	0.843	52.3	0.205	0.316	0.157	0.128	0.432	0.378	28.5	70.7	0.809	0.601	0.345
PHY 745WRF	0.770	21.8	0.621	2.51	19.9	0.902	52.0	0.208	0.287	0.143	0.123	0.369	0.353	25.6	73.7	0.721	0.554	0.297
STV 4892BR	0.936	25.2	0.621	2.56	16.4	0.884	51.9	0.205	0.307	0.147	0.125	0.417	0.312	29.2	70.0	0.730	0.579	0.358
TAM 01E-22	1.02	25.6	0.675	2.56	18.3	0.923	49.5	0.190	0.316	0.151	0.127	0.379	0.315	29.8	69.5	0.696	0.595	0.369
Overall mean	0.866	23.9	0.598	2.54	17.2	0.865	52.5	0.201	0.294	0.139	0.120	0.424	0.328	27.9	71.3	0.752	0.554	0.336

[z] Values represent best linear unbiased predictors (BLUP) of random effects.

[y] Sat = Total saturated fatty acids, Unsat = Total unstaturated fatty acids, Cpe = Total cycloprenoid fatty acids.

Of the nine environments included in the study, the dry land fields of Bella Mina, AL, in 2006, and Florence, SC, in 2007, produced the lowest fiber yields. Oils from these two environments were characterized by some of the lowest levels of 18:2 and relatively high levels of saturated fatty acids when compared with oils produced in other environments (Table 3). Both environments exhibited drought and high temperature stress during the growing season, as reflected by National Oceanic and Atmospheric Administration data for these areas and years (NOAA, Satellite and Information Service). Related to these trends, Stansbury et al. (1953) reported the iodine value of cottonseed oil (a measure of the degree of unsaturation) decreased with increased temperature and reduced rainfall, which was consistent with the compositional changes observed in these environments. Cyclopropenoid acids also appeared to be reduced in the seed from Bella Mina, AL, and Florence, SC (Fig. 2), which might indicate reduced activity of the enzymes associated with this part of the pathway.

A similar effect of environment on fatty acid profiles is known to occur in soybeans. Specifically, increased temperature and reduced moisture have been reported to decrease the relative proportion of 18:2 and 18:3 in soybean oil (Dornbos and Mullen, 1992; Wolf et al., 1982). Relative to this observation, Cheesbrough (1989) showed that elevated temperature reduced the activities of soybean FAD-II and FAD-III desaturases, with temperatures >35 °C resulting in essentially complete cessation of FAD-II activity. The decreased levels of 18:2 and 18:3 appeared to be compensated for by an increased level of 18:1(n-9) with little change in the level of 16:0 (Dornbos and Mullen, 1992; Wolf, et al., 1982). In cottonseed, decreased levels of 18:2 appeared to be compensated for by an increased proportion of several acids, including many saturated fatty acids. This suggests that there is a difference in the sensitivity of FAD-I activity with environment between soybeans and cottonseed.

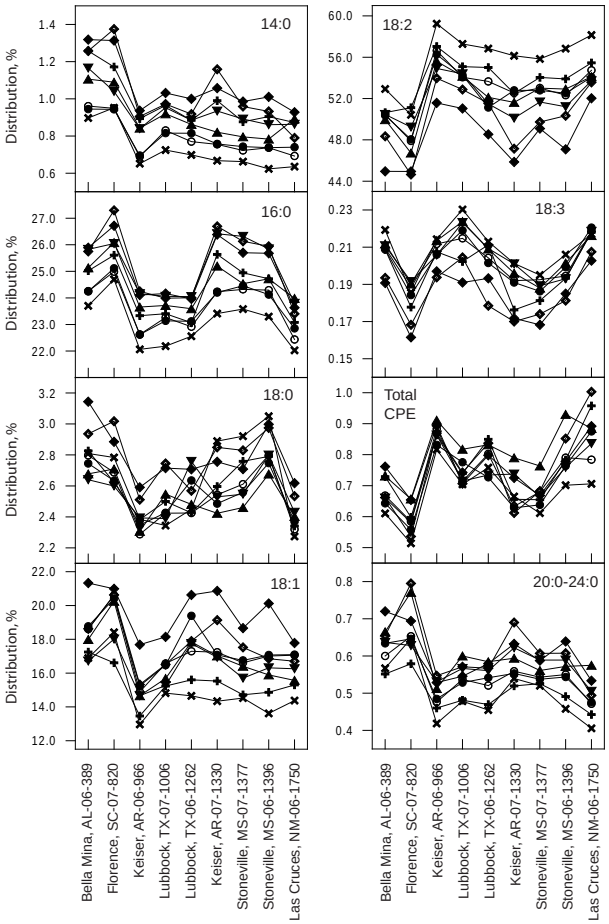

Figure 2. Variation in the percentages of selected cottonseed oil fatty acids with growing environment. Environments are ordered by increasing average lint yield, which together with the location and year are listed as part of the abscissa label. Genotypes shown are CS 37 (●), CS 45 (○), DP 143BG2RF (+), DP 555BR (×), FM 9063B2F (♦), FM 960B2R (◇), PHY 72 (▲), and STV 4892BR (▼).

Table 3. Environment means for cottonseed oil fatty acid components[z]

Environment (Loc-Yr)	14:0	16:0	16:1	18:0	18:1 (n-9)	18:1 (n-7)	18:2	18:3	20:0	22:0	24:0	cpe18:1	cpe19:1	Sat[y]	Unsat[y]	Cpe[y] acids	20:0-24:0	16/18 ratio
Belle Mina-2006	1.05	24.6	0.633	2.72	18.2	0.963	50.3	0.207	0.325	0.152	0.138	0.389	0.326	28.9	70.3	0.715	0.615	0.349
Keiser-2006	0.786	23.0	0.599	2.33	15.5	0.820	55.4	0.210	0.260	0.129	0.111	0.502	0.365	26.6	72.5	0.868	0.500	0.318
Las Cruces-2006	0.764	22.7	0.559	2.36	16.5	0.801	54.8	0.216	0.252	0.128	0.096	0.487	0.366	26.3	72.9	0.854	0.475	0.312
Lubbock-2006	0.828	23.2	0.650	2.48	17.6	0.882	52.9	0.207	0.278	0.136	0.111	0.451	0.349	27.0	72.2	0.800	0.525	0.322
Stoneville-2006	0.790	24.3	0.601	2.75	16.9	0.806	52.2	0.196	0.302	0.131	0.119	0.461	0.356	28.4	70.8	0.817	0.551	0.342
Florence-2007	1.03	25.3	0.640	2.64	19.4	0.964	48.5	0.184	0.341	0.161	0.144	0.329	0.275	29.6	69.8	0.603	0.647	0.363
Keiser-2007	0.854	24.8	0.589	2.54	17.6	0.867	51.2	0.186	0.308	0.141	0.122	0.396	0.312	28.8	70.5	0.708	0.572	0.352
Lubbock-2007	0.873	23.0	0.539	2.44	16.4	0.865	54.4	0.216	0.276	0.145	0.114	0.430	0.309	26.8	72.5	0.739	0.535	0.317
Stoneville-2007	0.816	24.7	0.574	2.63	16.7	0.819	52.4	0.187	0.305	0.131	0.128	0.374	0.293	28.7	70.6	0.666	0.565	0.348
Overall mean	0.866	23.9	0.598	2.54	17.2	0.865	52.5	0.201	0.294	0.139	0.120	0.424	0.328	27.9	71.3	0.752	0.554	0.336

[z] Values represent best linear unbiased predictors (BLUP) of random effects.

[y] Sat = Total saturated fatty acids, Unsat = Total unstaturated fatty acids, Cpe = Total cycloproprenoid fatty acids.

Although fatty acid composition of seeds grown in Bella Mina, AL, and Florence, SC, appear to have been influenced by weather conditions, relatively high levels of 16:0 were also observed in other environments, e.g., Stoneville, MS, in 2006 and 2007, and Keiser, AR, in 2007 (Fig. 2 and Table 3). This suggests other environmental factors (e.g., soil conditions or planting date) also influence cottonseed fatty acid profiles.

Associations among fatty acid components were identified from two sets of correlations based on either the environment or genotype BLUP means (Table 4). Many observed correlations appeared to have some foundation within fatty acid biosynthesis pathways. Significant positive correlations were generally observed between neighboring fatty acids when the two acids were removed from pathway branch points (Fig. 1). For example, levels of 20:0 and 22:0 were positively correlated as were levels of 22:0 and 24:0. These associations occurred regardless of whether correlations were based on genotype or environment means. A similar trend was apparent among adjacent pairs of unsaturated and cyclopropenoid fatty acids, i.e., levels of 18:2 and 18:3 and levels of cpe19:1 and cpe18:1, which were each positively correlated. For these component pairs, the correlations were stronger for variations based on environment compared with variations based on genotype (Table 4).

An inverse correlation was confirmed between 16:0 and 18:2, regardless of whether the variation was due to environment (R= -0.91**) or genetics (R= -0.75**). Lukonge et al. (2007) also observed a correlation between 16:0 and 18:2 in comparing cottonseed genotypes grown in a single environment. (Note: their correlation table (Table 3) and text indicated that this was a positive correlation, but analysis of their fatty acid data (Table 2) indicated this correlation was negative (R = -0.72) and that an error has occurred in the report.) Hence, cottonseed oils with lower levels of 16:0 tend to have higher levels of 18:2. As these two fatty acids represent greater than 70% of the total acids, the correlation reflects the dominating influence of this central part of the biochemical pathway. In this regard, there was essentially no correlation in the variation of the genotype means between the levels of 16:0 and 18:0, or between the levels of 18:0 and 18:1(n-9). An inverse association was observed between 18:1(n-9) and 18:2 both from environment and genotype means. This negative association indicates that FAD-II, which catalyzes the formation of 18:2 from18:1(n-9), has a strong deterministic influence on the distributions.

Table 4. Correlation among genotype (upper diagonal) and environment(lower diagonal) fatty acid component means[z,y]

Fatty acid	14:0	16:0	16:1	18:0	18:1 (n-9)	18:1 (n-7)	18:2	18:3	20:0	22:0	24:0	cpe18:1	cpe19:1	Sat[x]	Unsat[x]	Cpe[x] acids	20:0-24:0	16/18 ratio
14:0	-	0.91**	0.27	0.06	0.23	0.19	-0.81**	-0.34*	0.77**	0.68**	0.52**	-0.19	-0.03	0.92**	-0.92**	-0.14	0.75**	0.92**
16:0	0.60	-	0.03	0.02	0.07	-0.04	-0.75**	-0.25	0.79**	0.69**	0.49**	-0.16	-0.06	1.00**	-1.00**	-0.14	0.76**	1.00**
16:1	0.50	0.40	-	-0.07	0.59**	0.89**	-0.44**	-0.46**	0.26	0.31	0.37*	-0.23	0.10	0.06	-0.05	-0.11	0.32	0.09
18:0	0.52	0.80*	0.40	-	-0.06	-0.30	-0.02	-0.41*	0.22	-.02	-0.27	-0.23	-0.35*	0.11	-0.10	-0.32	0.06	0.02
18:1(n-9)	0.81**	0.72*	0.66	0.59	-	0.53**	-0.71**	-0.34*	0.53**	0.61**	0.51**	-0.28	0.41*	0.09	-0.09	-0.00	0.59**	0.11
18:1(n-7)	0.96**	0.51	0.62	0.40	0.84**	-	-0.34*	-0.20	0.13	0.26	0.50**	-0.25	0.07	-0.03	0.04	-0.14	0.26	0.01
18:2	-0.79*	-0.91**	-0.61	-0.77*	-0.94**	-0.77*	-	0.42*	-0.92**	-.89**	-0.68**	0.29	-0.23	-0.77**	0.77**	0.09	-0.93**	-0.78**
18:3	-0.24	-0.89**	-0.30	-0.58	-0.52	-0.21	0.71*	-	-0.39*	-.21	-0.02	0.37*	0.17	-0.30	0.28	0.34*	-0.29	-0.27
20:0	0.78*	0.95**	0.50	0.84**	0.83**	0.71*	-0.96*	-0.75*	-	0.92**	0.57**	-0.27	0.24	0.82**	-0.82**	-0.08	0.96**	0.80**
22:0	0.95*	0.57	0.42	0.42	0.83**	0.94**	-0.78*	-0.29	0.75*	-	0.72**	-0.19	0.43**	0.70**	-0.71**	0.07	0.98**	0.71**
24:0	0.84**	0.88**	0.51	0.75*	0.74*	0.76*	-0.89**	-0.65	0.96**	0.78*	-	-0.26	0.16	0.48**	-0.48**	-0.11	0.75**	0.51**
cpe18:1	-0.75*	-0.85**	-0.29	-0.61	-0.78*	0.70*	0.87**	0.71*	-0.89**	-0.77*	-0.88*	-	0.48**	-0.19	0.15	0.91**	-0.27	-0.16
cpe19:1	-0.62	-0.71*	-0.04	-0.43	-0.59	-0.56	0.67*	0.64	-0.74*	-0.70*	-0.76*	0.94**	-	-0.08	0.04	0.79**	0.30	-0.05
Sat[w]	0.67	0.99**	0.44	0.84**	0.75*	0.58	-0.93**	-0.84**	0.98**	0.63	0.92**	-0.87**	-0.71*	-	-1.0**	-0.17	0.78**	1.00**
Unsat[w]	-0.65	-0.99**	-0.45	-0.85**	-0.75*	-0.56	0.93**	0.84**	-0.97**	-0.61	-0.91**	0.84**	0.68*	-1.00**	-	0.12	-.78**	-1.00**
Cpe acids[w]	-0.71*	-0.81**	-0.20	-0.55	-0.72*	-0.66	0.81**	0.69*	-0.85**	-0.76*	-0.85**	0.99**	0.98**	-0.82**	0.79*	-	-0.04	-0.14
20:0-24:0	0.87**	0.90**	0.51	0.76*	0.84**	0.81**	-0.95**	-0.66	0.98**	0.85**	0.98**	-0.91**	-0.78*	0.93**	-0.92**	-0.87**	-	0.78**
16/18 ratio	0.62	1.00**	0.43	0.80**	0.74*	0.55	-0.92**	0.88**	0.96**	0.59	0.90**	-0.86**	-0.71*	1.00**	-0.99**	-0.81**	0.91**	-

[z] Pearson correlation coefficients among genotype and environment best linear unbiased predictors (BLUP) of random effects.

[y] * = $p < 0.05$, ** = $p < 0.01$

[x] Sat = Total saturated fatty acids, Unsat = Total unstaturated fatty acids, Cpe = Total cycloproprenoid fatty acids.

Synthesis of cpe19:1 is believed to occur by a two-step process from 18:1(n-9) (Bao et al., 2002; Yano et al., 1972). The first step in the pathway involves addition of a methyl group from *S*-adenosylmethionine to produce dihydrosterculic acid. The second step is presumed to be a desaturase reaction that converts dihydrosterculic acid into cpe19:1. Cpe18:1 is presumed to be formed from cpe19:1 by an α-oxidation process (Bao et al., 2002; Yano et al., 1972). Levels of cpe18:1 and cpe19:1 were positively correlated based on both genotype means (R=0.48**) and environment means (R=0.94**). Regardless of the source of variation, the positive association of cpe18:1 with cpe19:1 was the strongest effect observed, which supports the proposition that the shorter cyclopropenoid acid is formed from the longer cyclopropenoid acid.

Formation of 18:1(n-7), which was reported as a component of cottonseed oil by Radcliffe et al. (2001), occurs by two-carbon elongation from 16:1 (Shibahara et al., 1989). Positive correlations were observed between the relative levels of these acids. From the genotype means the correlation between these acids was R = 0.89**. From the environment means the correlation was also positive but less strong (R = 0.62).

Based on these associations, one can speculate on what cottonseed oil modifications might be possible among agronomic cotton genotypes. Lower levels of 16:0, 18:2, cpe18:1, and cpe19:1 and higher levels of 18:1 and possibly 18:0 would be ideal. Based on the correlations, however, this ideal distribution appears unlikely. As 16:0 and 18:2 were strongly inversely correlated, decreasing the level of 16:0 appears to lead necessarily to higher levels of 18:2. Hence, it appears unlikely both acids can be simultaneously reduced. Higher levels of 18:1 were correlated with lower levels of 18:2, so this change seems likely, but possibly with a concomitant increase in the level of 16:0. Hence, a reduction of 16:0 and an increase in 18:1 also does not appear favorable. Because cyclopropenoid fatty acids did not correlate strongly with any other fatty acid components, it may be possible to reduce their concentration without negatively influencing other compositional factors.

Of course, correlation analysis does not establish cause and effect, and these inferences are based on a small number of cotton genotypes representing a narrow range of cotton genetics. In addition, genetically modified cottonseeds have been reported with compositions that that do not support the correlations identified in the current study. Specifically, Liu et al. (2002) down-regulated FAD-II in cotton resulting in reduced levels of both 16:0 and 18:2. This difference emphasizes the complicated associations that likely exist among oilseed fatty acids, which can be influenced by processes that include not only acid synthesis but also transfer of acids between various carrier molecules and triglycerides, and transport mechanisms used to move glycerides between organelles and oil storage bodies. Consequently, analysis of a wider range of genetic material might significantly alter these associations.

Only a few studies have attempted to discuss variability in cottonseed fatty acid profiles, the source of this variation, or how fatty acid percentages correlate with each other. Lawhon et al. (1977) studied seed composition of eight glanded and eight glandless cotton genotypes produced at various locations. Only limited variation in fatty acid composition was reported and the glandless trait did not significantly affect seed oil composition (Table 5). Hamza et al. (1988), Nergiz et al. (1997), and Lukonge et al. (2007) studied fatty acid profiles of small numbers of genotypes (11, 16, and 24, respectively) grown at a single location for a single year (Table 5). As with the Lawhon et al. (1977) study, these studies showed fairly limited variation in fatty acid composition (Table 5). Yunusova et al. (1991) considered a handful of genotypes and their F1 progenies over two growing seasons (Table 5). Higher levels of saturated fatty acids occurred during the second year, which was characterized as having lower average humidity and higher temperature compared with the conditions of the first year. This is similar to our observation that higher temperatures and dryer growing conditions reduced 18:2 and increased saturated fatty acids. The effect, however, appeared variable among the studied genotypes, suggesting that a significant genotype x environment effect exists within their genotypes, which is contrary to our results. Yunusova et al. (1991) also reported that genotype L-78, a linter-free seed, had 16:0 and 18:2 levels of 43 and 26%, respectively, which is essentially reversed from the values expected for these acids in typical cottonseed oil. Hence, cotton germplasm may exist with substantially different oil properties. We note that these atypical levels of 16:0 and 18:2 reflect the same inverse correlation between these components that was found among our genotypes and environments and among the genotypes studied by Lukonge et al. (2007).

Table 5. Comparison of measured fatty acid profiles from the 2006 and 2007 National Cotton Variety Trials with prior surveys

Fatty acid	Measured values (this work)	CODEX standard for cottonseed oil	Lawhon et al. 1977[z]	Hamza et al. 1988[y,x]	Yunusova et al. 1991	Nergiz et al. 1997	Lukonge et al. 2007
14:0	0.56-1.4	0.6-1.0	0.6-1.5	0.7-1.2	0.2-0.6	0.67-1.08	0.62-0.93
16:0	19.6-27.6	21.4-26.4	17.6-26.0	23.0-25.5	20.7-43.2	20.1-26.8	20.6-25.1
16:1 (n-7)	0.43-0.79	nd-1.2	nr	0.9-1.5	0.8-3.2	0.82-1.23	0.41-0.59
18:0	2.0-3.2	2.1-3.3	1.9-2.5	2.1-2.9	0.9-4.4	1.87-2.37	0.22-2.79
18:1 (n-9)	12.8-22.2	14.7-21.7	15.0-19.2	16.7-19.8	11.3-26.9	14.0-17.6	15.2-18.5
18:1 (n-7)	0.69-1.1	-	nr	nr	nr	nr	nr
18:2	44.0-59.3	46.7-58.2	52.1-60.5	44.5-54.3	26.1-58.8	51.2-59.2	52.0 -57.2
18:3	0.15-0.25	nd-0.4	nr	nr	nr	tr-0.51	nd-0.17
20:0	0.20-0.45	-	nr	nr	nr	0.00-0.16	0.22-0.33
22:0	0.08-0.21	-	nr	nr	nr	nr	0.11-0.18
24:0	0.08-0.20	-	nr	nr	nr	nr	nd-0.23
cpe18:1	0.22-0.65	-	nr	nr	nr	nr	nr
cpe19:1	0.20-0.46	-	nr	nr	nr	nr	nr
%Sat[w]	23.0-32.8	-	20.4-29.0	nr	23.2-45.3	22.9-30.3	24.2-29.0
%Unsat[w]	66.6-76.1	-	70.0-79.6	nr	54.7-74.7	69.7-73.7	70.2-74.9
Cpe acids[w]	0.43-1.1	-	0.06-0.32	-	-	-	-
20:0-24:0	0.39-0.81	0.2-1.2	-	-	-	0.00-0.16[v]	-
16/18 ratio	0.259-0.408	-	0.215-0.359	-	0.274-0.824	0.268-0.395	0.276-0.354

[z] Reported a range of 0.0-0.8% of unidentified fatty acids.

[y] Profile was broken down by polar and storage lipids; listed values are for triglycerides, but similar profiles were reported for polar and total lipids.

[x] Reported a range of 0.0-1.8% for several additional minor fatty acids.

[w] Sat = Total saturated fatty acids, Unsat = Total unstaturated fatty acids, Cpe = Total cycloproprenoid fatty acids.

[v] Only 20:0 was reported.

The range of measured variation in the individual fatty acids in this work was similar to the range of variation reported in most prior studies (Table 5), with the exception of values for the Uzbek L-78 genotype (Yunusova et al., 1991). The range of values for individual fatty acids tended to be slightly broader than the range reflected in the Codex alimentarius trading standard for cottonseed oil (FAO/WHO Food Standards, 1999). Although genetics accounted for a significant amount of fatty acid variation, the overall range of variation was insufficient to indicate that breeding within this population would produce desirable changes in oil composition. Hence, efforts to breed cotton plants for improved oil properties will require a broader survey of cotton germplasm, perhaps including other *Gossypium* species, or possibly mutagenesis-based developmental efforts, which have been useful for modifying fatty acid profiles of other oilseeds (Fehr et al., 1991; Osorio et al., 1995).

In summary, current commercially acceptable cotton genotypes show some variation in fatty acid composition, and this variation is associated with both genetics and environment with minimal interaction between these effects. Because environment was found to affect seed oil composition, more work is needed to better delineate this influence. Finally, little documentation exits on the genetic association among oil and fatty acid composition with lint yield, fiber traits, and other useful traits, all of which must be considered when tailoring the cotton plant for value-added seed properties.

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