LIPIDS OF *Gossypium mexicanum*

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The surface lipids of the coat and the lipids of the kernel of the seeds of a wild form of the medium-fibered cotton plant *Gossypium mexicanum* var. *nervosum* have been studied in comparison with cultured species. Using the methods of CC, TLC, and GLC, and UV and mass spectrometry, in the cuticular lipids of *G. mexicanum* three classes of neutral lipids have been detected, and in the kernel lipids 10 classes of neutral lipids, 2 classes of glycolipids, and 7 classes of phospholipids. The structures of the epoxyacyl-, hydroxyacyl-, and diacylglycerols of the kernel have been established. The greatest differences between the seeds of the wild form of the cotton plant and the cultured species are the greater weight of the seed coat, the presence in it of a considerable amount of the 18:1 and of medium-molecular-weight fatty acids, and a higher level in the kernel of gossypol pigments, polar lipids, and acylglycerols containing the 18:2 acid.

One of the most effective methods for creating wilt-resistant varieties of the cotton plant is remote hybridization, which leads to a sharp change in the genetic interrelationships in the parasite–host system [1]. In the cotton plant of the species *Gossypium hirsutum*, a wild form of *G. mexicanum* var. *nervosum* has been isolated which is resistant to the pathogenic fungus *Verticillium dahliae* Kleb. By crossing it with varieties having different susceptibilities, the Tashkent-1 Tashkent-6, and other wilt-resistant varieties of medium-fibered cotton plant have been created [1]. There is information that the lipid components of the seeds [2] and of the seed coat [2, 3] participate in the formation of the resistance of the plant to diseases.

There is no information in the literature on the lipids of *G. mexicanum*.

We have investigated the surface lipids (SLs), the reserve lipids (RLs), and the cell lipids (CLs) of freshly gathered seeds of *G. mexicanum* in comparison with the lipids of the Tashkent-1 variety [4].

The seeds of the wild form were dark brown in color, ovoid, with a size of 0.7 × 0.4 cm; the weight of the husks of 1000 seeds was 20.5 g and the weight of 1000 seed kernels was 30.0 g.

The surface lipids of the coat were isolated by brief treatment of undamaged seeds with chloroform. For comparison we studied the SLs of the seeds of cotton plants of the Tashkent-1 variety. The yield of SLs on the weight of the seeds was 0.1% for *G. mexicanum* and 0.08% for Tashkent-1.

According to the results of TLC (systems 1, 2, and 3) the main component of the SLs of the two samples consisted of wax esters, while triacyl glycerols (TAGs) and free fatty acids (FFAs) were present in trace amounts.

The fatty acid compositions of the SLs of the two samples, determined by GLC, are given (wt.%) at the top of the next page.

As we see, the seed coats of *G. mexicanum* had somewhat higher levels of SLs than those of Tashkent-1, and these were enriched with unsaturated (mainly monoenoic) and medium-molecular-weight fatty acids.

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Acid	*G.* mexicanum	Tashkent-*1*
12:0	Tr.	—
13:0	2.8	
14:0	2,8	10.4
15:0	1.7	Tr.
16:0	18.4	35,2
16:1	10,3	9.1
17:0	4.9	5.3
18 0	6,2	3,0
18:1	13,2	5,2
19:0	4 6	Tr.
18 2	6 9	Tr.
20 0	6,9	11.9
21.0	1,6	12 0
22:0	11.7	7,9
Σ_{sat}	69 6	85.7
Σ_{unsat}	30.4	14.3

After the elimination of the SLs, the seeds were ground and the remaining lipids were extracted with a mixture of chloroform and methanol. The yield of RLs and CLs amounted to 18.2% of the weight of the seeds (or 30.6% of the weight of the kernels) for *G. mexicanum*, which was 1.4 times smaller than the level of these lipids in the variety Tashkent-1 (44.7% of the weight of the kernels).

The dark brown color of the lipids of the seeds of *G. mexicanum* and Tashkent-1 is due to gossypol pigments, which were eliminated by column chromatography (CC) on polyamide. As a result, the following were obtained (% on the weight of the seed lipids): reserve (or neutral) lipids — 89.6 and 94.3; gossypol pigments — 3.2 and 1.1; glycolipids (GLs) — 3.2 and 2.8; phospholipids (PLs) — 4 and 1.8, respectively. Thus, the amount of pigments and of cell lipids (GLs and PLs) in the seeds of the wild form was 10.4%, while in freshly gathered Tashkent-1 seeds their level was little more than half this.

The reserve lipids were analyzed in more detail. Rechromatography by CC on silica gel led to their separation into individual classes, and the amounts of these were determined gravimetrically. The composition of the RLs (% by weight) was: carbohydrates + sterol esters — 1.8; fatty acid methyl esters (FAMEs) — traces; triacylglycerols — 85.6; epoxyacyldiacylglycerols (EAGs) — 0.5; free fatty acids — 4.0; hydroxyacyldiacyl (HAGs) — 0.5; diacylglycerols (DAGs) — 4.0; sterols — 0.9; and monoacylglycerols (MAGs) — 2.7. With respect to the set of classes and their quantitative ratios, the RLs of *G. mexicanum* were close to those of medium-ripe seeds of the Tashkent-1 variety [4], but the level of HAGs with the fungitoxic 12-OH-9-18:1 acid [5] in them was substantially lower.

The sterol esters, mixed with the hydrocarbons, were saponified and the liberated sterols and alkanes were taken off as unsaponifiables and were separated by preparative TLC in system 3. Mass spectrometric analysis showed the presence in the hydrocarbons of the C-25, C-27, C-29, and C-31 paraffins with a predominance of the C-29 species. The sterols esterified by fatty acids and also those in the free form consisted, according to GLC, of 98% of β-sitosterol and 2% of campesterol.

The fatty acids were isolated from the saponifiable fraction of the sterol ethers and their composition was determined by GLC (Table 1). The acid composition of this class was distinguished by the highest level of the 16:0 species.

The structure of the TAGs was established from the results of hydrolysis by pancreatic lipase. The fatty acid composition of the 2-monoacyl glycerols (2-MAGs, see Table 1) obtained was close to that for the Tashkent-1 variety. The TAGs of *G. mexicanum* did not differ from the TAGs of ripe seeds of this variety with respect to the selectivity factor [6] of the 16:0, 18:1, and 18:2 acids for the sn-2 position and to the variety and quantitative ratio of the species (18 species) [7].

The structure of the EAGs was calculated by the use of a combination of the methods of alkaline hydrolysis, preparative TLC, GLC, acetolysis, and the mass spectrometry of trimethylsilyl derivatives (TMSs).

The composition and quantitative ratio of the epoxy acids ep-18:1 and ep-18:0 was obtained from the results of the GLC of their MEs (Table 1). A mass-spectrometric analysis of the TMS derivatives showed that the epoxy acids represented by the 12,13-epoxy-9-18:1 acid (the main species), the 9,10-epoxy-12-18:1 acid (traces), and the 9,10-epoxy-18:0 acid. The

TABLE]. Composition of the Fatty Acids of the Seed Lipids of *Gossypium mexicanum*

Acid	Seed lipid	Reserve lipids									GLs	PLs
		sterol esters	FAMEs	TAGs	EAGs	FFAs	DAGs	HAGs	MAGs	2-MAG		
12:0	—	—	—	—	—	--	—	—	0,5	—	—	—
14:0	0 6	3 1	—	1,0	0,8	2,1	0,6	0,3	0,7	0,5	0 8	0,5
15 0	—	1,5	—	—	—	—	Tr.	Tr.	0,7	—	—	—
16 0	19,1	33,3	20 4	23,5	14,9	30,0	21,2	18,8	27,5	2,5	23.8	21,1
16:1	1,1	2,5	6 3	0,8	0,8	1,8	1.5	1,9	4,7	1,5	0,6	1,0
17:0	—	2,1	—	—	—	—	—	Tr.	—	—	—	—
18 0	2,5	12,8	19,2	2,0	1.5	7,9	2,0	1,0	3,8		4,1	7,1
18 1	14,8	22,1	23,3	13,7	8,2	22,5	49,7	30.5	38,8	14.5	13.5	16,4
18.2	61,9	22,6	30.8	59 0	40.8	35,7	15,0	14 5	23.3	81,(	57.2	53,7
EP-18:1	-	—	—	—	31,0	—	—	—	—	—	—	—
EP-18:0	—	—	—	—	2,0	—	—	—	—	—	—	—
Hydroxyunsaturated FAs	—	—	—	—	—	—	—	33,0	—	—	—	—
$\Sigma_{sát}$	22.2	52.8	39,6	26.5	19,2	40,0	23.8	20,1	33.2	3.0	28,7	28,9
Σ_{unsat}	77,8	47,2	60,4	73.5	80,8	60,0	66,2	79,9	6>.8	97,0	71,3	71,1

same set of epoxides has been detected in the lipids of the Tashkent-1 variety of cotton plant [8, 9].

The fatty acids were isolated from part of the DAGs and HAGs by alkaline hydrolysis, and the composition of the unsubstituted fatty acids was determined by GLC (see Table 1). To calculate the species composition of the DAGs and HAGs and the structures of the hydroxy acids composing the HAGs, another part of these lipids was converted into TMS derivatives and their mass spectrum was recorded.

The mass spectrum of the TMS derivatives of the DAGs contained the M^+ peaks and the $(M^+ - 15)$, $(M^+ - 90)$, $(M^+ - 103)$, and $(M^+ - CH_2OCOR)$ fragments. An appreciable intensity of the M^+ peaks and the fragments mentioned was shown by the TMS derivatives of the 18:1-18:1 DAG (M^+ 692) and the 16:0-18:1 DAG (M^+ 666), while low intensities were shown by the 16:0-18:2 (M^+ 664) and 16:0-16:0 (M^+ 640) species. No fragments were detected in the mass spectrum that could have been assigned unambiguously to DAGs with different combinations of fatty acyl residues.

The HAGs were subjected to UV spectrometry in order to detect α-hydroxydienoic fatty acids with conjugated π-bonds, since we have identified acids with such a structure previously in a cultured cotton plant [8]. It was found that the HAGs of the wild form contained these acids in an amount comparable with that of the HAGs of moderately ripe seeds.

The mass-spectrometric analysis of the TMS derivatives of the HAGs showed that with respect to the remaining components the hydroxy acids of the HAGs of *G. mexicanum* did not differ basically from the HAGs of the Tashkent-1 seeds [10].

In the mass spectrum of the TMS derivatives of the HAGs, the peaks of ions were found that are characteristic for the breakdown both of TAGs [11] and of the TMS derivatives of hydroxy fatty acids [10]. The M^+ and $(M^+ - 90)$ ions corresponded to TAGs with two unoxidized acyl residues, mainly 18:1, 16:0, and 18:2 (see Table 1) and with one residue of the TMS derivatives of the 18:1, 18:0, 18:2, and 17:1 monohydroxy acids. The spectrum contained fragments of hydroxyacyl residues of the $[R(TMS)CO]^+$, $[R(TMS)COO]^+$, $[R(TMS)COOH]^+$, and $[R(TMS)\cdot COOH_2]^+$ types, which are also known for ordinary acyl radicals; fragments formed on the successive elimination of these residues from the sn-1 and sn-3-positions of the TMS derivatives of the TAGs; and characteristic breakdown fragments of the TMS derivatives themselves containing the CH_3-ends of the acid chains (m/z 187, 173, 227, 225, 215, 213, 211) [10], and also fragments of the type of $(M^+ - RCOOH - 187)$. The intense peaks of the $[M^+ - R(TMS)COOCH_2]$, $[M^+ - R(TMS)COO]$, and $[M^+ - R(TMS)CO]$ ions together with fragments of the type of $[R(TMS)CO + 74]^+$ and $[M(TMS)CO + 128]^+$ show that the hydroxy acids were present predominantly in the sn-3- and sn-1 positions and to a smaller degree in the sn-2-positions of the HAGs.

In the total gossypol pigments, the amount of gossypol itself was 19% (or 0.6% of the weight of the lipids). The chromatographic mobility of the gossypol pigments in TLC (system 4) and the qualitative reaction of the spot (R_f 0.75) corresponded to the behavior of a model

sample of the pigment isolated from the seeds of the cultured cotton plant. The other pigments (R_f 0.86 and 0.62 in system 4) were not identified.

The glycolipids and phospholipids were analyzed by TLC on silica gel using specific visualizing agents.

Of the classes of GLs we detected (system 5) digalactosyldiacylglycerols (R_f 0.55) and monogalactosyldiacylglycerols (R_f 0.68) with a predominance of the latter.

In the PLs, seven phosphorus-containing classes were detected by two-dimensional TLC in systems 6 (direction I) and 7 (direction II). Visually, their ratio was determined as PCs > PIs > PEs > lyso-PCs > N acyl-PEs > lyso-PEs > N-acyl-lyso-PEs, where PCs are phosphatidylcholines, PIs phosphatidylinositols, and PEs phosphatidylethanolamines.

The set of fatty acids in the lipids of the seeds of *G. mexicanum* is given in Table 1. A comparison of the figures in the table with those from the literature [12, 13] shows that the CLs of the wild form of the cultured variety do not differ appreciably with respect to the set of lipids. Acids of the TAGs, the FFAs, and the GLs of *G. mexicanum* have a higher level of the 18:2 acid but a lower level of the 18:1 acid and of saturated acids.

EXPERIMENTAL

UV spectra were obtained on a Hitachi spectrometer in hexane, and mass spectra on a MKh 1310 spectrophotometer at an energy of the ionizing electrons of 40-50 eV and a temperature of the ionization chamber of 90-100°C.

Gas-liquid chromatography was performed on a Chrom-4 chromatograph with a flame-ionization detector and a stainless-steel column filled with Chromaton N-AW-DMCS impregnated with 15% of Reoplex-400 or 17% of ethylene succinate for the MEs of the unsubstituted fatty acids; and filled with Chromaton N-AW-DMCS impregnated with 5% of SE-30 for the TMS derivatives of the MEs of the hydroxy acids. The column dimensions were 4 mm × 2.5 m and the temperature 198°C, or 220°C for the TMS derivatives of the MEs; the rate of flow of carrier gas (helium) was 0.62 kg/cm^2, that of H_2 60, and that of air 0.6 liter/min.

TLC on Silufol and silica gel was performed by the method described in [14] in the following solvent systems: 1) hexane–ether (9:1); 2) hexane–ether (8:2); 3) hexane–ether (7:3); 4) benzene–methanol (20:5); 5) chloroform–methanol–water (65:25:4); 6) chloroform–methanol–water (65:35:5); 7) chloroform–methanol–acetone–acetic acid–water (40:20:16:8:4); 8) chloroform–methanol–water (90:10:1); and 9) hexane–ether (5:5).

The seeds of *G. hirsutum* ssp. *mexicanum* var. *nervosum* 06422 from the autumn crop were supplied by A. G. Kas'yanenko (Department of General Genetics of the Cotton Plant, Academy of Sciences of the Tadzhik SSR).

The surface lipids were removed by three treatments of the seeds with hot chloroform for 1 min.

The reserve and cell lipids were extracted from the crushed seeds by steeping with chloroform–methanol (2:1) at room temperature for three days five times.

The carbohydrates were isolated from the evaporated lipid extracts by CC on Molselekt G-25 (Reanal), which before introduction into the column was wetted for 12 h in solvent system 8. A 3 × 10 cm column was used and the ratio of extract to adsorbent was 1:1.5. The lipids were eluted from the column with solvent system 8.

The neutral and polar lipids were separated by column chromatography on polyamide (1 mm) previously washed with acetone at a ratio of extract to adsorbent of 1:8 using a 3.4 × 106 cm column. The neutral lipids were eluted with hexane, the gossypol pigments with solvent system 9 and with diethyl ether, the glycolipids with acetone, and the phospholipids with methanol. The completeness of elution of each class was checked by TLC.

The column chromatography of the reserve lipids was performed as described in [14].

The lipids were identified by qualitative reactions, by a comparison of their chromatographic mobilities with those of model samples, by chemical transformations and by their UV and mass spectra. Epoxy compounds were detected with an ethanolic solution of picric acid [14]; glycolipids with α-naphthol; phospholipids with the Vas'kovskii [Vaskovsky] reagent with ninhydrin, and with the Dragendorff reagent [15].

Alkaline hydrolysis was carried out as described in [14], the acid methanolysis of glycolipids as in [12], the opening of epoxide rings as in [8], and pancreatic hydrolysis as in [7].

The fatty acids were esterified with diazomethane. The TMS derivatives of the hydroxy acids were obtained as described in [10]. The amount of free gossypol was determined by a handbook method [16].

SUMMARY

The seeds of a wild form of the cotton plant differ from the seeds of the cultivated species by a greater weight of the coat, by the presence in it of a considerable amount of 18:1 and medium-molecular-weight fatty acids, and by a higher level in the kernel of gossypol pigments, polar lipids, and acylglycerols containing the 18:2 acid.

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