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PHOSPHOLIPIDS OF THE MEDIUM-FIBER COTTON PLANT VARIETY 159-F

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We have investigated the phospholipids (PLs) of the seed kernels of the industrial medium-fiber cotton plant variety 159-F of introduction 2, 1972 harvest (sample 1), and "Elite" of the same variety, 1974 harvest (sample 2), collected in the Pakhtaabad region of Fergana oblast.

The seed kernels of the variety under consideration were characterized by the following indices [1] (%):

Index	Sample 1	Sample 2
Proportion of kernels	59.55	60.43
Moisture content	5.3	5.2
Oil content	38.42	39.03
Phosphorus content	0.98	0.98
Gossypol content	0.76	0.75

As can be seen from the figures given above, the main indices of the seed kernels of the samples studied are practically identical.

The ground seed kernels were defatted with petroleum ether and were freed from gossypol with acetone as described previously [2]; phospholipids were exhaustively extracted with a mixture of ether and methanol (2 : 1) [3]. The crude PL fraction was precipitated with acetone (0°).

To determine the distribution of the PLs in their isolation, all the lipid fractions (for sample 1) were checked with respect to yield and lipid phosphorus content (Table 1) and subjected to qualitative chromatography in a thin layer of silica gel (systems 1 and 2). The acetone-insoluble part was enriched with PLs, 2.57% P, and with carbohydrates (25%), while 12.3% of the lipid phosphorus passed into the acetone-soluble part. The carbohydrates were separated from the acetone-insoluble part and were determined by quantitative gel filtration on "Mol-Selekt" G-25 [4], the PLs being eluted with the solvent system chloroform–methanol–water (90 : 10 : 1), and the carbohydrates with water. The PLs of the acetone-soluble part were regenerated in a column containing silica gel, the neutral lipids being eluted with chloroform and the PLs with methanol. This part of the PLs was added to the bulk of the PLs from the acetone-insoluble part (see Table 1).

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TABLE 1. Distribution of Phosphorus in the Lipids of the Fractions of Samples 1, %

Fraction	Yield		Phosphorus content in the fractions	Distribution of the lipid phosphorus	
	on the kernel	on the total of all the fractions		on the kernel	on the sum of all the phosphorus
Isolation					
Petroleum-ether	30,2	76,8	0,0033	0,001	1,8
Acetone	3,9	9,9	0,141	0,0055	10,2
Chloroform–methanol	5,2	13,3	0,91	0,0478	88,0
Precipitation of the phospholipids with acetone					
Acetone-insoluble	1,6	4,1	2,57	0,0411	75,7
Acetone-soluble	3,6	9,2	0,187	0,0067	12,3
Purification of the acetone-insoluble part on "Mol-Selekt" G-25					
Phospholipid	1,2	3,1	3,27	0,0392	72,2
Purification of the acetone-soluble part by column chromatography SiO_2					
Chloroform	3,2	7,9	0,0098	0,0003	0,5
Methanol	0,28	0,70	2,31	0,0064	11,8
Total phospholipids					
Phospholipid	1,48	3,8	3,08	0,0456	84,0

TABLE 2. Fatty-Acid Compositions of the Lipid Fractions, %

Fatty acid	Petroleum ether extract		Acetone extract		Neutral lipids		Phospholipids	
	free lipids of the sample				bound lipids of the sample			
	1	2	1	2	1	2	1	2
12:0	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
14:0	1,2	1,3	2,4	1,9	2,3	1,9	1,7	2,7
16:0	24,5	23,6	23,8	22,9	23,2	25,5	29,6	30,3
16:1	1,1	1,4	5,4	5,1	3,3	1,4	2,4	4,8
18:0	2,2	2,2	2,7	2,9	3,5	1,4	3,2	2,5
18:1	19,4	19,3	27,1	26,3	19,3	19,0	18,3	14,7
18:2	51,6	52,2	38,6	41,0	48,4	50 8	44,8	45,0
Σ_s	27,9	27,1	28,9	27,7	29,0	28,8	34,5	35,5
Σ_u	72,1	72,9	71,1	72,3	71,0	71,2	65.5	64,5

Thus, taking the PLs in the petroleum-ether and acetone fractions into account, the yield of combined PLs for sample 1 was 1.76% and for sample 2 1.85%. Of the lipid phosphorus, 12.2% was contained in the petroleum-ether and acetone extracts the qualitative composition of which differed only slightly from that of the total PLs. Of the total amount of phosphorus in the seed kernels 5.5% was lipid phosphorus.

The qualitative and quantitative compositions of the PLs of both samples were determined by two-dimensional TLC on silica gel in solvent system 1 (direction I) and 2 (direction II). The PLs were identified by the usual methods. The quantitative compositions of the individual groups of PLs were determined after TLC by Tevekelov's method [5]. The results of the determinations are given below (%); X_1, X_2, X_3, X_4 – unidentified PLs):

PLs	Lyso-PLs	PI	PC	X_1	PE	X_2	X_3	X_4
R_f (syst. 2)	0.25	0.45	0.54	0.67	0,75	0.83	0.87	0.95
Sample 1	2.2	18.1	60.8	1.9	6.6	1.6	4.1	4.7
2	1.1	16.9	65.2	2.6	7.9	1.7	4.6	Tr.

The qualitative composition of the PLs of both samples of the cotton plant were similar, but appreciable differences were found in the quantitative amounts of the minor components: lyso-PC and X_4.

The main components in order of decreasing amount were phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidylethanolamine (PE), i.e., the same characteristics are observed as in other medium-fiber

TABLE 3. Position Distribution of the Fatty Acids, %

Fatty acid	Phosphatidylcholine			Phosphatidyl-ethanolamine			Phosphatidylinositol		
	total	position		total	position		total	position	
		1	2		1	2		1	2
12:0	—	—	—	1,4	—	1,9	2,5	—	—
14:0	0,7	2,2	—	1,1	1,4	1,5	2,5	—	1,3
16:0	23,2	42,8	3,4	23,1	55,7	3,4	32,8	73,2	4,4
16:1	—	—	—	0,9	—	1,2	2,9	—	1,9
18:0	2,3	4,6	—	1,0	3,9	—	4,3	10,3	—
18:1	24,2	20,8	27,8	12,8	5,8	14,0	7,5	6,0	8,9
18:2	49,6	29,6	68,8	59,7	33,2	78,0	47,5	10,5	83,5
Σ_s	26,2	59,6	3,4	26,6	61,0	6,8	42,1	83,5	5,7
Σ_u	73,8	40,4	96,6	73,4	39,0	93,2	57,9	16,5	94,3

[2, 6] and thin-fiber varieties of the cotton plant [4, 7]. In the variety investigated the amount of PC was 5-10% higher than in those mentioned, the PI was close to the mean figures, and the PE was considerably less.

By comparing the qualitative and quantitative indices of the PLs of the variety studied with other varieties of cotton plant grown in Uzbekistan [2, 4, 6, 7], we see that while the tissue PLs are qualitatively very similarly, they differ quantitatively. This is possibly one of the variety characteristics of the cotton plant, when the biosynthesis of the PLs of the cells of the plant tissues goes in a definite direction.

We have studied the total fatty-acid composition of all the lipid fractions (Table 2).

The qualitative composition of the fatty acids (FAs) of samples 1 and 2 was the same, but their quantitative composition differed somewhat. Among the unsaturated FAs the 18:2 acid predominated, and among the saturated the 16:0. The minimum amount of the 18:2 acid was found in the acetone fraction. The amount of the 16:0 fraction was a maximum in the total PLs and of the 18:1 acid in the acetone fraction. The total saturation was greatest in the PLs, which is harmony with the results of the work of many authors on plant phospholipids.

On the basis of the fact that the quantitative, qualitative, and fatty-acid compositions of the intralipid fractions of the two samples were fairly close, we made a detailed study of the FA distribution of the main phospholipids of sample 2.

By the usual methods of column chromatography on silica gel (eluents: chloroform, chloroform–methanol, and pure methanol) and rechromatography by TLC (system 2) we isolated homogeneous fractions of PCs, PEs, and PIs. They were all characterized by physical and chemical methods as known compounds.

Since a characteristic feature of these classes of natural substances for different species of plants is their fatty-acid composition, we studied the total composition and position distribution of the fatty-acid-radicals in their molecules, using enzymatic hydrolysis with kufi snake venom at pH 10.15 [8]. The results obtained are shown in Table 3.

The total unsaturation of the FAs in the PCs and PEs was the same, while in the PIs it was smaller by 16%. The amount of 18:1 acid in the PCs was twice that in the PEs and three times that in the PIs. The amount of the 18:2 acid in the PEs was 10% greater than in the PCs and PIs, and the amount of the 16:0 acids was greater by the same amount in the PIs than in the PCs and the PEs. In all the PLs the unsaturated acids, with a predominance of the 18:2 acid, were present in position 2. In the PCs and PEs the ratio of the 16:0/18:1 and 16:0/18:2 acids in positions 1 was approximately 1:1, and in the PIs the FAs were distributed more selectively between the two positions since there were 83.5% of saturated acids in position 1 and 94.3% of unsaturated acids in position 2. The 18:0 acid shows the highest specificity for the distribution of the fatty acids: in all the PLs it was present only in position 1.

From the results of the position distibution of the FAs in the PL molecules we determined statistically the possible molecular composition of the PLs (taking position isomerism into account, %):

Molecular species	PC	PE	PI
S-S'	0.3	2.2	1.6
S-S	1.4	1.9	3.1
U-S	1.7	2.7	1.0
U-U'	22.6	9.7	6.3
U-U	26.1	26.6	9.3
S-U	47.9	56.9	78.7

On the basis of the figures given above it may be concluded that the number of monounsaturated–monosaturated (S–U) molecules increases in the sequence from PC (49.6%) to PE (59.6%) and PI (79.7%), and the diunsaturated species (U–U) rise in the opposite sequence: PI (15.6%), PE (36.3%), PC (48.7%).

EXPERIMENTAL

The solvents were prepared by generally accepted methods [9]. The characteristics of the cotton plant were determined by standard methods [1]. For chromatography we used type KSK silica gel [100-150 mesh for column chromatography (CC) and < 150 mesh for thin-layer chromatography (TLC)]. Solvent systems for TLC: 1) chloroform–methanol–water (65 : 35 : 5); 2) chloroform–methanol–25< ammonia (14 : 6 : 1).

The phosphorus in the lipid samples were determined by the gravimetric method [1].

The quantitative compositions of the individual groups of PLs were determined by Tevekelov's method [5] after their separation by two-dimensional TLC. The compositions of the fatty acids were determined by analyzing their methyl esters on a UKh-2 chromatograph at 196°C with a 2.0-m column. The stationary phase was 17% of polyethyleneglycol succinate on "Celite" 545, and the carrier gas was helium.

Enzymatic hydrolysis was performed with phospholipase A_2 from kufi snake venom at pH 10.15 [8]. The time of enzymatic hydrolysis at 27°C was 0.5, 48, and 96 h for the PC, PE, and PI, respectively. The end of the reaction was checked by TLC in system 2.

SUMMARY

1. The qualitative and quantitative compositions of the PLs of an industrial medium-fiber cotton plant of variety 159-F (1972 and 1974 harvests) have been considered. It has been shown that the total PL amounted to 1.76 and 1.85% and consisted of 60-65% PC, 16-18% PI, 6-8% PE, and 10-14% of combined minor components, respectively.

2. The distribution of the lipid phosphorus has been studied during the isolation of PLs by selective solvents. The lipid phosphorus amounted to 5.5% of the total phosphorus in the seed kernels.

3. The qualitative and quantitative compositions of the fatty acids of the individual lipid fractions and of the individual PCs, PIs, and PEs have been determined. The position distribution of the fatty acids of the main groups of PLs has been established and a possible molecular composition has been given.

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