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MOLECULAR COMPOSITION OF COTTONSEED PHOSPHATIDYLCHOLINES

A. Sh. Isamukhamedov, L. A. Shustanova, and S. T. Akramov

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Biological membranes consist mainly of proteins and lipids, and the bulk of the latter are composed of phospholipids, of which phosphatidylcholine (PC) predominates. The structure and a number of important properties of biomembranes depend on the molecular structure of the PC.

In recent years, the structural elements of biomembranes, particularly the phospholipids (PLs) of animal origin, have been studied extremely intensively, but investigations devoted to the PLs of plants and their molecular structure are still inadequate; consequently, the analysis of plant PLs is of great interest.

We have reported [1-3] possible molecular forms of the main groups of PLs of the cotton plant determined by calculation on the basis of the position distribution of the fatty acids in their molecules. In the present paper we give the results of experimental investigations of the fine molecular structure of the PC of the seed kernels of the cotton plant of variety S-6029 [4]. The total fatty-acid composition and position distribution of the fatty acid radicals of the glyceride part of the PC molecule of this variety of cotton plant has been reported previously [3].

The phosphatidylcholines were studied by means of the scheme shown. For better separation into molecular types, the PC was converted by the action of phospholipase into diglyceride (DG) and also into monoacetyldiglyceride (MADG) derivatives. The DG and MADG were separated according to their degree of unsaturation on argentized plates in systems 1 and 2. The yields of the individual fractions are given in Tables 1 and 2.

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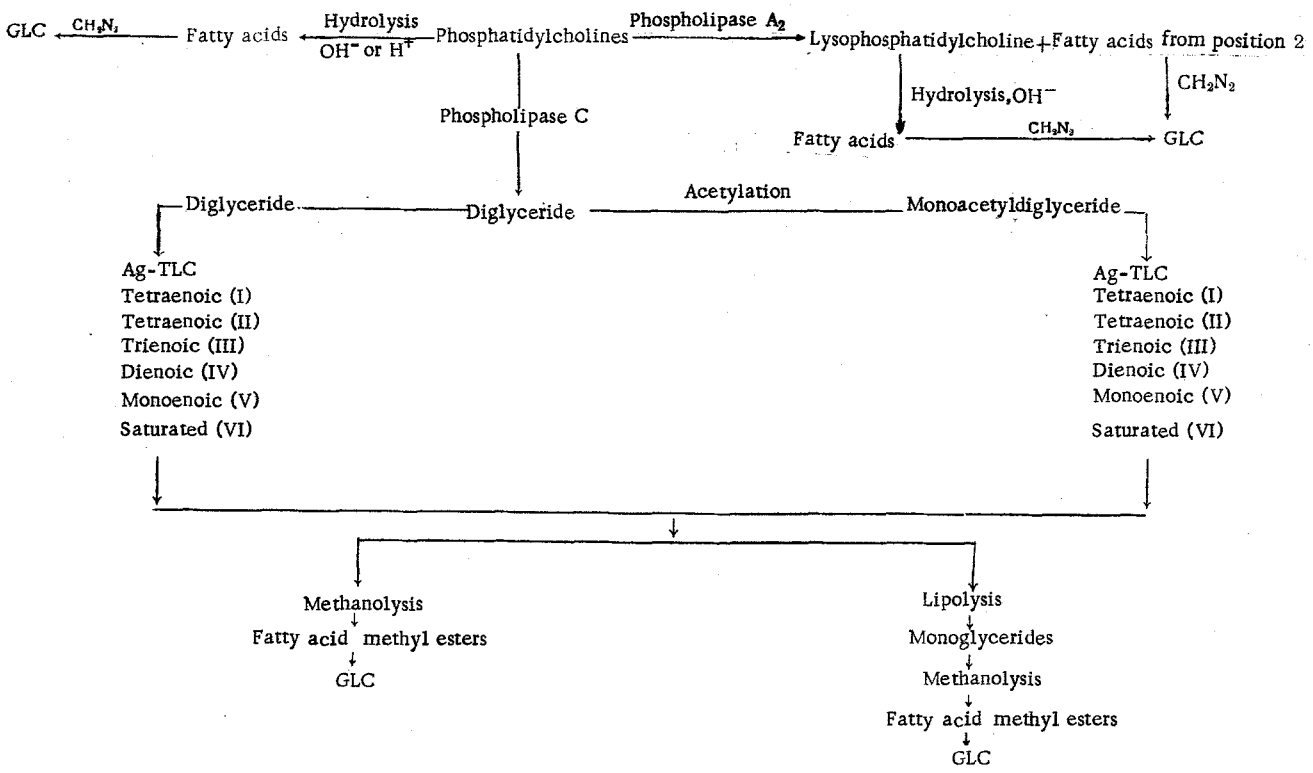

An aliquot of each subfraction was subjected to methanolysis to determine the total fatty-acid composition, and the remainder was subjected to lipolysis to identify the fatty acid in position 2 of the glycerol residue. The lipolysis was carried out with the aid of the pancreatic lipase of the porcine pancreatic gland. The zone of the monoglycerides were taken off and subjected to methanolysis, and the resulting methyl esters were analyzed by the GLC method. The mean results of three determinations are given in Tables 1 and 2.

In spite of the fact that visually the DGs and MADGs investigated were clearly separated into six zones according to their degree of unsaturation, the results of the fatty-acid analyses of each subfraction showed that they were not individual substances but were enriched with definite types of compounds: fractions I and II proved to consist of tetraenes, III trienes, IV dienes, V monoenes, and VI saturated compounds (the tetraenes, trienes, etc., contain the DGs or MADGs in which the total numbers of double bonds in the fatty-acid radicals amount to four, three, etc.).

Knowing the yields of the fractions and the compositions of the fatty acids in position 2, we checked the total amount of each acid in this position. The results were close to those obtained in the hydrolysis of the native PC by phospholipase A_2 [3], and therefore the lipolysis took place specifically. On the basis of these facts we found the amounts of fatty acids in position 1 (see Tables 1 and 2).

The experimental results on the total and position distribution of each fatty acid in the individual subfractions were confirmed by calculation. They were close to the total and position distribution of the fatty acids in the native PCs. It follows from this that the process of separating the DGs and MADGs on argentized plates does not affect the position distribution of the fatty acids and their quantitative content.

Let us consider each subfraction of DGs and MADGs in more detail.

The Tetraenic Fraction I with a yield of 4.0-3.5% contains only two acids, 16:0 and 18:2, the 18:2 acid being localized exclusively in position 2. Thus, the fraction consists of two species – 18:2–18:2 and 16:0–18:2, amounting to 3.0-2.7 and 1.0-0.8%, respectively.

The Tetraenic Fraction II amounted to 16.5-14.4%. Here the 18:2 acid is uniformly distributed over the two positions and amounts to about 90% of all the acids, so that the main form is 18:2–18:2, more than 50% of the total amount of this species being concentrated in this fraction.

The Trienic Fraction III (24.6-18.9%) mainly contained the 18:2 and 18:1 acids, the 18:1 acid predominating in position 1. The main species of this fraction are 18:1–18:2 and 18:2–18:1 – 10.3 and 10.3%–, which corresponds to more than half of all the trienes.

TABLE 1. Composition and Position Distribution of the Fatty Acids in Positions 1 and 2 of the Subfractions of the Diglycerides Obtained by Ag–TLC and Lipolysis (wt. %)

Fraction	Diglyceride	Fatty acid								Σ S; 29,8	Σ U 70,2
		10:0; 1,8	12:0; 1,2	14:0; 0,8	16:0; 24,3	16:1; 0,7	18:0; 1,7	18:1; 23,1	18:2; 46,4		
I (4,0)	total	—	—	—	12,5	—	—	—	87,5	12,5	87,5
	1	—	—	—	25,0	—	—	—	75,0	25,0	75,0
	2	—	—	—	—	—	—	—	100,0	—	100,0
II (16,5)	total	—	—	—	3,6	1,8	—	7,2	87,4	3,6	96,4
	1	—	—	—	5,4	1,8	—	7,7	85,1	5,4	94,6
	2	—	—	—	1,8	1,8	—	6,7	89,7	1,8	98,2
III (24,6)	total	—	1,2	—	4,8	1,6	—	37,0	55,4	6,0	94,0
	1	—	0,8	—	8,0	2,0	—	41,5	47,7	8,8	91,2
	2	—	1,6	—	1,6	1,2	—	32,5	63,1	3,2	96,8
IV (33,4)	total	—	1,5	—	37,5	—	2,1	18,3	40,6	41,1	58,9
	1	—	3,0	—	71,4	—	4,2	18,4	3,0	78,6	21,4
	2	—	—	—	3,6	—	—	18,2	78,2	3,6	96,4
V (13,8)	total	—	2,9	2 9	42,0	—	4,3	41,2	6,7	52,1	47,9
	1	—	—	1,5	80,6	—	8,6	8,6	0,7	90,7	9,3
	2	—	5,8	4,3	3 4	—	—	73,8	12,7	13 5	86,5
IV (7,7)	total	23,4	—	5,2	48,0	—	5,2	13,0	5,2	81,8	18,2
	1	7,8	—	10,4	63,6	—	10,4	7,8	—	92,2	7,8
	2	39,0	—	—	32,4	—	—	18,2	10,4	71 4	28,6

Note. The yields of the subfractions (wt. %) are given in parentheses; S – saturated; U – unsaturated fatty acids.

TABLE 2. Composition and Position Distribution of the Fatty Acids in Positions 1 and 2 of the Subfractions of the Monoacetyldiglycerides Obtained by Ag–TLC and Lipolysis (wt. %)

Fraction	Monoacetyldiglyceride	Fatty acid								Σ S; 31,4	Σ U; 68,6
		10:0; 1,8	12:0; 0,4	14:0; 1,0	16:0; 26,5	16:1; 0,4	18:0; 1,7	18:1; 23,8	18:2; 44,4		
I (3,5)	total	—	—	—	12,9	—	—	—	87,1	12,9	87,1
	1	—	—	—	25,8	—	—	—	74,2	25,8	74,2
	2	—	—	—	—	—	—	—	100,0	—	100,0
II (14,4)	total	2,8	—	—	4,2	1,4	—	4,9	86,7	7,0	93,0
	1	2,1	—	—	7,6	—	—	—	90,3	9,7	90,3
	2	3,5	—	—	0,7	2,8	—	9,8	83,2	4,2	95,8
III (18,9)	total	7,5	—	—	4,8	1,0	—	35,9	50,8	12,3	87,7
	1	9,7	—	—	7,0	2,0	—	62,8	13,5	16,7	83,3
	2	5,3	—	—	2,6	—	—	9,0	83,1	7,9	92,1
IV (35,5)	total	—	—	1,4	36,9	—	2,5	17,0	42,2	40,8	59,2
	1	—	—	—	69,4	—	5,0	9,2	16,4	74,4	25,6
	2	—	—	2,8	4,4	—	—	24,8	68,0	7,2	92,8
V (20,9)	total	—	1,4	2,4	38,2	—	2,8	43,1	12,1	44,8	55,2
	1	—	0,4	1,5	73,6	—	5,6	13,9	5,0	81,1	18,9
	2	—	2,4	3,3	2,8	—	—	72,3	19,2	8,5	91,5
VI (6,8)	total	—	1,5	—	51,5	—	3,0	19,0	25,0	56,0	44,0
	1	—	3,0	—	70,7	—	6,0	10,2	10,1	79,7	20,3
	2	—	—	—	32,3	—	—	27,8	39,9	32,3	67,7

The Dienic Fraction IV had a yield of 33.4-35.5%, i.e., 1/3 of the initial sample. The 18:0 acid is paired mainly with the 18:2 acid in 18:0–18:2 species. The 16:0–18:2 molecule species of this fraction amounts to 2/3 of the total amount of this species.

The Monoenic Fraction V with a yield of 13.8-20.9% consists mainly of the 16:0 and 18:1 acids, the 16:0–18:1 species making up the bulk of this fraction and more than half the total amount of this species.

The Saturated Fraction VI amounted to 7.7-6.8%, the main saturated acid being the 16:0 acid, and in the DG also the 10:0 acid, which is localized in both positions; the main species of the samples investigated were 16:0–16:0 and 16:0–10:0.

Analysis of the results obtained shows that the total number of species was 46 for the DG and 43 for the MADG (Table 3). The main molecular species of the DG and MADG were the diunsaturated (U–U) and the mono-

TABLE 3. Subfraction Composition and Total Molecular Compositions of Cotton Plant Phosphatidylcholines

Phosphatidylcholine	II		III		IV		V		VI		Total	
	DG	MADG	DG	MADG	DG	MADG	DG	MADG	DG	MADG	DG	MADG
10:0—10:0	—	+	—	+	—	—	—	—	0,2	—	0,2	+
12:0—10:0	—	—	—	—	—	—	—	—	0,3	—	0,3	—
16:0—10:0	—	+	—	+	—	—	—	—	2,0	—	2,0	+
18:0—10:0	—	—	—	—	—	—	—	—	0,3	—	0,3	—
12:0—12:0	—	—	+	—	—	—	—	+	—	—	+	+
14:0—12:0	—	—	—	—	—	—	+	+	—	—	+	+
16:0—12:0	—	—	+	—	—	—	0,6	0,4	—	—	0,6	0,4
18:0—12:0	—	—	—	—	—	—	0,1	+	—	—	0,1	+
12:0—14:0	—	—	—	—	—	—	—	+	—	—	—	+
14:0—14:0	—	—	—	—	—	—	+	+	—	—	+	+
16:0—14:0	—	—	—	—	—	0,7	0,4	0,6	—	—	0,4	1,3
18:0—14:0	—	—	—	—	—	+	0,1	+	—	—	0,1	+
10:0—16:0	—	+	—	0,1	—	—	—	—	0,1	—	0,1	0,1
12:0—16:0	—	—	+	—	+	—	—	+	0,3	+	0,3	+
14:0—16:0	—	—	—	—	—	—	+	+	—	—	+	+
16:0—16:0	—	+	+	+	0,9	1,1	0,5	0,5	1,7	1,7	3,1	3,3
18:0—16:0	—	—	—	—	0,1	+	+	+	0,3	0,1	0,4	0,1
16:1—12:0	—	—	+	—	—	—	—	—	—	—	+	—
16:1—16:0	—	—	+	+	—	—	—	—	—	—	+	+
18:1—10:0	—	—	—	0,7	—	—	—	—	0,2	—	0,2	0,7
18:1—12:0	—	—	0,2	—	—	—	0,1	0,1	—	—	0,3	0,1
18:1—14:0	—	—	—	—	—	0,1	0,1	0,1	—	—	0,1	0,2
18:1—16:0	—	—	0,2	0,4	0,2	0,2	+	0,1	0,1	0,2	0,5	0,9
10:1—16:1	—	+	—	—	—	—	—	—	—	—	—	+
12:0—16:1	—	—	+	—	—	—	—	—	—	—	+	—
16:0—16:1	—	+	+	—	—	—	—	—	—	—	+	+
10:0—18:1	—	+	—	0,2	—	—	=	—	0,1	—	0,1	0,2
12:0—18:1	—	—	+	—	0,2	—	—	+	0,1	+	0,3	+
14:0 -18:1	—	—	—	—	—	—	0,1	0,1	—	—	0,1	0,1
16:0—18:1	0,1	0,1	0,7	0,1	4,4	6,1	8,1	11,1	1,0	1,4	14,3	18,8
18:0—18:1	—	—	—	—	0,2	0,4	0,9	0,9	0,1	0,1	1,2	1,4
18:2—10:0	—	0,5	—	0,2	—	—	—	—	—	—	—	0,7
18:2—12:0	—	—	0,2	—	—	—	+	+	—	—	0,2	+
18:2—14:0	—	—	—	—	—	0,2	+	+	—	—	+	0,2
18:2—16:0	0,3	0,1	0,2	0,1	+	0,3	+	+	—	0,2	0,5	0,7
16:1—16:1	—	—	+	—	—	—	—	—	—	—	+	—
18:1—16:1	—	—	0,1	—	—	—	—	—	—	—	0,1	—
16:1—18:1	—	—	0,2	+	—	—	—	—	—	—	0,2	—
18:1—18:1	0,1	—	3,3	1,1	1,2	0,8	0,9	2,1	0,1	0,2	5,6	4,2
10:0—18:2	0,8	0,2	—	1,5	—	—	—	—	+	—	0,8	1,7
12:0—18:2	—	—	0,1	—	0,8	—	—	+	0,1	+	1,0	+
14:0—18:2	—	—	—	—	—	—	+	+	—	—	+	+
16:0—18:2	—	1,0	1,2	1,1	18,6	16,7	1,5	3,0	0,5	2,0	22,8	24,6
18:0—18:2	—	—	—	—	1,2	1,2	0,2	0,2	—	—	1,4	1,4
18:2—16:1	0,3	0,4	0,2	—	—	—	—	—	—	—	0,5	0,4
18:2—18:1	0,9	1,3	3,8	0,3	0,2	1,5	+	0,9	—	0,2	4,9	4,2
16:1—18:2	0,3	—	0,3	0,4	—	—	—	—	—	—	0,6	0,4
18:1—18:2	1,2	—	6,5	10,0	4,8	2,2	0,2	0,6	+	0,3	12,7	13,1
18:2—18:2	12,5	10,8	7,4	2,7	0,8	4,0	+	0,2	—	0,3	23,7	20,7

Note: Subfraction I consists of two species: 16:0—18:2 (1.0 and 0.8%) and 18:2—18:2 (3.0 and 2.7%, respectively). DG) diglyceride; MADG) monoacetyldiglyceride.

saturated—monosaturated (S—U). The U—U molecule species were represented by the 18:2—18:2 (23.7 and 20.7), 18:1—18:2 (12.7 and 13.1%), 18:2—18:1 (4.9 and 4.2%), and 18:1—18:1 (5.6 and 4.2%) compounds and amounted to 46.9 and 42.2% of the total amount of species. The (S—U) molecules are paired in the species 16:0—18:2 (22.8 and 24.6%) and 16:0—18:1 (14.3 and 18.8%), making up 37.1 and 43.4%, respectively, of the total amount. The combined U—U and S—U amounted to 84.0 and 85.6% of the total amount of PC species.

Thus, the experimental conditions that we selected make it possible to determine the molecular structures of the PCs of the cotton plant in the form of their DG and MADG derivatives.

A comparison of the experimental results on the molecular structures of the PCs of the cotton plant with the calculated figures shows that their amounts are higher than the calculated amounts – 46 and 43% against 40% [3], but only slight differences are found in the quantitative contents of the individual species. On the whole, however, the results of the two methods are close and give an idea of the molecular structure of the PLs.

EXPERIMENTAL

All the solvents used were treated by the usual methods [5]. The solvents were distilled off from the samples in a rotary evaporator in an atmosphere of nitrogen at a temperature not exceeding 40°C.

The GLC analysis of the fatty acid methyl esters was performed on a UKh-2 instrument with a thermal conductivity detector at 196-197°C in a copper column 2 m × 4 mm filed with Celite 545 (80-100 mesh); the stationary phase was 17% of polyethyleneglycol succinate, and the carrier gas was helium.

For TLC we used treated type KSK silica gel [3] (150-200 mesh) mixed with 5% of gypsum and 2.0-2.5 parts of a 15% aqueous solution of silver nitrate. The plates were dried first at room temperature and then in a drying chest at 110-120°C for 20-30 min. The systems of solvents for the mobile phases in TLC were: 1) benzene–methanol (92:8); 2) petroleum ether–diethyl ether–ethanol (40:10:1.5); 3) diethyl ether–petroleum ether (9:1) [6]; 4) diethyl ether–petroleum ether (3:7) [6]; and 5) chloroform–methanol–water (65:25:4) [7].

Homogeneous PC was obtained as described previously [3].

Hydrolysis with Phospholipase C from Clostridium perfringens. The phospholipase was obtained under the direction of G. F. Shemanova (I. I. Mechnikov All-Union Scientific-Research Institute of Vaccines and Sera, Moscow). To a solution of 140 mg of PC in 30 ml of ether was added 1.5 ml of Tris buffer with pH 8.6 in which was dissolved 0.4 mg of the enzyme and 0.1 ml of 20% $CaCl_2$. The time of complete hydrolysis was 2-2.5 h. The course of the reaction was followed by TLC in systems 4 and 5. After the end of the reaction, the ethereal solution was washed with water (2 × 10 ml), dried over anhydrous sodium sulfate, and concentrated, and the yield of diglycerides was determined – 95-97%.

Hydrolysis with Pancreatic Lipase. The lipase was obtained from fresh pancreatic gland of pigs by the method of Markman et al., [6]. To 10 mg of diglycerides or monoacetyldiglycerides were added successively 0.5 ml of Tris buffer, pH 8.6, 10 mg of lipase, 0.1 ml of 1 M $CaCl_2$ solution, and 0.2 ml of 0.1% sodium deoxycholate solution. The time of hydrolysis was 30 min. The hydrolysis products were separated by TLC in system 3. The isolated monoglycerides were subjected to methanolysis [8].

Acetylation of the Diglycerides. Variant 1 [9]. A solution of 100 mg of the diglycerides in 0.5 ml of pyridine was treated with 2.5 ml of acetic anhydride and the reaction mixture was carefully shaken and was thermostatted at 40°C for 2.5 h, after which it was left overnight at room temperature. Yield 85%.

Variant 2. To 100 mg of the diglycerides was added 1 ml of piperidine, and then the flask was cooled in a beaker containing ice, and 3 ml of acetyl chloride was added dropwise. A white precipitate formed was filtered off and washed with cold ether (3 × 10 ml). The combined ethereal filtrate was washed with water (3 × 50 ml), dried over anhydrous sodium sulfate, and evaporated.

The monoacetyldiglycerides were purified by TLC in system 4, taken up from the zone of localization, and eluted with chloroform–methanol (1:1); the solvent was eliminated and the yield was determined – 95%.

The Separation of the PCs Derivatives into Molecular Species According to Unsaturation was effected on argentized plates – in the case of the diglycerides, in system 1, and in the case of the monoacetyldiglycerides in system 2. On one plate with dimensions of 12 × 18 cm was deposited 10 mg of the sample in the form of a chloroform solution. After separation and evaporation of the solvent, the plates were sprayed with a 0.4% aqueous solution of Rhodamine 6G. The zones of separation of the substances were detected in UV light, and their boundaries were traced round with a needle. The individual fractions were taken off, transferred to a 30 × 800 mm column, and eluted with 250 ml of chloroform – methanol–water (1:1:0.1 by volume).

The solutions were evaporated in a rotary evaporator, to each of the concentrated fractions (2-3 ml) was added 20 ml of ether, and they were treated with water (4 × 10 ml) to eliminate the Rhodamine 6G and the silver nitrate and were dried over sodium sulfate, after which the solvent was distilled off and the yields of the subfraction were determined.

Methanolysis. A sample of the DGs, MADGs, or the monoglycerides (5 mg) was mixed with 0.5 ml of a 5% solution of HCl in methanol [8] and methylation was carried out in a sealed tube at 100°C for 2 h. The methyl ethers of the fatty acids were transferred into ether (20 ml), and the solution was washed with water (3 × 5 ml) and dried with sodium sulfate. After the elimination of the ether, the residues were analyzed by GLC.

SUMMARY

The diglyceride and monoacetyldiglyceride derivatives of the phosphatidylcholines of the seed kernels of the cotton plant of variety S-6029 have been separated into individual subfractions in a thin layer of silica gel impregnated with silver nitrate.

The total fatty-acid compositions of the individual subfractions and the position distributions of the fatty acids in the glyceride moiety of the molecules have been determined, and on this basis the molecular compositions of the phosphatidylcholines have been established: for the DGs 46 species and for the MADGs 43 species. It has been shown that in the cotton plant two types of molecular species are synthesized (mainly to the extent of 85%): Disaturateds and monosaturated–monosaturateds, the disaturateds being characterized by a greater diversity of species but a low amount.

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THE SEED OIL OF Catalpa. I

L. N. Andrianova, A. L. Markman, and I. U. Yusupova

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The decorative plant catalpa has a light and soft wood highly resistant to decay which has long been used as a material for railway sleepers, posts, and underwater structures [1].

The seed oil of catalpa has not been studied sufficiently deeply and, apparently, for this reason has not yet found a use. We have investigated samples of the seed oil from five species of catalpa: southern catalpa (C. bignonioides Walt. [C. syringaefolia]), northern catalpa (C. speciosa Word ex Engelm.), Chinese catalpa (c. ovata G. Don), the hybrid teas catalp (C. bignonioides × ovata), and Ducloux catalpa (C. duclouxii Dode [C. fargesi duclouxi]), which are cultivated in the Soviet Union (the towns of Kamyshin and Lipetsk) and in botanical gardens: the Main Botanical Garden (Moscow), those of Nikitskii (Yalta), Tashkent, Stavropol', and Voronezh, and the Central Republican Botanical Garden of the Academy of Sciences of the Ukrainian SSR (Kiev).

The oil content of the catalpa seeds varies between 21.2 and 36.7% (Table 1), which is not inferior to those of the oil crops most widely used in the Soviet Union for example, the oil content of linseed is 26.9-47.2%,

TABLE 1

Geographic site	Amt. of oil in the catalpa seeds, %				
	northern	southern	Chinese	teas	ducloux
Tashkent	29,9	—	—	—	—
Yalta	—	21,2	21,9	—	36,7
Stavropol'	36,1	—	—	—	—
Kamyshin	31,8	—	—	31,9	—
Kiev	33,1	34,3	26,3	—	—
Voronezh	—	29,9	—	—	—
Lipetsk	—	30,3	25,6	—	—
Moscow	—	10,1	—	—	—

Kalinin Polytechnic Institute. Moscow Branch of the All-Union Scientific-Research Institute of Fats.

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