DEPENDENCE OF THE COMPOSITION OF THE LIPIDS OF THE SEEDS OF *Plectranthus glaucocalyx* ON THE CONDITIONS OF GROWTH

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A change in the geographical growth zone and in the density of the soil has a substantial influence on the amount of lipids in the seeds of *Plectranthus glaucocalyx* Maxim. and their degree of unsaturation, and also on the structure of the triacylglycerols.

The chemical composition of plants depends substantially on seasonal and soil-climatic conditions. This also applies to their lipids.

The dependence of the composition of the lipids on the temperature has been studied in greatest detail. The degree of unsaturation of a fat is lower in climatic zones with higher mean annual temperatures. This tendency is preserved for plants of a single climatic zone when the mean temperature of the vegetation period of one year differs from the temperature of another year [1].

A change in temperature is reflected not only on the degree of unsaturation of the lipids but on their accumulation [1, 2] and structure. Thus, in peanut seeds, with a decrease in the total amount of the 18:1 acid in the triacylglycerols the amount of this acid in the sn-1 and sn-2 positions, but not in the sn-3 position, decreases [3].

The factors affecting the amount and composition of neutral lipids of various plant organs include the intensity of the light [2], the supply of moisture from the soil [4] and its salinity [5], the season [6], and the growth site [1, 3].

The amount and composition of the polar lipids in the rhizomes of *Iris* species change with a deficiency of O_2 (anoxia) in the soil [7].

However, results on the nature of the changes in the lipid composition as a function of seasonal conditions are contradictory [1, 2].

The aim of our work was to investigate the changes in the lipid composition of the introduced species *Plectranthus glaucocalyx* Maxim., family Labiatae, under the influence of various soils and climatic conditions. There is no information in the literature on the lipids of this species of plant.

The plants and seeds of the wild-growing *Plectranthus*, collected in the autumn of 1975 in the Far East, were planted in freshly plowed soil of the Laboratory for the Introduction of Medicinal Plants of the F. N. Rusanov Botanical Garden of the Academy of Sciences of the Uzbek SSR (Tashkent) in an open sunny position. Watering was carried out 11-13 times during the vegetation period, depending on the meteorological conditions of the year. The basic phenological characteristics of the *Plectranthus* are given in Table 1, from which it can be seen that the climate of the introduction zone (Central Asia) had no influence on the development of the plant. Over six years, the periods of growth and development of the introduced plant coincided with the seasonal rhythm of the weather. However, a considerable change in the height of the plant was observed, which is connected with a change in the density of the soil. In 1979, when the soil was not loosened, the plant on the dense soil and, consequently, with less aeration and a smaller water permeability of the soil, had the minimum height and the leaves wilted. In the same year, after the end of the vegetation period the plant was replanted into freshly plowed soil.

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TABLE 1. Phenology of *Plectranthus glaucocalyx*

Year of observation	Day and month									Height of the plant, cm
	spring sprouting	budding	last flowers				ripening of fruit		end of investigation	
			beginning	mass	end of blossoming	last flowers	first	mass		
1976	20.III	8.V	28.V	2.VI	15.VI	11.VII	22.VI	10.VII	11.X	120*
1977	20.III	14.V	29.V	20.V	20.VI	11.VII	4.VII	16.VII	20.IX	140
1978	21.III	17.V	28.V	5.VI	18.VI	11.VII	29.VI	12.VIII	18.IX	145
1979	29.III	15.V	29.V	6.VI	19.VI	11.VII	15.VII	25.VII	16.IX	110
1980	29.III	14.V	30.V	10.VI	19.VI	11.VII	13.VIII	25.VIII	20.IX	140
1981	27.III	8.V	18.V	20.V	10.VI	16.VI	1.VII	20.VII	20.IX	150

*The maximum height of the plant in the wild is 150 cm.

TABLE 2. Lipid Compositions of Samples 1-3 of the Seeds of *Plectranthus glaucocalyx*

Class of lipids	R_f	Percent on the total weight		
		1	2	3
1. Hydrocarbons	0.98*	1,0	0,9	1,3
2. Sterol esters	0,94	1,0	1,2	
3. Fatty acid methyl esters	0,77	0,2	0,3	1,8
4. Unidentified	0.75	0,3	0,2	
5. Triacylglycerols	0 60	88.4	85,3	83,1
6. Free fatty acids	0,39	1,8	2,5	2,7
7. Esters of triterpene acids	0,35	0,7	1,3	1,5
8. Diacylglycerols	0,29	0,9	1,6	1,8
9. Sterols	0,27	2,0	2,6	3,1
10. Chlorophylls together with an unidentified class	0,20	2,9	2,9	3,2
11. Monoacylglycerols	0,08	0,8	1,2	1,5

*Chromatographic mobilities: 1-10 on Silufol in system 1; 11, in system 2.

In the following year (1980), the height of the plants increased by 30 cm, and in 1981 it reached 150 cm, which corresponds to the maximum height of the stem of the wild-growing species. In these years the plants were distinguished by the development of the epigeal part, and no signs of wilting were observed.

We studied the lipid composition of three samples of seeds of the *Plectranthus* growing under different conditions: sample 1) seeds gathered in 1981 from wild plants; 2) seeds collected in 1981 from introduced plants growing in freshly plowed soil; and 3) seeds collected in 1979 from depressed introduced plants that had grown in dense soil. The seeds of sample 1 were taken for comparative analysis.

The yields of lipids amounted to (% on the mass of the seeds): for sample 1) 24.8; 2) 10.5; 3) 5.3. Consequently, the oil content of the introduced *Plectanthrus* was less than half that of the wild plant.

Under conditions of inadequate aeration and water permeability of the soil, the oil content was halved again.

The lipids were separated and analyzed by the usual methods.

As can be seen from Table 2, the lipid compositions of the seeds of *P. glaucocalyx* were represented by 12 classes. A change in the region of growth and and soil conditions did not affect its qualitative indices.

The quantitative changes in samples 2 and 3 as compared with 1 are expressed in a lower amount of triacylglycerols (TAGs) and an increase in the amount of intermediate products in the synthesis of the TAGs: free fatty acids (FFAs) and mono- and diacylglycerols (MAGs and DAGs). The amount of lipophilic substances (hydrocarbons, sterol esters, and free sterols) increased simultaneously. A reduced aeration of the soil changed the quantitative composition to a somewhat greater degree than a change in the geographical zone. These changes can

TABLE 3. Fatty Acid Compositions of the Acyl-Containing Fractions of the Seeds *Plectranthus glaucocalyx* (%, GLC)

Fractions of the samples		Acid*															
		12:0	13:0	14:0	15:0	16:0	17:0	18:0	18:1	18:2 (9, 12)	18:2 (5, 6)	18:3	20:0	22:0	x	Σ_{sat}	Σ_{unsat}
Total lipids																	
Sample	1	—	—	Tr.	—	3,0	—	1,6	6,9	24,7		63,8	—	—	—	4,6	95,4
	2	—	—	Tr.	—	4,3	—	1,3	14,8	30,5		49,1	—	—	—	5,6	94,4
	3	—	—	0.1	—	5,3	—	2.0	20,1	30.6		41,9	—	—	—	7,4	92,6
Sterol esters																	
Sample	1	1,2	—	1,4	0,5	18.7	—	6,5	9,3	30,9		12,7	13,5	0,8	4,5	47,1	52,9
	2	1,0	—	0,6	Tr.	34,8	—	7,6	7,2	25,8		13.2	6,0	0,6	3,2	53,8	46,2
FAMEs																	
Sample	1	4,0	3,4	7,2	4,6	14,4	5,8	2,1	19,5	13,4		5,6	5,7	5,6	8,7	61,5	38,5
	2	2,6	Tr.	2,1	Tr.	12,7	0,4	5,7	19,9	26,5		11,6	5,0	6,2	7,3	42,0	58,0
TAGs																	
Sample	1	Tr.	Tr.	Tr.	Tr.	2,1	—	Сл.	5,0	17,0	5,0	70,9	Tr.	Tr.	—	2,1	97,9
	2	Tr.	Tr.	Tr.	Tr.	3,9	—	Сл.	13,3	27,1	2,3	53,4	Tr.	Tr.	—	3,9	96,1
	3	Tr.	Tr.	Tr.	Tr.	5,1	Tr.	1,4	19,0	13,2	19,1	42,2	Tr.	Tr.	—	6,5	93,5
FFAS																	
Sample	1	0,2	Tr.	0,5	0,1	12,4	Tr.	4,2	12,4	23,8	6,1	40,0	0,3	Tr	—	17,7	82,3
	2	Tr.	Tr.	Tr.	Tr.	10,6	Tr.	3,6	21,2	30,0	3,9	30,7	Tr.	—	—	14,2	85,8
DAGs																	
Sample	1	Tr.	Tr.	0,1	—	4,9	Tr.	2,5	7,9	33,7	5,5	45,4	Tr.	—	—	7,5	92,5
	2	Tr.	Tr.	0,1	Tr.	7,5	Tr.	2,6	19,5	34,0	2,4	33,9	Tr.	—	—	10,2	89,8
MAGs																	
Sample	1	Tr.	Tr.	0,4	Tr.	10,9	Tr.	3,2	15,7	29,1	3,4	37,3	Tr.	—	—	14,5	85,5
	2	Tr.	Tr.	0,4	Tr.	12,3	—	4,1	21,7	30,7	1,3	29,5	Tr.	—	—	16,8	83,2

*The 11:0 acid was detected in trace amounts in the FFA, DAG, and MAG fractions, and the 16:1 acid in trace amounts in all the fractions.

TABLE 4. Distribution of the Acids in the Triacylglycerols of *Plectranthus glaucocalyx*

Position	Sample	Acid, moles-%					
		16:0	18:0	18:1	18:2 (9, 12)	18:2 (5, 6)	18:3
sn-1	1	6,2	Tr.	7,3	19,8	4,7	62,0
	2	11,4	Tr.	16,0	25,0	2,8	44,8
sn-2	1	—	—	6,3	25,8	2,2	65,7
	2	0,9	—	19,1	33,6	1,3	45,1
	3	0,5	—	26,6	36,3		36,6
sn-3	1	0,7	Tr.	1,1	5,1	8,1	85,0
	2	0,3	Tr.	4,2	22,1	3,1	70,3

be considered as adaptations of the plant at the level of lipid metabolism to the changed conditions of growth.

Six classes of samples 2 and 3 and the TAGs of sample 1 were analyzed in detail.

From the acyl-containing lipids we isolated the fatty acids which, like the free fatty acids, were methylated with diazomethane and analyzed by GLC. The natural fatty acid methyl esters (FAMEs) were analyzed by GLC without treatment.

With the aid of TLC on silica gel containing 30% of $AgNO_3$ in system 3, the 18:2(5,6) acid — laballenic acid — was detected in the methyl esters of the acids isolated from the MAGs, DAGs, TAGs, and FFAs. Its amounts were calculated as described previously [8].

By the GLC method, in the MEs of the saturated acids isolated by Ag^+-TLC from the fractions mentioned above we identified the minor acids from 12:0 to 15:0, and 17:0, 20:0, and 22:0. The same acids were detected in appreciable amounts in the FAMEs and the acid fractions of the sterol esters (Table 3).

The presence of the 18:2(5,6) acid was also confirmed by the corresponding fragments (12:0, pentanedicarboxylic acids) from the periodate-permanganate oxidation of the MEs of the acids isolated from the lipids.

In view of the small amounts of the sterol ester and FAME fractions, no confirmation of the presence of laballenic acid in them was made.

The presence in the lipids of the seeds of *Plectranthus glaucocalyx* of laballenic acid, which is characteristic mainly for the oils of Labiatae rich in linoleic acid, appears unusual.

The compositions of the acids of the total lipid material and the individual acyl-containing classes of *P. glaucocalyx* are given in Table 3.

It follows from Table 3 that the sterol esters, the FAMEs, and the FFAs are far more diverse in compositions and include more saturated acids than the TAGs.

We have reported previously that in the seeds of plants of the family *Labiatae* growing under the usual conditions the free acids contain a larger amount of saturated components than those esterified in the TAGs [9]. A deterioration of the soil conditions leads an enrichment of the unesterified acids with unsaturated components (Table 3).

In the main lipids of the introduced plant the amount of unsaturated acids had fallen in comparison with the wild variety. A still greater change in the degree of unsaturation of the lipids took place under the influence of the inadequate aeration of the soil. Of the unsaturated acids, the greatest quantitative changes were undergone by the 18:1 and 18:3 acids. Thus in the TAGs of the introduced species the amount of the 18:1 acid was 2.7 times greater and that of the 18:3 acid and 1.3 times smaller than in the TAGs of the wild-growing plant.

The fact that the unusual laballenic acid is concentrated in the FFAs of the neutral lipids of the seeds of some species *Labiatae* has been reported previously [10]. In sample 1, this acid was again found mainly in the free state. However, a weak aeration and a low water permeability of the soil, causing a state of stress in the plant, led to an eightfold increase in the amount of the 18:2(5,6) acid included in the TAGs (samples 2 and 3, Table 3).

The increase in the synthesis and in the degree of binding of the 18:2(5,6) acid in the TAGs is apparently a specific response to unfavorable conditions of growth.

We have previously reported the inclusion of laballenic acid in the extreme position of the TAGs of the seeds of two species of *Phlomis*, belonging to the family *Labiatae* [11]. Since there is no information on the specific distribution of the 18:2(5,6) acid in the sn-1 and sn-3 positions of the TAGs in the literature, it appeared of interest to obtain a quantitative estimate of the distribution of this unusual acid in the TAGs and also to determine the influence of the growth site on the structure of the TAGs.

The structures of the TAGs of samples 1 and 2 were determined by the method of stereospecific analysis and that of sample 3 by pancreatic hydrolysis. When the fatty acid MEs isolated from the 2-MAGs and the lysophosphatidylphenols by the Ag^+-TLC method were analyzed, laballenic acid was found in them. After the preparative Ag^+-TLC of these methyl esters and their subsequent GLC analysis, the amounts of the 18:2(5,6) acid in each of the three positions of sn-glycerol were calculated. Information on the distribution of the acid in the TAGs of *P. glaucocalyx* according to the geographical growth zone in given in Table 4.

Laballenic acid, while esterifying all three positions of the glycerol enriches the sn-3 position to the greatest degree. The nature of its distribution does not depend on the growth site.

The ordinary acids in the TAGs of *P. glaucocalyx* are distributed in the following way. Saturated acids are included mainly in the sn-1 position. The 18:2 acid esterifies the central position predominantly, and, of the two extreme positions, mainly sn-1. The 18:3 acid occupies the sn-3 position predominantly. The nature of the distribution of these acids is retained with a change in the geographical zone of growth, in spite of the change in their total amount in the initial acids. The nature of the distribution of the 18:1 acid changes, however: In the TAGs of the wild *Plectranthus* the 18:1 acid enriches the sn-1 position, and in the introduced species with an increase in the amount of 18:1 acid in the initial TAGs (13%) this acid predominates in the sn-2 position. The specificity of the distribution of the 18:1 acid in the central position of the TAGs of the seeds of Central Asian species of *Labiatae* has been shown by us previously [12].

Thus, the growth site and a reduced aeration of the soil have a substantial influence on the amount, degree of unsaturation, and structure of the TAGs of the seeds of *Plectranthus glaucocalyx*.

EXPERIMENTAL

UV spectra were taken on a Hitachi spectrometer in hexane. Gas-liquid chromatography was performed in a Chrom-4 instrument with a flame-ionization detector using a 2.5 m × 4 mm column filled with 17% of ethylene succinate on Chromaton N-AW at 202°C with a pressure of the carrier gas (helium) of 0.78 kg/cm^2.

The seeds of the wild *Plectranthus glaucocalyx* were collected in the Bol'shekhekhtsirzskii Reservation, Khabarovsk krai, and that of the introduced plant in the Tashkent Botanical Garden.

Adsorption chromatography was carried out as described by Sanders [3]. The following solvent systems were used for TLC: 1) hexane–diethyl ether–CH_3COOH (80:20:1); 2) heptane–methyl ethyl ketone–CH_3COOH (43:7:0.5), with two runs; and 3) C_6H_6.

The esters of the triterpene acids were identified by comparing their chromatographic mobilities with the acetate of ursolic acid, as model, and also from their specific colorations after treatment with 50% H_2SO_4 followed by heating.

On the basis of their UV spectra, the chlorophylls were identified as chlorophylls *a* and b [13]. The hydrolysis of all the acyl-containing fractions other than the sterol esters was carried out as described previously [8], while the sterol esters were saponified under the conditions given by Kates [14]. Periodate-permanganate oxidation was done by a known method [8].

The structures of the TAGs were determined by stereospecific analysis and Brockerhoff's method with our modification of the stage of phosphorylating the acylglycerols [11].

SUMMARY

1. Changes have been detected in the lipid composition of the seeds of *Plectranthus glaucocalyx* Maxim. under the influence of the conditions of growth. In the seeds of the introduced (Central Asian) variety of the plant, as compared with the wild (Far Eastern) variety the oil content and the amount of unsaturated acids in the lipids had fallen, and there was a change in the structure of the triacylglycerides.

2. Weak aeration and a low water permeability of the soil also caused a fall in the oil content of the seeds of the introduced species *Plectranthus* and in the total degree of unsaturation of its lipids, with an increase in the amounts of the 18:1 and 18:2(5,6) acids in them.

3. In the TAGs of *Plectranthus glaucocalyx*, the 18:2(5,6) acid is distributed over all three positions with a preference for sn-3. In the case of the introduced plant, a change in the nature of the distribution of the 18:1 acid in the TAGs of the seeds, as compared with the wild variety, was observed but the distribution of the other acids did not change.

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ISOLATION OF PHOSPHATIDYLINOSITOL FROM SOYBEAN PHOSPHATIDES

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A method is described for isolating phosphatidylinositol (PI) from a lipid extract of soybean phosphatides. The method includes the separation of the extract with the aid of a Büchner funnel and alumina into two fractions — choline-containing phospholipids and a PI fraction — and the purification of the latter on a column of DEAE-cellulose. As a by-product, phosphatidylethanolamine with a chromatographic purity of 95-96% is obtained from the PI fraction.

Known methods for the isolation of phosphatidylinositol (PI) [1-9] are complex, laborious, and give a low yield of product, since they usually include a fairly large number of operations (complex extraction, solvent fractionation, dialysis, conversion into the salt form, and column chromatography using one or more columns) and in a number of cases [1, 3, 4] do not permit pure PI to be obtained, which makes additional rechromatography of its fractions necessary. The method which we propose is comparatively simple and permits chromatographically pure PI to be obtained in high yield (80% of its initial amount in soybean phosphatides). Preliminary results have been published in an Inventor's Certificate [10].

Brain lipids [1, 3, 5, 8] are most frequently used to obtain PI, but sometimes the lipids of the liver [7] and the kidneys [4]. All these sources contain a considerable amount of phosphatidylserine (PS), which seriously complicates the isolation of the PI in the pure form [1, 3, 4, 6, 8]. We have isolated the PI from commercial soybean phosphatides obtained from Far Eastern and American varieties of soybean which contain no PS and a fairly large amount of PI. The results of an analysis of the phospholipids (PLs) and the fatty acids (FAs) of the PI from soybean phosphatides are given in Table 1. They differ somewhat from those found in the literature [9, 11, 12]. Thus soybeans and soybean phosphatides (azolectin) contain PS, but no diphosphatidylglycerol (DPG), diphosphatidylinositol (DPI), or an unknown PL of low polarity at the zone of the front have been found. However, in these papers there are statements of the presence of an unknown PL in the zone of the start but similar in its chromatographic behavior to DPI. In our opinion, the appearance of DPI in a phosphatide extract is due to the presence in the soybean paste of lipoproteins which after their treatment with acetone (see the Experimental part) liberate DPI, since denaturation of the protein takes place. The fatty acid composition of the PI that we have isolated likewise differs somewhat from the composition given in the literature [9]. Thus, the amount of unsaturated acids was considerably higher in our material (70% as compared with 41%), although these differences are considerably less when they are compared with figures for the FAs of the PIs isolated from turnips and apples (70% as compared with 66 and 58%, respectively) [13, 14]. In our opinion, the most probable reason for such discrepancies is to be found in the procedure for treating the initial raw material — more severe in the case of the azolectin [9] and mild in the isolation of the phosphatides from the Far Eastern and American varieties of soybean.

The phosphatidic acid (PA) found in the soybean phosphatides in large amount (9-20%) complicates the isolation of the PI. With the aim of removing it, for chromatography we used Al_2O_3 (see the Experimental part), which has the property of strongly binding PA. As a result it was possible to obtain a fraction of choline-containing PLs (phosphatidylcholine (PC), lyso-PC, neutral lipids, glycolipids) and a PI fraction (PI, DPI, phosphatidylethanolamine (PE), N-acyl-PE, phosphatidylglycerol (PG), DPG, an unknown PL, glycolipids) uncontaminated by PA. To elute the first fraction we used the $CHCl_3$—CH_3OH (1:1) system, and for the second the $CHCl_3$—CH_3OH—1% aqueous CH_3COONH_4 (1:1:0.3) system.

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