INFLUENCE OF THE DEGREE OF RIPENESS OF COTTON SEEDS ON THEIR LIPID COMPOSITION

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The influence of the degree of ripeness of the seeds of the cotton plant on their lipid composition has been studied. It has been established that as the seeds ripen their lipid composition becomes simpler, and in the developing cotton seeds the biosynthesis of triglycerides takes place with the participation of the mono- and diacylglycerides as intermediates.

A detailed study of the biosynthesis of triacylglycerols and of the nature of the metabolism of lipid classes in the seeds of higher plants has begun comparatively recently. In such crops as soybean [1], safflower [2], peanut [3], etc., considerable changes in the lipid composition of the seeds according to their degree of ripeness has been detected. As a result of the investigations of more than 10 species of plants, an experimental confirmation has been obtained of a pathway for the biosynthesis of triacylglycerols proposed previously including a stage of the formation of intermediate mono- and diacylglycerols [4].

Cotton seeds have been the least studied in relation to lipid biosynthesis. Individual investigations performed in this direction have been limited simply to a study of the dynamics of the accumulation of oils and fatty acids [5]. The present paper gives the results of an investigation of the metabolism of lipid classes in ripening seeds of the cotton plant of the variety Tashkent 1.

According to our preliminary results, in the first ten days from the moment of flowering lipids are synthesized slowly in the seeds of this plant but the fatty acids of the total lipids are the most diverse in composition [6]. The following ten days of development (20 days from the moment of flowering) are characterized by intensive oil formation. In this period about 46% of the lipids on their relative amount in the ripe seeds accumulates. The remainder of the lipids is synthesized mainly in the course of a 30- to 40-day ripening period. In this process, the composition of the fatty acids simplifies.

For the chemical analysis of the lipids we selected seeds from the 10-, 35-, and 70-day (ripe seeds) phases of development. The characteristics of the seeds are given below:

Index	Days from the moment of flowering		
	10	35	70
Water of 100 seeds, g	1.11	4.32	12.51
Moisture content, %	88.0	56.6	5.1
Oil content, % on the absolutely dry matter	3.1	14.2	22.5
Amount of lipids in 100 seeds, g	0.03	0.61	2.81

As we see, as the seeds ripen the amount of lipids increases simultaneously with the increase in the weight of the seeds, the rate of oil formation being the highest in the period between the 10th and 35th days from the moment of flowering. In the second phase of develop-

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TABLE 1. Change in the Lipid Compositions of Cotton Seeds during Ripening

Class of lipids	Days from the moment of flowering					
	10		35		70	
	wt.%	mg/100 seeds	wt.%	mg/100 seeds	wt.%	mg/100 seeds
1. Unidentified	6,1	1,8	Tr.	Tr.	—	—
2. Hydrocarbons	10,0	3,0	0,3	1,8	0,2	5,6
3. Sterol esters	8,2	2,4	Tr.	Tr.	—	—
4. Fatty acid methyl esters (MEs)	1,5	0,1	Tr.	Tr.	—	—
5. Triacylglycerols (TAGs)	9,1	2,7	83,6	510,0	92,5	2599,2
6. Diol lipids	Tr.	Tr.	—	—	—	—
7. High-molecular-weight fatty alcohols	1,5	0,1	Tr.	Tr.	Tr.	Tr.
8. Free fatty acids (FFAs)	12,1	3,7	3,3	20,1	0,8	22,5
9. Epoxyacylglycerols (EAGs)	Tr.	Tr.	0,9	5,5	0,5	14,0
10. Sterols	10,6	3,1	2,1	12,8	1,6	45,0
11. Diacylglycerols (DAGs)	9,1	2,7	3,6	22,0	—	—
12. Hydroxyacylglycerols (HAGs)	Tr.	Tr.			2,4	67,4
13. Monoacylglycerols (MAGs)	12,1	3,7	1,9	11,6	—	—
14. Polar lipids (PLs)	19,7	5,9	4,3	26,2	2,0	56,2

ment lipids are synthesized only half as fast. These results are in harmony with the corresponding figures for the seeds of other crops [7].

The total lipids of the three samples were separated by column chromatography (CC) into individual classes of compounds. The lipids were identified by means of qualitative reactions, chromatographic mobilities, and comparison with model samples, and by IR, UV, PMR, and mass spectroscopy. Details of the class compositions of the samples are given in Table 1.

In the lipids of the 10-day seeds, 14 classes of compounds were detected, including, a class of nonpolar lipids which could not be identified. In the middle seed-ripening period (35 days from the moment of flowering), the total lipids lacked diol lipids. The lipids of the ripe cotton seeds consisted of eight classes of compounds. In addition, cottonseed oil contains the specific pigment gossypol. Since gossypol is not desorbed from silica gel quantitatively, its amount in the total lipids was determined indirectly. There was no gossypol in the lipids of the 10-day seeds, 0.03% of it in the 30-day seeds, and 0.57% in the ripe seeds.

As can be seen from Table 1, the lipids of the seeds of an early degree of ripeness are the most complex in composition, containing more than 30% of lipophilic components (hydrocarbons, sterols and their esters, fatty alcohols), almost 20% of polar lipids, and only 9% of TAGs.

The free acids are not the determining class of the lipids of the extract of the 10-day seeds, as assumed previously [4], and they are comparable in amount with the MAGs.

During ripening, a rapid accumulation of TAGs takes place and even in the lipids of the 35-day seeds they amount to 84% of the total lipid extracts. The weight content of the other classes of compounds, with the exception of gossypol, HAGs, and EAGs, falls, although the absolute amount of FAAs, sterols, MAGs, and PLs continues to increase as the mass of the seeds increases. By the moment of ripening, the absolute amount of PLs has doubled in comparison with their amount in the 35-day oil. The same results were obtained in the study of the class lipid composition of peanut and cacao seeds [3, 8].

In the ripe seeds, the main class of lipids consisted of the TAGs, triacylglycerols containing epoxy and hydroxy acids had reached their maximum level (2.9%), and MAGs and DAGs were absent. The highest absolute amounts of MAGs and DAGs were recorded in the seeds of the medium-ripe seeds.

Thus, in the developing seeds of the cotton plant, as well, the biosynthesis of the TAGs takes place with the participation of MAGs and DAGs as intermediate compounds.

The metabolic role of the other classes of compounds present in lipid extracts of early-ripe seeds is not completely clear, but the results given in Table 1 show that in the early stage of the biosynthesis of the lipids the proportion of acids bound to the alcohol groups of sterols and of mono- and polyhydric alcohols is substantially higher than the amount of fatty acids present in the free form. It is possible that part of the free acids is used by the plant in the early stages of the development of the seeds with the formation of the intermediate classes of lipids fulfilling certain functions in this period.

Little is yet known about the physiological activity of the free fatty acids and their esters in the period of the intensive increase in the weight of the seeds; nevertheless, the glycolipid "brassin" has been assigned to a new group of phytohormones [9]. It has been reported that the natural fatty acid MEs in minor amounts in some animal and plant tissues may act as promotors of tissue growth stimulators [10].

It must be mentioned that this is the first time that fatty acid MEs have been detected in unripe cotton seeds. The fact that they are native compounds was confirmed by extracting the lipids from a sample of the 10-day seeds with a mixture of solvents containing no methanol.

The quantitative compositions of the hydrocarbons, fatty alcohols, EAGs, HAGs, and sterols isolated from the combined lipid extracts of the 10- and 35-day seeds were the same as in the lipids of the ripe seeds [11]. The fatty acid compositions of the lipids of the free samples are discussed in the following paper [12].

In a mass spectrometric study of the alcoholic part of the TAGs of the 10-day seeds, in addition to the trihydric alcohol glycerol, trace amounts of a dihydric alcohol — ethylene glycol — were also detected, which indicates the presence of diol lipids in the lipids of the early-ripe seeds. Because of the very small amounts, it was impossible to isolate the diol lipids.

EXPERIMENTAL

The seeds of the cotton plant of different degrees of ripeness were collected from an experimental plot of the "Leninskii put'" kolkhoz [collective farm], Tashkent province, in 1979.

The conditions of recording the IR, UV, PMR, and mass spectra and the conditions for performing TLC and CC have been described previously [10].

The total lipids from the seeds were extracted with a mixture of chloroform and methanol (2:1) at room temperature, the solvent was driven off in a rotary evaporator, and the lipids were recrystallized from hexane or petroleum ether (40-60°C).

The sterol esters were identified from their mobilities in TLC by comparing their R_f values with those of esters of β-sitosterol and by the qualitative reaction with 50% H_2SO_4. After severe hydrolysis with a 20% methanolic solution of KOH for 24 h, two products were obtained — fatty acids and the total sterols, the qualitative composition of which was, according to their mass spectrum, similar to that of the free sterols [11].

The fatty acid methyl esters were identified by their mobilities on TLC in comparison with a marker and by mass spectroscopy. Mass spectrum (130°C, 40 eV, 0.5 mA), m/z: 214, 228, 242, 268, 270, 292, 294, 296, 298 (M^+); 183, 197, 211, 237, 239, 261, 263, 265, 267 ($M^+ - 31$); 182, 196, 210, 236, 238, 260, 262, 264, 266 ($M^+ - 32$); 74.

Diol Lipids. The isolation and identification of the alcohol moiety was carried out as described by Vaver et al. [13]. The combined alcohols obtained consisted of glycerol and ethylene glycol. Mass spectrum (140°C, 40 eV, 0.5 mA), m/z: 92.62 (M^+); 74 ($M^+ - 18$); 57; 61 ($M^+ - 31$); 44 ($M^+ - 18$); 43 ($M^+ - 31 - 18$); 43 ($M^+ - 1 - 18$); 31 ($M^+ - 31$) [14].

Polar lipids were eluted from the column by methanol from all the samples of oil. The qualitative reaction of this fraction for the phospholipids was negative [15]. The lipids from the 10- and 35-day samples of seeds gave a positive qualitative reaction for glycolipids

[16], the coloration of the PLs of the 10-day seeds being more intense. IR spectrum, ν_{max}^{KBr}: 3600-3200 m, 3050 m, 2920 s, 2850 m, 1730 s, 1100 s. After alkaline hydrolysis, fatty acids were obtained.

CONCLUSION

The influence of the degree of ripeness of cotton seeds on the composition of their lipids has been studied. It has been established that the lipids of the early-ripe seeds are the most complex in composition: at the moment of ripening the lipid composition has simplified.

It has been observed that in the developing seeds of the cotton plant the biosynthesis of triacylglycerols takes place with the participation of monoacyl- and diacylglycerols as intermediates.

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