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PHOSPHOLIPIDS OF GERMINATING SEEDS OF A COTTON PLANT OF THE TASHKENT-1 VARIETY

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Phosphatidylinositol with a nontraditional position distribution of the fatty acids has been detected in germinating cotton seeds.

Usually, in phospholipid (PL) molecules of living organisms the sn-2 position is esterified by unsaturated fatty acids (FAs) [1]. However, there are exceptions [2-7]. Dyatlovitskaya [7] considers that a change in the structure of the lecithins of tumor cells is due to disturbances of their biosynthesis.

We have previously, for the first time, detected in 24-hour flowers of a cotton plant of the 159-F variety a phosphatidylinositol (PI) with an unusual distribution of the FAs [8] which appears in the transitional time of development of the flower [9]. PI possesses a broad spectrum of biological action [10, 11]. By analogy with ordinary phosphatidylcholine (PC) and the thrombocyte-activating (platelet-activating) factor (PAF – an alkyl-acyl PC in the sn-2 position of which low-molecular-mass FAs are localized [12], which possesses a powerful and many-sided action [13]), it may be assumed that a PI with an unusual position distribution of the FAs should differ substantially in biological activity from ordinary PI. In the ontogenesis of a plant, one of the main transitional moments is the time from the state of dormancy (ripe seeds) to the stage of development (germinating seeds). By analogy with cotton flowers, we assumed that during the germination of cotton seeds a PI with a nontraditional position distribution of the FAs appears in their cell membranes.

The composition of the PLs and the position distributions of the FAs in the main PLs of ripe cotton seeds of the Tashkent-1 variety have been studied previously [14, 15]. According to the literature, the change in the PLs was studied from ripe seeds to 10- to 18-day shoots (samples were taken every 2-3 days) [16-18], and the FA composition was determined either in the total lipids or in the combined PLs at the same intervals [18-20]. The position distributions of the FAs in the individual PLs of the germinating cotton seeds were not studied.

We have investigated the dynamics of the composition of the FAs of the total lipids during the germination of cotton seeds of the Tashkent-1 variety under standard conditions in the interval from 0 to 96 h, assuming that the transitional period in the development of the cotton plant is reflected in the pattern of the dynamics of the FAs (Table 1).

The greatest change in the quantitative level of the FAs was observed in the interval from 6 to 24 h. We selected this interval — namely, 19-hour germinating seeds – to study the PLs, and we studied the PLs of ripe seeds as control (Table 2).

The results that we obtained for the ripe seeds differ somewhat from those given in the literature [14, 15], while for the germinating seeds the differences are more considerable [16-18], apparently because of different times of taking samples. The ripe seeds contained eight classes of PLs, in the total of which 68.2% consisted of phosphatidylcholine, and 27.3% of PI. In the total PLs of the germinating seeds we determined 13 classes of PLs of which the main ones were phosphatidic acid, 42.9%, and PI, 32.3%. In the germinating seeds the amount of PC had fallen by more than 50% in comparison with the ripe seeds.

From the total PLS of the ripe and germinating seeds we isolated the PC and PI in individual form and determined the position distributions of the FAs in them by enzymatic hydrolysis with phospholipase A_2 (Table 3). In the ripe seeds, the traditional position distribution of the FAs was observed in the PC and PI. In the PC of the germinating seeds the position

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TABLE 1. Fatty-Acid Composition of the Total Lipids of Germinating Cotton Seeds of the Tashkent-1 Variety, %

FA	Hours from the beginning of germination								
	0	2	4	6	8	24	48	72	96
16 : 0	28.8	31.4	30.6	30.8	38.3	31.1	24.1	33.4	26.9
18 : 0	1.3	Tr.	0.5	Tr.	1.3	Tr.	3.8	Tr.	2.1
18 : 1	17.1	15.1	15.1	17.0	18.9	14.3	21.8	22.9	20.1
18 : 2	52.8	53.5	53.8	52.2	41.5	54.6	50.3	43.7	50.9
Σ_s	30.1	31.4	31.1	30.8	39.6	31.1	27.9	33.4	29.0
Σ_u	69.9	68.6	68.9	69.2	60.4	68.9	72.1	66.6	71.0

TABLE 2. Composition of the Phospholipids of Ripe and Germinating Cotton Seeds of the Tashkent-1 Variety

Phospholipids	R_f		Seeds, %	
	syst. 1	syst. 2	ripe	19-hour germinating
Unident.	0.25	0.00	0.5	7.0
Lysophosphatidylcholine	0.25	0.35	0.7	0.6
Lysophosphatidylinositol	0.19	0.38	0.2	—
Phosphatidylinositol	0.30	0.50	27.3	32.2
Phosphatidylcholine	0.50	0.55	68.2	10.7
Phosphatidic acid	0.25	0.80	0.9	42.9
Phosphatidylethanolamine	0.57	0.68	2.1	2.7
Phosphatidylglycerol	0.65	0.78	0.1	1.3
Unident.	0.10	0.17	—	1.1
Unident.	0.17	0.47	—	0.4
Unident.	0.72	0.69	—	0.6
Unident.	0.75	0.77	—	0.3
Unident.	0.80	0.82	—	0.1
Unident.	0.86	0.85	—	0.1

TABLE 3. Composition and Position Distribution of the Fatty Acids in the Phosphatidylcholines and Phosphatidylinositols of Ripe and 19-hour Germinating Seeds of a Tashkent-1 Cotton Plant

FA	Phosphatidycholine			Phosphatidylinositol		
	tot.	position		tot.	position	
		sn-1	sn-2		sn-1	sn-2
			Ripe seeds			
14 : 0	0.8	0.8	0.8	0.2	0.4	—
Unident.	—	—	—	0.3	0.6	—
16 : 0	27.5	50.3	4.7	46.4	72.4	20.4
18 : 0	Tr.	Tr.	Tr.	2.9	5.8	Tr.
18 : 1	24.8	15.8	33.8	19.7	17.9	21.5
18 : 2	46.9	33.1	60.7	30.5	2.9	58.1
Σ_s	28.3	51.1	5.5	49.5	78.6	20.4
Σ_u	71.7	48.9	94.5	50.5	21.4	79.6
			19-hour germinating seeds			
14 : 0	0.6	1.2	—	0.9	0.7	1.1
Unident.	—	—	—	0.7	0.9	0.5
16 : 0	22.8	38.6	7.0	50.0	44.6	55.4
Unident.	—	—	—	1.0	—	2.0
18 : 0	Tr.	Tr.	Tr.	1.6	1.7	1.5
18 : 1	26.3	24.0	28.6	29.4	34.7	24.1
18 : 2	50.3	36.2	64.4	16.4	17.4	15.4
Σ_s	23.4	39.8	7.0	52.5	47.0	58.0
Σ_u	76.6	60.2	93.0	47.5	53.0	42.0

distribution of the FAs was close to that of the ripe seeds, with only an 11% increase in the amount of unsaturated FAs in the sn-1 position, mainly due to oleic acids.

In the PI of the the 19-hour germinating seeds 58.0% of saturated FAs, mainly palmitic acid (55.4%) was detected in the sn-2 position and 53.0% of unsaturated acids, mainly oleic (34.7%) and lineolic (17.4%) in the sn-1 position.

Thus the hypothesis of the appearance of a PI with a nontraditional position distribution of the FAs in the transitional period of the development of the cotton plant has been confirmed completely. Since, as in our case, a deviation from the traditional position distribution of FAs in PLs has been detected in various healthy organisms [2-9], this phenomenon can hardly be regarded as a deviation from the norm.

EXPERIMENTAL

A cotton plant of the Tashkent-1 variety was grown in a thermostat at 26°C. The total PLs were isolated as described in the literature [21, 22]. The qualitative and quantitative compositions of the PLs were determined after two-dimensional TLC by the method of [23]. First direction – system 1: chloroform–methanol–25% ammonia: (10:4:1); second direction – system 2: chloroform–methanol–acetic acid–water (10:4:1:1). The individual PLs were isolated by column chromatography, with subsequent purification by TLC in systems 1 and 2, using silica gel as sorbent [21].

Enzymolysis with phospholipase A_2 was conducted as in [21]. Methyl esters were analyzed on a Chrom-41 chromatograph with a flame-ionization detector: steel column (2.5 × 4 mm [sic]); stationary phase poly(ethylene glycol) succinate (17%) on Celite-545 (80-100 mesh); thermostat temperature 197°C; evaporator temperature 250°C; carrier gas helium.

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