The main unsaturated acids of the chlorella lipids contain the first double bond in the Δ^9 position.

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STUDY OF THE PHOSPHOLIPIDS OF VARIOUS ORGANS OF *Crambe amabilis* ACCORDING TO THE VEGETATION PERIODS

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The known studies of phospholipids according to the vegetation periods of plants are devoted mainly to the changes in the phospholipid complex in the process of the ripening of the seeds of certain plants [1-6]. Our aim was to study the change in the fatty-acid and fractional compositions of the phospholipids of various organs of *Crambe amabilis* according to the vegetation phases of the plant.

The plants were collected in 1977 in the Bostanlyskii region of the Tashkent oblast (environs of Burchmulla). The combined phospholipids (PL's) from the various organs of the plant [7, 8] were freed from accompanying carbohydrates [9] and were analyzed in a thin layer of silica gel in solvent systems 1 and 2. From their chromatographic mobilities, the phospholipids with R_f 0.4 and 0.98 (in system 1) and 0.3 and 0.9 (in system 2) were assigned, respectively, to the phosphatidylglycerols (PG's) and the phosphatidic acids (PA's), which are widely distributed in the leaves of plants [1, 2]. The amounts of the individual PL's (Table 1) were determined from the phosphorus contents [10] of the corresponding spots on the chromatogram [11].

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TABLE 1. Amounts and Fractional Compositions of the Phospholipid Complexes in Various Organs of Crambe amabilis According to the Vegetation Phases

Date of collection	Phase of development and plant organ	Yield of PL's, % on the weight of the air-dry raw material	Amount of phosphorus in the total phospholipids, %	Quantitative compositions of the individual phospholipid fractions, %								
				PC's	PI's	PE's	PS's	Lyso-PC's	N-Acyl-PE's	N-Acyllyso-PE's	PG's	PA's
2.04.77	Leaf rosettes	0,26	2,5	2,3	29,5	13,6	22.7	—	9,3	4,5	4,5	13,6
16.04	Before budding epigeal part	0,31	2,3	16,0	26,2	16,0	10,5	—	9,0	4.0	4,9	13,4
30.04	Budding											
	epigeal part	0,28	1,9	21,9	24,8	20.1	7,3	2,3	6,7	3,1	4,0	9,8
	buds	0,6	2,4	35,0	22,1	20,1	5,7	4,4	4,1	3,1	2,3	3 2
	Flowering											
14.05	flowers	0,63	2,6	37,2	20,2	21,0	5,4	4,3	4,5	3,2	2,0	2,2
	stems	0,1	2,2	24,0	25,2	23,0	5,4	2,0	4,4	4,0	5,5	6,5
	leaves	0 15	1,8	23,0	33,4	25,2	—	1,0	3,5	2,6	3.0	8,3
29.05	Incipient fruit-bearing											
	unripe seeds	0,8	2,9	40,0	21,4	23,8	4,7	4,3	3,7	2,1	—	—
	stems	0,07	2,5	25,3	25,4	23,4	3,1	3,0	4,4	3,4	6,0	6,0
	leaves	0,13	2,0	17,5	31,3	21,3	—	1,9	5,5	4,2	8,3	10,0
12.06	Fruit bearing											
	unripe seeds	0,85	3,3	48,8	18,1	21,3	3,1	3,9	2,8	2.0	—	—
	stems	0,05	2,7	24,9	23,1	23,9	3,0	3,0	5,7	4,0	6,3	6,1
	leaves	0,12	1,9	18,1	30,9	22,1	—	2,0	5,0	3,9	8,0	10,0
26.06	Fruit bearing											
	unripe seeds	0,95	3,3	50,0	17,0	20,1	3,1	4,5	2,9	2,4	—	—
	stems	0,04	2,7	24,5	23,5	23,0	3,0	3,1	5,9	4,2	6,6	6,2
	leaves	0,08	2,0	18,0	30,0	23,0	—	2,5	4,9	3,0	9,9	8,7
10.07	End of fruit-bearing											
	ripe seeds	1,1	3,5	55,6	15,3	20,0	2,9	1,5	2,5	2,2	—	—

It can be seen from Table 1 that during the vegetation process the amount of total phospolipid complex varies greatly. In particular, the amount of PL's in the leaves and stems decreases from the beginning of budding (0.28%) and reaches a minimum at the end of the vegetation period, while during this period an intensive accumulation of PL's in the buds begins, reaching a maximum at the moment of full ripeness of the seeds (1.1%). It may be concluded from this that the PL's are present in all the plant organs investigated throughout the vegetation period and in the period of the full ripeness of the seeds they are localized almost completely in them.

We may note that in all the stages of the development of the plant there is approximately twice as much phospholipids in the leaves as in the stems.

The individual phospholipid fractions in the plants studied also change during its growth. As is well known, the phosphatidylcholines (PC's), phosphatidylinositols (PI's), and phosphatidylethanolamines (PE's) are the main components of the total phospholipids of all seeds studied [4, 9, 12], including those of Cr. amabilis [13]. Table 1 shows that during the development of the plant the amount of PC's increases from 2.3 to 55.6% and of PE's from 13.6 to 20.2% and the amount of PI's decreases nonuniformly – from 29.5 to 15.3%. The amount of phosphatidylserines (PS's), of N-acyl-PE's, and of their lyso analogs falls up to the period of complete ripeness of the seeds, the most considerable variations being observed in the PS's: in the early stages of development they make up 22.7%, and in the period of full ripeness of the seeds 2.9%. It is interesting that the PS's disappear from the leaves completely from the moment of flowering of the plant. The great fall in the amount of PS's, the rise in the amount of PC's, and the less considerable change in the amount of PE's in the process of development of the plant suggest to us the idea that these PL's are transformed into one another, and the results obtained confirm one of the possible routes for the synthesis of PC's as the following: phosphatidylserines – phosphatidylethanolamines – phosphatidylcholines [14].

Conversely, the amounts of PG's and PA's in the leaves are less than those in the stems. We did not detect them in the seeds, possibly because of their small amount, which agrees with the results of other workers [1, 14]. The variation in the amounts of PG's and PA's in the period of the growth of the plant, and also their absence in the unripe seeds (period of intensive oil formation) and in the ripe seeds can probably be explained by the use of these substances in metabolic reactions involved in the biosynthesis of triglycerides [15].

TABLE 2. Fatty-Acid Compositions of the Total Phospholipids Obtained from Various Organs in Various Vegetation Periods of Crambe amabilis

Phase of development and plant organ	Fatty acid												
	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:1	Σ_S	Σ_U
Leaf rosettes	2,0	2,8	0,8	38,0	1,8	4,0	7,0	7,6	36,0	—	—	47,6	52,4
Before budding													
epigeal part	1,9	0,9	0,7	18,6	0,8	1,5	12,0	6,4	50,0	—	7,2	23,6	76,4
Budding													
epigeal part	—	—	0,7	26,7	1,8	2,6	6,6	8,8	49,6	—	3,2	30,0	70,0
buds	0,8	0,7	0,3	23,8	1,1	4,8	5,1	14,4	46,6	—	2,4	30,4	69,6
Flowering													
flowers	0,6	0,7	0,4	24,0	0,9	3,0	5,4	20,2	40,8	1,1	3,2	29,4	70,6
stems	2,9	—	—	23,3	3,2	2,8	10,8	12,1	44,9	—	—	29,0	71,0
leaves	8,4	—	—	14,3	2,9	1,3	6,2	7,4	42,2	—	17,3	24,0	76,0
Incipient fruit-bearing													
unripe seeds	1,1	0,7	0,5	25,0	3,6	3,0	8,6	20,7	30,5	3,8	2,5	34,1	65,9
Incipient fruit bearing													
stems	11,1	—	—	23,9	3,3	4,6	8,3	11,1	37,7	—	—	38,6	61,4
leaves	5,9	2,4	2,2	15,6	1,9	1,5	10,8	12,9	31,9	—	14,9	27,6	72,4
I. Fruit-bearing													
unripe seeds	1,3	0,5	0,2	27,2	1,6	2,6	12,0	26,1	22,3	3,0	3,2	35,1	64,9
stems	3,2	—	—	28,0	3,5	4,2	8,8	14,4	37,9	—	—	35,4	64,6
leaves	2,3	2,1	—	24,6	2,6	4,0	15,6	12,3	27,8	—	8,7	33,0	67,0
II. Fruit-bearing													
unripe seeds	1,8	1,1	0,6	20,0	1,4	2,5	16,5	26,0	20,5	3,6	6,0	29,6	70,4
stems	5,2	—	—	28,0	3,0	6,2	17,6	13,3	26,7	—	—	39,4	60,6
leaves	4,4	1,2	1,2	20,3	1,7	2,4	20,9	8,8	28,9	—	10,2	29,5	70,5
End of fruit-bearing													
ripe seeds	2,0	0,6	0,4	16,4	0,8	0,7	27,5	28,1	14,4	2,5	6,6	22,6	77,4

Our results confirm that phosphatidic acids are widely distributed in nature and possibly play the role of intermediates in glycerolipid metabolism [16].

Lysophosphatidylcholines (lyso-PC's) appear in the budding period and their amount decreases up to the moment when the seeds are ripe; during the growth of the plant their amount in the leaves is smaller than in the stems. The main amount of lyso-PC's is localized in the seeds.

Each set of total PL's was deacylated by alkaline saponification, and the liberated fatty acids were analyzed in the form of their methyl esters by the GLC method (Table 2). The results show that among the saturated acids of the total PL's of all the organs of Cr. amabilis investigated palmitic acid ($C_{16:0}$) predominates, its amount decreasing nonuniformly from 38% in the leaf rosette phase to 16.4% at the end of the vegetation period. Up to the period of fruit-bearing, among the unsaturated acids linolenic ($C_{18:3}$) predominated in all the samples of the total PL's its amount decreasing nonuniformly up to this stage. From the moment of fruit-bearing, the amount of linolenic acid decreased considerably with a simultaneous increase in the amounts of linoleic ($C_{18:2}$) and oleic ($C_{18:1}$) acids.

In the period of the complete ripeness of the seeds, in the total PL's among the unsaturated acids oleic and linolenic predominated almost equally. Arachidic acid ($C_{20:0}$) was detected in the flowering phase only in the flowers, and from the moment of incipient fruit bearing to the stage of complete ripeness of the seeds it was also found in the seeds; its amount was relatively stable in the process of development of the plant. Erucic acid ($C_{22:1}$) was absent from the stems and its main amount was localized in the leaves.

At the beginning of the development of the plant, the total amount of saturated (Σ_S) and the total amount of unsaturated (Σ_U) acids were, respectively, 47.6% and 52.4%. The amount of unsaturated acids in the ripe seeds fell to 22.6%, and the unsaturated acids rose to 77.4%. One of the reasons for this phenomenon is possibly that at the beginning of the vegetation period a considerable part of the total PL's consists of PC's (see Table 1), the molecules of which contain, in esterified form, predominantly the saturated acids [17-19].

EXPERIMENTAL

The solvents used were purified and rendered absolute by known methods [20]. The PL's from the leaves were isolated by the method of Kates and Eberhardt [7] and those from the stems and leaves by Folch's method [8].

The total PL's were purified as described previously [9]. The qualitative and quantitative composition of the total PL's were determined by two-dimensional TLC in a fixed layer of silica gel in the following systems: 1) chloroform–methanol–water (63:35:5), and 2) chloroform–methanol–ammonia (65:35:5). The PL's were identified with the aid of color reactions and by the characteristic functional groups for the varieties determined.

The fatty acids of the phospholipids were isolated by alkaline saponification (5% KOH in methanol, room temperature). The mixture of fatty acid methyl esters was separated by GLC. Conditions for GLC: UKh-2 chromatograph with a katharometer in a copper column 2500 × 4 mm containing 18% of polyethylene glycol succinate on Celite-545 (80-100 mesh) at 197°C with helium as the carrier gas. The pressure of helium at the outlet was 2.5 atm. We also used a Khrom-4 instrument with a flame-ionization detector and a copper column (2500 × 3 mm) filled with 15% of Reoplex-400 on Chromaton N-AW-DMCS (60-80 mesh). The temperature of the column was 205°C and of the evaporator 255°C. The rate of flow of the carrier gas (helium) was 40 ml/min.

Isolation of the Total Phospholipids from the Leaves [7]. Freshly comminuted leaves (23 g) were treated with hot isopropanol (115 ml) for 3-5 min. The hot mixture was filtered, and the residue on the filter was washed first with hot isopropanol and then with chloroform–isopropanol (1:1) and with chloroform. The combined filtrates were evaporated in a rotary evaporator to dryness. The residue was dissolved in chloroform (50 ml), and the resulting solution was washed several times with water. The chloroform solution was evaporated to dryness. Yield 0.034 g.

SUMMARY

The changes in the fatty-acid and fractional compositions of the phospholipid complexes of various organs of Crambe amabilis have been studied according to the vegetation periods.

It has been established that throughout the vegetation period phospholipids are present in all organs, and at the stage of complete ripeness of the seeds they are almost wholly localized in the seeds. In the process of the development of the plant considerable qualitative and quantitative changes take place in the fractional and fatty-acid compositions of the phospholipids obtained from various organs of the plant.

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