PHOSPHOLIPIDS OF *Phaseolus aureus*. I

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The mung bean (*Ph. aureus* Roxb., family Leguminosae) is a valuable protein plant which is widely cultivated in Uzbekistan. There is information in the literature on the phospholipids of *Ph. aureus* grown in India [1]. For the purposes of the search for suitable plant materials for obtaining lecithins, in the present paper we give the analytical characteristics of the phospholipids (PLs) of some plants of the family Leguminosae, including the mung bean. For the latter we have shown the total yield of combined PLs (1.5%), and also the amounts of nitrogen, phosphorus, choline, and fatty acids in them and their iodine numbers. We have studied the phospholipid complex of the seeds of the mung bean of variety Pobeda-104 obtained from the Central Asian experimental station of VIR [All-Union Scientific-Research Institute of Plant Breeding].

The PLs were extracted with chloroform–methanol (2:1) from the seeds that had been defatted with acetone [2]. The extract obtained consisted of a dark green resinous mass containing a considerable amount of pigments, carbohydrates, amino acids, and neutral lipids. It was freed from carbohydrates by gel filtration in chloroform–methanol–water (90:10:1) through Molselekt G-25 [3].

The sugars eluted from the Molselekt by methanol were analyzed by paper chromatography in the butanol — pyridine — water (6:4:3) system. Glucose and fructose were identified. Pigments and neutral lipids were eluted by column chromatography of the crude total PLs on silica gel (eluents: acetone and chloroform). The yield of purified total PLs was 1.3%, and their phosphorus content was 3.6%. Two-dimensional chromatography in system 1 and 2 revealed the presence of eight phospholipids, and six of them were identified. The quantitative distribution of the components of the total material and their R_f values in direction I were as follows: phosphatidylcholines (PCs), R_f 0.5, 56%; phosphatidylethanolamine(PEs), R_f 0.7, 20.8%; phosphatidylinositols (PIs), R_f 0.2, 14.1%; N-acylphosphatidylethanolamines (N-acyl-PEs), R_f 0.95, 3.2%; N-acyl-lyso-phosphatidylethanolamines (N-acyl- lyso-PEs), R_f 0.85, 2.5%; lysophosphatidylcholines (lyso-PCs), R_f 0.1, 1.7%; x_1-PL, R_f 0.15, 1.2%; and x_2-PL, R_f 0.9, 0.5%.

It must be mentioned that a higher content of PEs than of PIs is untypical for plant PLs [4].

Homogeneous fractions of the PLs obtained by the column chromatography of the total material followed by preparative TLC were subjected to acid hydrolysis. In addition to glycerol, the hydrolyzates of the PCs and lyso-PCs were found to contain choline, those of the PIs inositol, and those of the PEs, N-acyl-PEs, and their lyso analogs contained ethanolamine. The fatty acids of the total PLs and of the individual fractions were split out by alkaline hydrolysis and were analyzed in the form of esters by GLC (Table 1).

The fatty acids (FAs) of the total and the individual PLs consisted of a set of 12-13 acids. With respect to increasing degree of unsaturation, the PLs formed the following sequence: N-acyl-PEs → lyso-PCs → PEs → PIs → PCs → N-acyl-lyso-PEs.

The total PLs contained trace amounts of the 21:0 acid, which was present wholly in the N-acyl-PEs in amide-bound form. The distribution of the FAs between position 1 and 2 was established from a study of the products of enzymatic hydrolysis (see Table 1).

It follows from the results obtained that the total unsaturation of position 1 was almost the same for the PEs and the PIs, and the unsaturation of position 2 was the same in all the fractions. In the PCs the distribution of the FAs was less specific than in the PEs and PIs, which is possibly due to the comparatively small degree of unsaturation of the ordinary PC molecule.

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TABLE 1. Compositions of the Total Phospholipids of the Seeds of the Mung Bean of Variety Pobeda-104 and of their Individual Fractions

Fraction	Fatty acid													
	10:0	12:0	14:0	15:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	21:0	ΣS	ΣU
Total phospholipids	0,3	0,5	0,4	Tr.	26,4	Tr.	0,9	Tr.	4,0	2,8	47,6	17,1	Tr.	32,5	67,5
Phosphatidylcholines															
Total	2,3	1,2	0,7	0,7	22,4	0,7	0,7	Tr.	3,9	3,4	50,2	13,8	—	31,9	68,1
Position 1	4,4	2,7	1,6	1,5	37,9	1,7	1,6	Tr.	7,9	4,0	29,2	7,5	—	7,6	42,4
Position 2	3,6	2,4	1,8	1,7	5,2	1,5	1,4	Tr.	2,3	3,3	59,3	17,5	—	18,4	81,6
Phosphatidylethanol-amines															
Total	2,4	1,2	0,8	0,8	38,1	1,9	Tr.	2,0	3,4	3,0	38,9	7,5	—	46,7	53,3
Position 1	4,3	3,2	2,2	2,4	59,7	1,7	2,0	Tr.	7,5	3,4	13,6	Tr.	—	81,3	18,7
Position 2	—	—	—	—	10,4	—	3,1	1,7	4,8	8,8	63,2	8,0	—	18,3	81,7
Phosphatidylinositols															
Total	2,2	1,6	0,7	0,6	31,4	0,8	0,9	—	5,1	2,6	36,1	18,0	—	42,5	57,5
Position 1	,0	2,8	1,4	1,3	59,9	1,0	1,6	—	8,9	2,4	11,8	3,9	—	80,9	19,1
Position 2	1,6	1,9	2,9	2,1	5,6	1,9	1,8	—	2,2	3,2	50,0	26,8	—	18,1	81,9
N-Acyl-PEs															
Total	3,9	2,3	3,5	3,3	18,5	Tr.	3,7	Tr.	6,7	7,9	22,4	17,1	10,7	52,6	47,4
O-Acyls	0,9	0,6	1,3	1,0	28,5	2,2	1,1	Tr.	3,5	2,9	46,7	11,3	Tr.	36,9	63,1
N-Acyls	7,5	2,4	2,1	2,2	11,3	3,0	2,8	3,2	4,3	5,2	11,6	33,4	11,0	43,6	56,4
N-Acyl-lyso-PEs															
Total	0,8	0,5	1,1	0,7	19,7	1,3	0,9	0,6	4,3	6,8	48,2	15,1	—	28,0	72,0
O-Acyls	0,9	0,3	0,7	0,7	7,4	0,8	0,6	Tr.	2,2	4,8	59,3	22,3	—	12,8	87,2
N-Acyls	0,6	0,5	1,1	1,2	10,1	1,9	1,4	1,3	3,6	6,3	51,5	20,5	—	18,5	81,5
Lysophosphatidyl-cholines	9,7	2,1	2,0	1,6	25,9	3,3	1,9	Tr.	6,1	7,4	33,5	6,5	—	49,3	50,7

In determining the possible molecular composition of the main PLs, we based ourselves on the experimental results of the position distribution of the fatty-acid radicals in their molecules and the calculation method [5]. As a result, 92 species were calculated for the PCs, 88 for the PIs, and 67 for the PEs, of which 73, 67, and 48, respectively, were present in amounts of less than 1%. These molecular species of the PLs investigated were formed mainly from the minor acids. The dominating species in all cases were formed from different combinations of the 16:0, 18:1, 18:2, and 18:3 acids.

On summing separately the saturated and unsaturated species, we obtained the following group compositions of the diglycerides of the PCs, PEs, and PIs;

	PCs	PEs	PIs
Saturated—saturated	10.2	14.8	14.7
Saturated—unsaturated	8.2	3.5	3.4
Unsaturated—saturated	34.6	66.5	66.3
Unsaturated—unsaturated	47.0	15.2	15.6

The group compositions of the PEs and the PIs were almost identical, as was to be expected from the overall information on the position distribution of the saturated and unsaturated FAs. The PCs contained a considerably larger amount of unsaturated—saturated and unsaturated—unsaturated species, which is due to the greater unsaturation of the PC molecules as compared with the PE and PI molecules.

The analysis of the N-acyl-PEs and N-acyl-lyso-PEs was carried out by the method of Bomstein [6]. It can be seen from Table 1 that the N-acyl-lyso-PEs form the most unsaturated section of the total PLs. On both cases, the saturated FAs were present predominantly in the amide-bound form. In the NMR spectrum of the N-acyl-lyso-PEs there was a multiplet at 5.3 ppm (>CH—O—CO—R), the presence of which confirmed the considerable unsaturation of the O-acyls and the structure of the PLs investigated as 2-acylglycerylphosphoryl-N-acylethanolamines [7]. On the basis of the fatty-acid composition of the lyso-PCs it may be assumed that the latter are 1-acylglycerophosphorylcholines.

EXPERIMENTAL

TLC was performed with KSK silica gel having a particle size of up to 125 μm, and column chromatography with the same material having a particle size of up to 50 μm. Two-dimensional chromatography was carried out in the following solvent systems: 1) chloroform—methanol—

ammonia (65:35:5) and 2) chloroform—acetone—methanol—acetic acid—water (40:20:10:10:3).

The alkaline hydrolysis of the PLs was performed in 10% methanolic KOH solution at room temperature [8]. Chromatograms of the methyl esters of the fatty acids were taken on a UKh-2 chromatograph at a column temperature of 198°C using a column 2.5 m long containing 70% of PGS on PEGS on Celite-545 (60-80 mesh). The peaks of the fatty acid methyl esters were identified from their relative retention times using the linear dependence of the logarithms of these magnitudes on the number of carbon atoms [9, 10].

The acid hydrolysis of the PLs was carried out in sealed tubes in 3 N (or, in the case of the PIs, 6 N) HCl at the boiling point of the bath for 24 h. Amines and polyols were identified with markers in the 2% ammonia—methanol (2:3) system. The enzymatic hydrolysis of the main PL fractions were carried out with the aid of phospholipase A_2 from the venom of the Azerbaidzhan kufi in tris buffer with pH 10.4 at 37°C. The partial deacylation of the N-acyl-PEs and their lyso analogs was performed in 0.1 N KOH in methanol at 37°C for 80 min. The NMR spectrum of the N-acyl-lyso-PEs was recorded on a JNM-4H 100/100 MHz instrument in deuterochloroform solution.

SUMMARY

The phospholipid complex of the seeds of the mung bean of variety Pobeda-104 has been investigated. It was found that the amount of phosphatidylethanolamines was greater than that of the phosphatidylinositols.

The fractional and fatty-acid compositions of the total phospholipids were determined. It was established that the 21:0 acid was localized completely in the N-acyl-phosphatidylethanolamine fraction, and this exclusively in the amide-bound form.

On the basis of the results of analysis of the fatty-acid composition and the NMR spectrum, the structure of 2-acyl-glycerophosphoryl-N-acyl-ethanolamines is proposed for the N-acyl-lysophosphatidylethanolamines.

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