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ELECTROPHORETIC AND AMINO ACID COMPOSITIONS OF THE PROTEINS OF MAIZE LINES DIFFERING IN THE ANTHOCYAN COLORATION OF THE SEEDS

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The electrophoretic and amino acid compositions of the proteins of lines of maize differing in the anthocyan coloration of their seeds have been investigated. The molecular masses of the quantitatively main components ranged from 11 to 42 kDa for the albumins and from 15 to 81 kDa for the globulins. Differences between the lines were observed in the levels of components with molecular masses of from 11 to 31 kDa. The amino acid compositions of the zeins of the lines studied were characterized by high levels of leucine, glutamic acid, aspartic acid, alanine and serine, and those of the other groups of proteins by high levels of arginine, glutamic acid, and glycine.

We have previously studied the electrophoretic compositions of the zeins of various lines and forms of maize cultivated in Uzbekistan [1]. It was observed that the zeins of maize seeds differing with respect to their anthocyan coloration contain dissimilar electrophoretic components which can be used as markers. Continuing investigations in this direction, we have obtained three lines of maize homogeneous in the electrophoretic composition of the zeins and differing in the anthocyan coloration of the seeds.

The ancestor of these lines is a maize with grains of different colors, predominantly of hybrid origin. Seeds from this cob were separated into three groups with different seed colorations and were sown to obtain the next generation. We used seeds of the third generation homogeneous with respect both to the morphological characteristics of the plants and to the electrophoretic spectra of the zeins.

The lines obtained were designated as L-1 (with white seeds), L-2 (with golden yellow seeds), and L-3 (with a dark anthocyan coloration of the seeds). The albumins and globulins of the seeds of these maize lines were represented by a multitude of components with molecular masses of from 10 to 95 kDa (Fig. 1). The main difference between the albumins and the globulins consisted in the fact that polypeptides with molecular masses of 81, 71, 62, 52, and 34 kDa were present as the main components in the globulin fraction and as minor components in the albumins. A distinguishing feature of the albumins was the high level of components with molecular masses of 42 and 11 kDa, which were minor components in the globulin fraction. The differences mentioned were common for the albumins and globulins of all the maize samples studied. There were definite differences between the maize lines studied in the compositions of both the albumin and the globulins. Thus, in the L-1 albumins 24 components were detected, in the L-2 albumins, 22, and in the L-3 albumins, 21. Of them, 18 components were common for all the lines. In L-3, with the anthocyan coloration, a number of the components present in L-1 and L-2 were absent.

Analogous differences were observed in the composition of the globulins. Common for all the lines were 15 electrophoretic components. For the remaining components, partial similarity was observed between L-1 and L-2, L-1 and L-3, and L-2 and L-3. The molecular masses of the individual components ranged from 11 to 31 kDa. Consequently, polymorphism between the lines was found in the composition of the relatively low-molecular-mass proteins.

The proteins of the maize seeds were represented mainly by zeins, albumins, and globulins. The amount of zeins could reach 50% of the total proteins and, therefore, it is on the amino acid composition of this group of proteins that the nutritional value of maize as a whole substantially depends. An amino acid analysis of the zein fraction showed that these proteins were

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TABLE 1. Amino Acid Compositions of the Seed Proteins of Various Maize Lines (mol.-%)

Amino acid	Zeins			Albumins + globulins		
	L-1	L-2	L-3	L-1	L-2	L-3
Aspartic acid	9.00	10.93	9.82	5.65	3.96	7.56
Threonine	4.64	3.60	3.49	2.78	1.17	2.70
Serine	6.87	9.07	9.34	4.58	3.08	5.51
Glutamic acid	13.97	16.96	17.39	17.32	14.65	13.84
Proline	2.98	0.97	1.29	5.75	4.57	9.78
Glycine	0.62	0.27	0.64	11.00	7.94	12.36
Alanine	11.61	8.87	9.34	7.03	3.56	5.96
Cystine	–	–	–	–	–	–
Valine	3.03	3.93	4.34	3.10	2.21	3.13
Methionine	–	–	–	–	–	1.51
Isoleucine	2.89	2.06	2.20	0.95	0.58	1.16
Leucine	29.65	35.87	37.41	2.76	2.34	3.65
Tyrosine	2.13	1.82	0.91	1.35	1.13	3.40
Phenylalanine	6.68	2.27	1.86	1.67	1.91	1.39
Lysine	2.32	2.06	1.15	3.24	2.77	5.21
Histidine	0.85	1.13	0.64	–	4.88	6.66
Arginine	2.75	0.16	0.21	32.92	45.25	16.18
Acidic	22.97	27.89	27.27	22.97	18.61	21.40
Basic	5.92	3.35	2.00	36.16	52.29	28.06
Acid/alk.	3.88	8.33	13.64	0.64	0.36	0.76
Essential	50.06	50.92	51.09	14.5	15.86	25.41

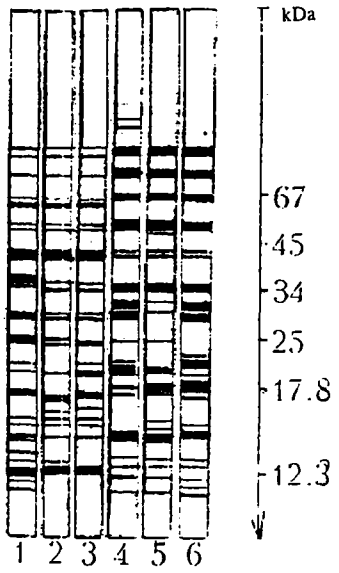

Fig. 1. Sketch of electrophoregrams of the albumins (**1**)-(**3**) and globulins (**4**)-(**6**) of seeds of various maize lines: *1, 4*) L-1; *2, 5*) L-2; *3, 6*) L-3.

rich in such amino acids as leucine, glutamic acid, aspartic acid, serine, and alanine. The sulfur-containing amino acids methionine and cysteine were almost indetectable in hydrolysates of these proteins. The sum of the essential amino acids, with the exception of methionine, amounted to 50 mol.-%. From their amino acid compositions, these proteins were hydrophobic, their leucine content reaching 37 mol.-%.

To determine the amino acid composition of the remaining group of proteins, the residual flour after threefold extraction with 70% ethanol was used to isolate the total proteins of this group. Their amino acid composition differed greatly from that of the zeins with respect to the levels of proline, glycine, alanine, isoleucine, and arginine.

A high level of arginine was characteristic for these proteins; in the case of L-2 it amounted to 45 mol.-%. The amount of essential amino acids was highest for the L-3 line, with the anthocyan coloration of the seeds. Thus, the three lines of maize obtained from a single cob differed with respect to the electrophoretic compositions of the zeins, the albumins, and the globulins. Their spectra can be used as marker characteristics of these lines. The amino acid compositions of the proteins of each line are also individual, which is explained by the different sets of polypeptides composing them.

EXPERIMENTAL

For the isolation of the proteins we used maize-seed flour that had been defatted with diethyl ether. The zein fraction was extracted with 70% ethyl alcohol for 2 h at a flour–solvent ratio of 1:10 (weight/volume). The extraction was repeated three times. The extract was clarified by centrifugation in a TsLR-1 centrifuge at 5000 rpm for 30 min, and the clear supernatant was dried in a vacuum thermostat at 60°C. From the residual flour after the removal of the zeins we isolated successively the albumin fraction — with distilled water — and the globulin fraction — with a solution of sodium dodecyl sulfate.

The electrophoretic study of the proteins was conducted by Laemmli's method [2]. As markers for determining the molecular masses of the polypeptides we used bovine serum albumin (76 kDa), egg albumin (45 kDa), chymotrypsinogen (25 kDa), myoglobin (17.8 kDa) and cytochrome C (12.3 kDa) from Serva Feinbiochemica.

For the analysis of their amino acid composition, a total fraction of albumins and globulins was isolated with a 10% solution of sodium chloride from the residual flour after the elimination of the zeins. The extract was clarified by centrifugation at 5000 rpm for 30 min and was subjected to dialysis against distilled water to eliminate Cl^- ions, and the total proteins were precipitated with cold acetone at a ratio of extract to acetone of 1:1. The precipitate of total proteins was collected by centrifugation and was dried in a vacuum desiccator after being washed successively with acetone (3-4 times) and diethyl ether (3-4 times).

To determine their amino acid compositions, the proteins were hydrolyzed in sealed tubes in the presence of 20% HCl at 105°C for 24 h. After evaporation, the hydrolysate was analyzed on a Biotronik System LG-2000 amino acid analyzer.

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