3. The strain of common rue callus tissue obtained is of great interest as a model of the study of details of the biogenesis of the acridone alkaloids.

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VARIETY CHARACTERISTICS OF SOYBEAN SEEDS IN RELATION TO PROTEIN AND OIL CONTENTS AND ACTIVITIES OF PROTEINASE INHIBITORS

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The protein and oil contents and the activity of proteinase inhibitors in six varieties of soybean have been studied. It has been found that the specific amidase activity of trypsin inhibitors ranges from 170 to 320 nominal units. Electrophoretic results indicates the presence in the water-soluble fraction of seven or eight components possessing inhibitor activity in relation to trypsin and chymotrypsin.

Proteins capable of suppressing the activity of a number of proteinases of animals and microorganisms have been found in soybean seeds [1-3]. Although these protein inhibitors are being studied intensively their functions in plants have so far remained obscure.

In the present paper we give the results of a comparative investigation of the activities of trypsin and chymotrypsin inhibitors in the seeds of a number of varieties of the soybean. The characteristics of protein and oil contents and the ratio of trypsin inhibitors to chymotrypsin inhibitors are given. Since the proteinase inhibitor proteins consist of a combination of protein, we have analyzed their component compositions with the aid of electrophoresis. In addition, we have attempted to evaluate the influence of geographical-ecological factors on the activity of the protein inhibitors in the soybean varieties studied.

As can be seen from Table 1, the protein content of the seeds ranged from 44.4 to 37.8%. The amplitude of variability was ±6.6%. The highest amount of protein was found in the variety Rannyaya-10 (44.4%), and the lowest in the variety Éra (37.8%).

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TABLE 1. Protein and Oil Contents and Specific Activities of Protein Inhibitors in the Seeds of a Number of Soybean Varieties

Variety of soybean	Protein content, %	Oil content, %	Protein:oil ratio	Sum of protein + oil	Specific amidase activity of the trypsin inhibitors per 1 mg of soybean protein	Ratio of soybean protein to trypsin (μg/μg)	Specific activity of proteinase inhibition in 1 mg of soybean protein, mg/min				Ratio of trypsin inhibitors to chymotrypsin inhibitors
							trypsin		chymotrypsin		
							in 1 mg of soybeam protein	% inhibition	in 1 mg of soybean protein	% inhibition	
Rannyaya-10	44,4	21.6	2.05	66,0	320	1,27	1,50	90.6	0,33	44,4	4,3:1
Fakel	43,4	20,8	2,08	64,2	220	1,81	1,40	76,0	0,23	30,0	6,0:1
Volna	41,4	22,5	1,95	66,6	270	1,49	1,56	91,0	0,46	60,0	3,4:1
Bystritsa	40,6	20,3	2,00	60,9	230	1,71	1,60	91,0	0,40	51,0	4,0:1
Plamya	38,5	24,5	1,57	63,0	210	1,79	1,80	93,0	0,47	54,0	3,8:1
Éra	37,8	21,5	1,75	59,3	170	2,38	1,46	89,3	0,34	53,0	4,3:1

Another important index — the oil content — reached 24.5-20.3% in the varieties studied, the amplitude of variability being ±4.2%.

The variety Plamya had the highest oil content (24.5%) and the variety Bystritsa the lowest (20.3%).

The protein:oil ratio was the highest in the varieties Rannyaya-10 and Volna and lowest in the varieties Éra and Bystritsa.

The specific amidase activity of trypsin inhibitors in 1 mg of inhibitor protein in the soybean varieties studied ranged from 170 to 320 nominal units (Fig. 1).

According to these indices, the given varieties can be divided into three group: 1) varieties with a high amidase activity of the trypsin inhibitors — Rannyaya-10 and Volna — in which the ratio of protein to enzyme (μg of inhibitor protein to μg of trypsin) amounted to 1.27:1 and 1.49:1, respectively;

2) with a mean specific amidase activity of the trypsin inhibitors — Bystritsa, Plamya, and Fakel — in which the ratio of protein to enzyme was 1.71:1, 1.79:1, and 1.81:1, respectively; and

3) with a low specific amidase activity of the trypsin inhibitors — Éra — for which the ratio of protein and enzyme contents was 2.38:1.

It must be mentioned that the degree of inhibition of the proteinase activity of trypsin in all the varieties studied with the exception of Fakel, had only slight differences, ranging between 89.4 and 93%.

The greatest chymotryptic activity among the varieties studied was exhibited by Volna, Plamya, and Bystritsa, and the least by Fakel and Rannyaya-10.

Thus, the highest specific activity of the proteinase inhibition of trypsin and chymotrypsin was characteristic of the varieties Plamya, Volna, and Bystritsa, and the lowest of the variety Fakel.

The variety Fakel had the highest ratio of trypsin inhibitors to chymotrypsin inhibitors — 6:1. For the other varieties, the difference with respect to this index was slight, with the exception of Volna (3.4:1).

A comparative study of the relative electrophoretic mobilities (REMs) of the proteins inhibiting trypsin and chymotrypsin showed that the varieties studied separated mainly into seven or eight components (Fig. 2). This makes it possible to assume that the water-soluble fraction contained several components capable of inhibiting trypsin and chymotrypsin. Furthermore, some of the components, the REMs of which were 0.96, 0.88, 0.86, 0.82, 0.67, 0.56, 0.45, and 0.34, were found in practically all the varieties studied. Differences were detected only with respect to the components having REMs of 0.60, 0.26, 0.40, 0.73, and 0.76.

It must be mentioned that the components found frequently in the soybean varieties studied indicate a high stability of the biosynthetic apparatus of these proteins in the phylogenesis of a soybean culture and differences appear as an adaptation reaction in the form of the corresponding proteins in dependence on the genetic features of the variety and the conditions of the medium.

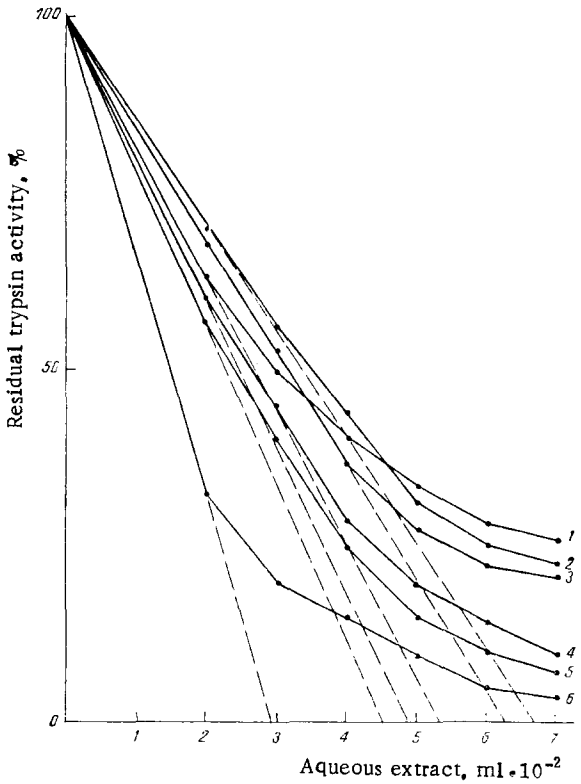

Fig. 1. Inhibition of the amidase activity of trypsin (C = 20 μg) by an extract of water-soluble soybean proteins (pH 8.2, temperature 25°C). Soybean varieties: 1) Fakel; 2) Éra; 3) Plamya; 4) Bystritsa; 5) Volna; 6) Rannyaya-10.

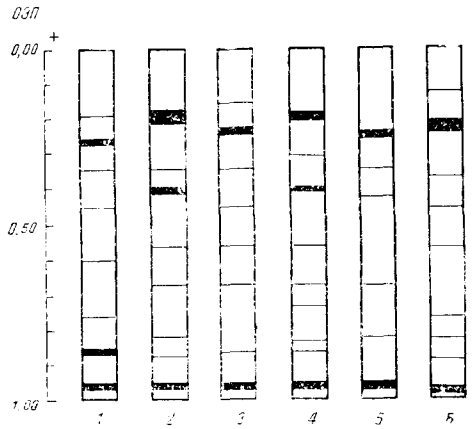

Fig. 2. Sketch of electrophoretograms of the water-soluble soybean proteins possessing inhibitory activity. Soybean varieties: 1) Rannyaya-10; 2) Fakel; 3) Volna; 4) Bystritsa; 5) Plamya; 6) Éra.

The opinion exists that the conditions of growth of some pulses in geographically distinct regions scarcely affect the change in the amount of the total nitrogen of the cotyledons and of its component forms and the protein fractions [4, 5]. In view of this, it was interesting to determine how certain biochemical indices are realized — in particular, the amount of inhibitors in the seeds of a given variety of soybean according to geographical-ecological conditions.

As can be seen from Fig. 3A, B, and C, the inhibition of the proteinase activity of trypsin in the soybean varieties Amurskaya-41, Khersonskaya-2, and Khersonskaya-6 under the conditions of Tadzhikistan, although only slight, was greater than the same index under the

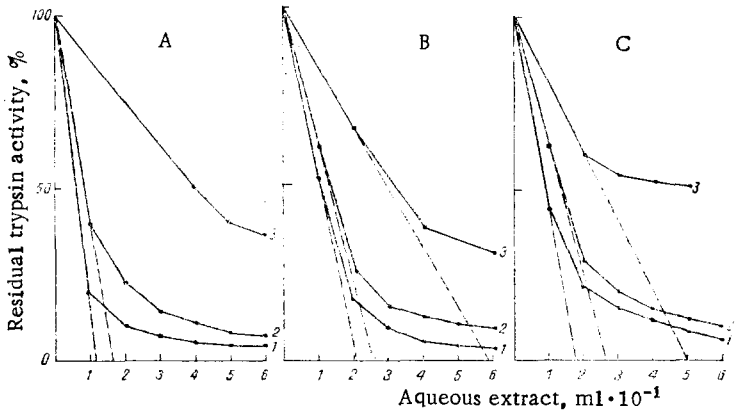

Fig. 3. Inhibition of the proteolytic activity of trypsin (C = 25 μg) and chymotrypsin (C = 10 μg) by an extract of water-soluble soybean proteins (pH 7.8; temperature 40°C): A. 1) Under the conditions of Tadzhikistan, Amurskaya-41; 2) under the conditions of the Far East, Amurskaya-41; 3) under the conditions of the Far East, Amurskaya-41 (with chymotrypsin). B. 1) Under the conditions of Tadzhikstan, Khersonskaya-2; 2) under the conditions of Kherson, Khersonskay-2; 3) under the conditions of Kherson, Khersonskaya-2 (with chymotrypsin). C. 1) Under the conditions of Tadzhikstan, Khersonskaya-6; 2) under the conditions of Kherson, Khersonskaya-6; 3) under the conditions of Kherson, Khersonskaya-6 (with chymotrypsin).

conditions of the Far East and of Kherson. This may be due to the fact that under the conditions of Tadzhikistan, where the climate is hotter, there is an increased accumulation of inhibitor protein, although in the qualitative aspect the differences are insignificant.

EXPERIMENTAL

Ripe soybean seeds of the 1977 harvest were obtained in the variety section of the Farming Institute of the Ministry of Agriculture of the Tadzhik SSR.

We used trypsin (Czechoslovakia) that had been twice recrystallized, having a specific activity with respect to BAPA (N-benzoyl-d,l-arginine p-nitroanilide) of 8.1 μmol/min and with respect to casein of 22.9 mg/h, and chymotrypsin that had been twice recrystallized, with a specific activity with respect to casein of 21.6 mg/h.

An aqueous extract of the proteinase inhibitor was obtained and the specific amidase activity of the soybean seed trypsin inhibitors was determined by means of a method which we have published previously [2]. The amount of protein in the aqueous extracts was determined by Lowry's method [6], and that in the soybean seeds by the micro-Kjeldahl method. The specific activity of the proteinase inhibition of trypsin and of chymotrypsin was determined by the method of Sumathi and Pattabiraman [7], and the calculations were made as described by Pleshkov [9].

Disk electrophoresis was performed by a known method in 7.5% polyacrylamide gel at pH 8.3 [9]. The time of electrophoresis was 3 h.

SUMMARY

1. It has been found that the protein and oil contents and the activity of the trypsin and chymotrypsin inhibitors, and also their ratio, in the seeds of several varieties of soybeans may reach considerable magnitudes.

2. A comparative study of the relative electrophoretic mobilities of the protein inhibitors of trypsin and chymotrypsin in the soybean varieties studied show that they consist basically of seven or eight components.

3. The amount of trypsin inhibitors in the seeds of a given variety of soybean varies insignificantly as a function of the ecological-geographical conditions of growth, although this index is more considerable under the conditions of Tadzhikistan.

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ISOLATION AND PROPERTIES OF TRANSFERRIN MESSENGER RNA FROM RAT LIVER

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Highly purified transferrin mRNA characterized by electrophoretic and sedimentational homogeneity has been obtained from rat liver, with a sedimentation coefficient of 20S and a molecular weight of 0.86 MD. In a system consisting of a lysate of rabbit reticulocytes the Tf-mRNA programs the synthesis of an immunoreactive precursor of transferrin with a molecular weight of 82 kD. More than 50% of the nucleotide sequence of Tf-mRNA is present in the paired state.

Transferrin (Tf) is the main carrier of iron in the organism of vertebrates and is a donor of iron for such vitally important hemoproteins as hemoglobin, the cytochromes, catalase etc. Genetic anomalies in the structure and function of Tf may play an important role in the pathogenesis of such hereditary diseases as hemochromatosis and atransferrinemia. Consequently, progress in their study must be linked primarily with the existence of information on the structure of the normal Tf gene of mammals, the sequence of stages in the expression of the Tf gene, and the molecular organization and functional activity of Tf mRNA. At the same time there is no information in the literature on the molecular structure and mechanisms of the expression of the TF gene of Man and other mammals.

In the course of a number of years we have been investigating the mechanism of the expression of the transferrin gene in the rat liver. This protein is synthesized in the membrane-bound polyribosomes of the liver [1, 2] and then passed through a complex pathway of intracellular transport and post translational modification which procede the secretion of the mature protein into the bloodstream [2].

We have isolated from rat liver highly purified transferrin mRNA characterized by electrophoretic, sedimentation, and functional homogeneity. The main structural and functional characteristics of this mRNA and also the results of experiments on its reverse transcription have been described previously [3-5].

In the present paper we give details of the isolation of the Tf mRNA and some of its physicohemical parameters.

The messenger RNA coding transferrin belongs to the predominant class of mRNAs of rat liver cells. It has been shown previously that its concentration amounts to about 7000 molecules per hepatocyte [3]. In spite of such a high level of Tf-mRNA in the liver cells, the isolation and purification of transferrin mRNA is a complex task. Table 1 shows the results

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