PROTEIN CONTENT, ACTIVITY, AND ELECTROPHORETIC COMPOSITION OF THE INHIBITOR-CONTAINING FRACTION OF PEA SEEDS

V. I. Domash, O. S. Koroleva, and G. N. Kotlovskaya

UDC 577.15/17:633.358

The amounts of protein and the activities of the proteinase (trypsin) inhibitors of 16 varieties of pea have been studied. The amount of protein ranged from 19.3 to 25.2% and the amidase activities of the trypsin inhibitors from 18 to 40.8 IU. It was found that the electrophoretic spectrum of the inhibitor-containing fraction of the pea seed protein consisted of 12-16 components with molecular masses of 14-89 kDa. Features of the electrophoretic spectrum of some varieties of pea have been established.

Natural proteinase inhibitors are widely distributed in the vegetable kingdom. The seeds of legumes, cereals, and the Solanaceae are particularly rich in them. It is assumed that these proteins play the role of regulators of proteolytic enzymes, have a protective function, and are also one of the antifeeding factors [1-4]. In the literature, a particularly large number of publications has been devoted to the soybean – the crop richest in inhibitors. There is only a small number of investigations relative to trypsin inhibitors in pea seeds. The variation in the level of activity of trypsin inhibitors as a function of the type and variety, the year, and the growth site has been studied. It has been shown that the deviation from the maximum level with a variation in these factors does not exceed 25% [5, 6].

We have made a comparative investigation of the amounts of protein, the activities of protein inhibitors of proteinases (trypsin) in the seeds of 16 seed and fodder varieties of pea and the qualitative electrophoretic compositions of some of them differing in their inhibitor contents.

As can be seen from Table 1, the amount of protein in the pea seeds ranged from 19.3 to 25.2%. The highest level of protein was in the variety Fitotron 1 – 25.2% – and the lowest in the variety Sokol 2 – 19.3%.

The amidase activities of the trypsin inhibitors ranged from 18.4 to 40.8 IU. The highest activities were characteristic of such varieties as Orpela, Streletskii 11, Streletskii 21, Ramonskii, Sokol 2, Straus, and Truzhenik. Moderate levels of these proteins were present in the varieties Orientir, Aist, Batyr, and others, and low levels in Smaragd, Orlovskii 3, and Malinovka.

The activities of the inhibitors in these varieties were, respectively, about 25 and 15.2-19.7 IU. The specific amidase activities of the trypsin inhibitors in the pea varieties studied ranged from 66 to 189 arbitrary units.

It can be seen from Fig. 1, which shows the inhibition of the amidase activity of trypsin by a weak salt extract from pea seeds that a linear relationship between the decrease in the trypsin activity and the amount of inhibitor extract added appeared within narrow inhibition limits. A deviation from linearity in the pea varieties investigated is observed after the binding of 50% of the trypsin.

As our investigations showed, a weak correlation exists between the activity of the trypsin inhibitors and the total amount of protein in pea seeds ($r = 0.25$), which agrees with the results of other authors [7]. But at the same time, a high correlation ($r = 0.82$) exists between the inhibitory activity and the oil content of pea flour.

V. F. Kuprevich Institute of Experimental Botany, Belorussian SSR Academy of Sciences, Minsk. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 243-247, March-April, 1989. Original article submitted June 27, 1988; revision submitted September 14, 1988.

 0009-3130/89/2502-0210 $12.50 © 1989 Plenum Publishing Corporation

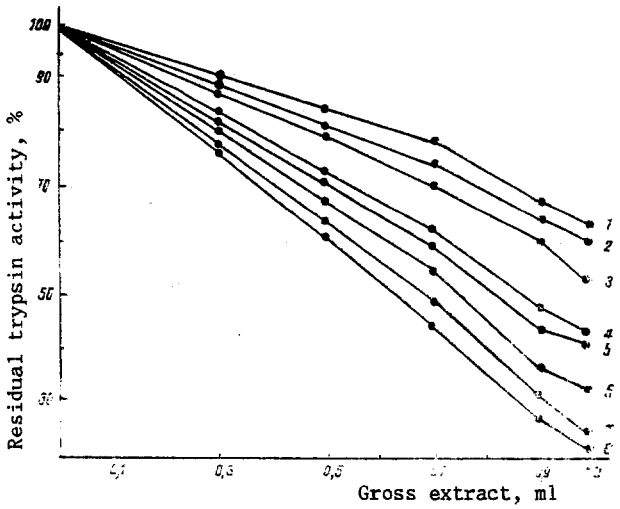

Fig. 1. Inhibition of the amidase activity of trypsin (C = 20 μg) by the inhibitor-containing fraction of pea seed proteins (pH 8.2, 25°C) of the following varieties: 1) Smaragd; 2) Orlovskii 3; 3) Sever; 4) Aist; 5) Mir; 6) Straus; 7) Sokol 2; 8) Truzhenik.

TABLE 1. Amounts of Protein and Activities of the Trypsin Inhibitors in the Seeds of Different Varieties of Pea

Variety	Protein content, % on the absolutely dry mass	Activity of trypsin inhibitors, IU/g of absolutely dry mass	Specific activity of inhibitors, IU/mg of pea protein
Sokol 2	19,3	32,0	166
Orlovskii 3	24,5	18,4	75
Orpela	21,5	40,8	189
Mir 3	23,3	29,0	124
Malinovka	19,8	19,7	99
Orientir	19,8	24,6	124
Smaragd	23,1	15,2	66
Streletskii 11	20,8	33,7	162
Streletskii 21	22,6	39,7	175
Aist	23,3	25,4	109
Straus	22,1	31,5	142
Sever	22,4	20,7	92
Truzhenik	21,5	33,1	154
Fitotron 1	25,2	28,6	113
Batyr	23,8	25,1	105
Ramonskii	23,0	33,8	147

The results of an investigation of the electrophoretic spectra of the inhibitor-containing fractions of pea seed proteins showed the presence, in the main, of 12-16 components with molecular masses (MMs) of from 14 to 89 kDa, which correspond to relative electrophoretic mobilities (REMs) of 0.77-0.08 (Fig. 2). Characteristic for the varieties studied are major and minor components with MMs of about 14, 17.8, 21.8, 23, 26, 33.8, 35.8, 38.9, 40, 53, 58, 66, 70, 85, and 89 kDa.

The best-defined zones on the electrophoretograms are the zones with MMs of about 14, 21, 35, and 85 kDa, which correspond to REMs of 0.77, 0.62, 0.43, and 0.10. In the varieties investigated the proportion of proteins with a MM of 85 kDa was about 5%, of those with a MM of 35 kDa to 16-27%, and of proteins with a MM of about 21 kDa to 4-10% on the total amount of protein.

The largest in the quantitative respect was the zone of proteins with a molecular mass of about 14 kDa and less. It made up about 30-39% of the total amount of components. An exception was the variety Orlovskii 3, for which this zone amounted to about 20%. It must be mentioned that this variety also had the lowest trypsin-inhibitor activity.

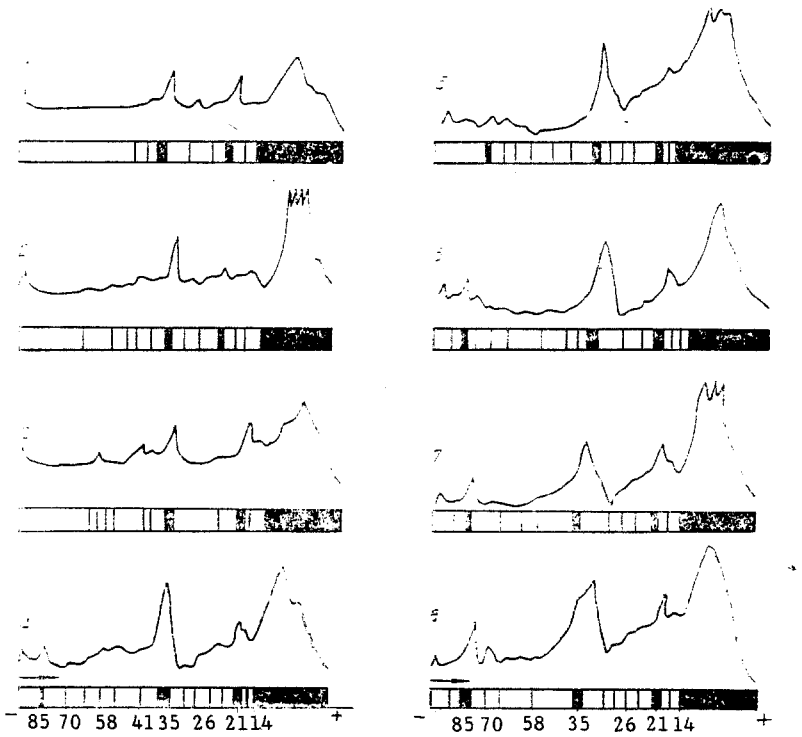

Fig. 2. Densitograms and patterns of electrophoretograms of the inhibitor-containing fractions of trypsin-inhibiting proteins of pea seeds of the following varieties: 1) Straus; 2) Smaragd; 3) Orlovskii 3; 4) Aist; 5) Mir 3; 6) Truzhenik; 7) Sever; 8) Sokol 2. The molecular masses are given in kilodaltons (kDa).

On comparing with one another the electrophoretic spectra from the different varieties of pea it must be pointed out that the absence of a zone with a molecular mass of about 85 kD was characteristic for the varieties Straus, Smaragd, and Orlovskii 3. These varieties also differ in their morphophysiological characteristics.

Certain variety differences were also observed relative to the minor components in the range of molecular masses between 89 and 21 kDa. But, on the whole, as our investigation showed, the electrophoretic compositions of the inhibitor-containing fractions of the pea seed proteins were characterized by polypeptides with, in the main, the same electrophoretic mobilities but with different quantitative ratios of the individual zones.

The similar component compositions of the inhibitor-containing fractions of the proteins in the varieties of pea investigated that we have observed indicate a stability of the protein synthesizing system in this crop and the observed differences were apparently due to genetic features of the varieties.

EXPERIMENTAL

Ripe seed and fodder peas of the 1987 harvest were obtained from All-Union Institute of Leguminous and Grain Crops.

The amidase activities of the trypsin-inhibiting proteins were determined with the aid of the synthetic substrate N^{α}-benzoyl-DL-arginine-p-nitroanilide (BAPA) by Gofman's method [8]. The inhibitors were extracted with 0.2 M NaCl. The activities of the trypsin inhibitors have been expressed in terms of an arbitrary inhibitor unit (IU), corresponding to the amount of inhibitor inhibiting the cleavage of 1 μmole of the substrate in 1 min under standard conditions.

For electrophoresis we used the inhibitor-containing fractions of the proteins, which were obtained by extracting weighed amounts of flours with 0.2 M NaCl in a ratio of 1:10 for 2 h and centrifugation at 7000 rpm for 20 min. In the supernatant liquid, the acid-labile, ballast, proteins were precipitated with 0.3 N HCl, and it was recentrifuged. The

supernatant liquid, containing the trypsin-inhibiting proteins was concentrated and used for analysis.

The proteins were separated in a AVGÉ-1 apparatus made in the Khiiu Kalur experimental laboratory in 15% polyacrylamide gel containing 0.1% sodium dodecyl sulfate by Laemmli's method [9]. Electrophoresis was conducted in Tris-glycine buffer, pH 8.3, at a voltage of 250 V and a current strength of 25 mA per plate for 2.5 h. The plates are fixed in 7% TCA for 30 min and were stained with a 0.1% solution of Coomassie R-250 in 10% acetic acid for an hour. The excess of dye was washed out with ethanol–acetic acid–water (1:1:8). The gels were scanned on a LKB 2202 densitometer. Densitograms were recorded by a DKS 4-003 recorder. In the determination of the molecular masses (MMs) the following marker proteins were used: myoglobin (MM 17,800), ovalbumin, (MM 45,000), and chymotrypsinogen (MM 25,000).

The amounts of proteins in the seeds were found by Kjeldahl's method (total nitrogen × 6.25).

Trypsin from Spofa (Czechoslovakia) and BAPA from Fluka (Sweden [sic]) were used. The other reagents were of domestic production.

SUMMARY

1. Variety differences have been detected with respect to the amounts of protein in peas which range from 19.3 to 254.2%, and in the levels of activity of the trypsin inhibitors, which range from 18 to 40.8 IU.

2. The electrophoretic system of each of the inhibitor-containing fractions of pea seed proteins consists of 12-16 components with molecular masses of 14-89 kDa. The bulk consists of components with a molecular mass of about 14 kDa.

3. Features of the electrophoretic spectra of the inhibitor-containing fractions of the proteins of a number of pea varieties have been established.

LITERATURE CITED

1. V. V. Mosolov, Protein Inhibitors as Regulators of Proteolysis Processes [in Russian], Nauka, Moscow (1983), pp. 4-32.
2. M. Laskowskii and J. Kato, Annu. Rev. Biochem., 49, 593 (1960).
3. C. A. Ryan, Annu. Rev. Plant Physiol., 24, 173 (1973).
4. M. P. Chernikov, E. Ya. Stan, and M. É. Lyaiman, Zh. Vses. Khim. Ob-va im. D. I. Mendeleeva, 23, No. 4, 739 (1978).
5. I. M. Vaisblai, Izv. Akad. Nauk SSSR, Ser. Biol. Nauk, No. 6, 840 (1978).
6. I. M. Vaisblai, Izv. Akad. Nauk SSSR, Ser. Biol. Nauk, No. 1, 26 (1979).
7. V. Kh. Muskhinov and V. V. Khangil'din, Problems of Genetics and Selection in the Urals [in Russian], Sverdlovsk (1977), p. 72.
8. Yu. Ya. Gofman and I. M. Vaisblai, Prikl. Biokhim. Mikrobiol., 11, No. 5, 777 (1975).
9. U. K. Laemmli, Nature (London), 227, No. 5259, 680 (1970).