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PROTEIN CONTENT, ACTIVITY AND AMINO ACID COMPOSITION OF PROTEINASE INHIBITORS OF SEEDS OF SOME VARIETIES OF PEA AND THEIR HYBRIDS

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The results are given of a study of the quantitative content of protein in the seeds of some pea varieties and mutants, the activity of the total inhibitor proteins, and correlations of their activity with the protein content of the seeds and the amino acid compositions of the proteinase inhibitors. Considerable differences have been found in the amounts of a number of amino acids of the protein inhibitors of parental varieties and mutants of the pea, the amounts of serine, glutamic acid, alanine, and valine correlating positively with the inhibitor activity.

Considerable amounts of proteins capable of acting as effective inhibitors of proteolytic enzymes of living organisms — trypsin and chymotrypsin — have been found in the seeds of legumes (soybean, pea, bean, lupin, etc.) [1-4].

Various functions are ascribed to proteinase inhibitors in plants. It is considered that they may play the role of reserve proteins or regulators of the activity of proteolytic processes preventing the premature breakdown of the reserve proteins [5]. Proteins inhibiting trypsin and chymotrypsin are capable of suppressing the activity of the proteases of a number of harmful insects and phytopathogenic microorganisms thereby protecting plants from damage [6]. In addition, these proteins find use in the elucidation of the mechanism of the action of specific enzymes [7] and in practical medicine [8].

At the same time, it is considered that the presence of inhibitors in a grain, particularly when they have a high activity, considerably lowers their nutrient value and impairs the technological properties of the proteins of cultivated plants [9]. Therefore, in the selection of agricultural crops directed to increasing protein content one of the criteria of the quality of the seeds is the amount and activity of proteins that are proteinase inhibitors.

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TABLE 1. Protein Content, Activity, and Amino Acid Composition of the Protein Inhibitors of the Seeds of a Number of Varieties and Their Hybrids

Amino acids, mg per 100 mg of inhibitor protein	Mutant K-5	888	900	990	Ramonskii 77	940	Falenskii 42	829	K-2004	K-2	419	Torsdag
Lysine	7,13	8,30	7,80	7,36	5,66	7,07	6,93	7,80	8,01	7,36	5,66	6,89
Histidine	1,92	1,02	2,05	0,91	1,96	1,83	2,50	2,11	2,55	2,27	1,91	1,98
Arginine	17,77	9,99	13,18	11,10	20,15	14,66	16,64	10,67	6,62	14,14	23,13	10,52
Asparagine	16,41	17,03	17,15	19,25	23,14	17,49	12,62	17,93	14,74	16,87	25,20	14,97
Threonine	3,17	3,79	3,77	3,50	3,66	3,36	3,61	3,47	4,41	4 55	2,57	4,70
Serine	4,30	4,99	4,86	4,95	3,70	4,59	4,16	4,66	4,99	4,23	3,26	4,45
Glutamic acid	21,11	22,26	20,22	21,48	17,74	20,67	19,56	19,83	22,17	19 23	16 08	22,27
Proline	4,70	4,09	3,65	5,01	6,11	4,95	8,04	4,82	4,88	3,59	3,75	3,46
Glycine	6,11	6,92	6,46	6,22	4,55	6,24	4,44	6,34	6,04	6,18	4,48	6,19
Alanine	4,30	6,32	6,33	5,91	3,66	5,24	4,16	9,37	5,22	4,73	4,11	4,95
Cysteine	1,30	1,44	2,30	1,33	0,58	0,88	1,94	1,46	1,16	1,09	1,14	1,11
Valine	3,79	3,43	3,01	3,62	2,23	4,30	3,47	3,09	5,80	4,64	2,40	5 69
Methionine	0,51	0,36	0,32	0,18	0,44	0,12	0,83	0,43	0,05	0,03	0,20	0,07
Isoleucine	0,51	0,60	0,64	0,72	0,53	0,59	2,08	0,54	2,32	2,05	0,45	2,35
Leucine	1,87	2,41	2,37	2,35	1,65	2,12	3,05	2,22	3,02	2,82	1,55	3,09
Tyrosine	2,60	3,49	3,20	3,08	2,18	2,94	2,64	3,03	3,71	3,05	2,04	3 46
Phenylalanine	2,49	3,55	2,69	3,02	2,05	2,88	3,33	2,22	4,30	3,18	2,08	3,84
Protein content in the seed, %	27,5	26,6	26,2	25,4	30,7	26,2	28,5	26,2	29,5	33,0	31,5	30,2
Inhibition of the amidase activity of trypsin per 1 mg of protein/min, arb. units	6,25	6,05	4,27	2,64	1,93	4,34	4,09	4,10	5,48	4,75	5,49	5,48
Inhibition of the proteinase activity of chymotrypsin per 1 g of protein/min, arb. units	1,41	1,31	1,19	0,91	0,66	1,16	1,37	1,30	1,50	1,22	0,74	1,48

TABLE 2. Correlations Between Inhibitor Activity, Protein Content, and Amounts of Individual Amino Acids in Pea Seeds

Variety and line of pea	Amount of protein in the seed, %	Activities of inhibitors, arb. units		Total activity IT + ICh, arb. units
		of trypsin	of chymotrypsin	
Ramonskii 77	30,7	1,93	0,66	2,59
Falenskii 42	28,5	4,09	1,37	5,46
Torsdag	30,2	5,48	1,48	6,96
K-2	33,0	4,75	1,22	5,97
K-2004	29,5	5,28	1,50	6,78
K-5	27.5	6,25	1,41	7.66
990	24,5	2,64	0.91	3,55
829	26,2	4,10	1,30	5,40
900	26,2	4,27	1,19	5.46
940	26,2	4,34	1,16	5,50
419	31,5	5,49	0,74	6,23
888	26,6	6,65	1,31	7,96

Variety and line of pea	Amino acid, mg per 100 mg of protein					
	serine	glutamic acid	proline	glycine	alanine	valine
Ramonskii 77	4,70	16,10	7,08	8,12	5,45	2 56
Falenskii 42	5,26	17,61	9,27	7,85	6,16	3,99
Torsdag	5,54	19,52	3.91	10,55	7,15	6,26
K-2	5,23	16,98	4,08	10,73	6,87	5,16
K-2004	6,07	19,28	5,40	10.33	7,89	6,35
K-5	5,37	18.91	5,40	10,70	6,36	4,27
990	6,06	18,77	5,61	10,65	8,50	3,96
829	5,88	17,86	5,53	11,19	8,95	3,49
900	5,94	17,76	4,10	11,16	9,21	3,30
940	5,58	18,88	3,79	11,05	9,14	2,63
419	4,20	14,77	4,39	8,05	6,21	2,75
888	6,10	19,36	4,53	11,80	9,04	3,76

The task of our investigation was the study of the quantitative content of protein in the seeds of several varieties and mutants of the pea, the activity of the total inhibitor proteins, and a correlation of their activity with the protein content of the seeds and the amino acid composition of the proteinase inhibitors. Since mutant peas (K-2, K-5, K-2004) were used as the mother plants in hybridization, we attempted to analyze the influence of a mutant gene of the manifestation of the above-mentioned indices.

As can be seen from Table 1, the amount of protein in the seeds of the mutant and hybrid pea varieties studied varied widely: from 25.4 (line 990) to 33% (mutant K-2). It must be mentioned that the majority of hybrids considered were inferior in their protein content to both parental forms. It has been reported previously [10] that in the inheritance of the protein content of seeds of pea hybrids that have been produced, a low protein content is dominant, and therefore in selection for increased protein content it was recommended to select parental pairs with a high level of this index. As an example we can give the combination of parents of line 419 (31.5% of protein).

An investigation of the proteolytic action of trypsin and of chymotrypsin in the presence of the inhibitor proteins isolated from the pea varieties and hybrids studied showed that their inhibitor activities varied within wide limits; from 1.93 to 6.65 arb. units for trypsin and from 0.66 to 1.50 arb. units for chymotrypsin. At the same time, it must be mentioned that a high activity of inhibitors with respect to trypsin is almost always accompanied by a high activity with respect to chymotrypsin (for K-5, 6.25 and 1.41 arb. units for trypsin and chymotrypsin, respectively, for K- 2004, 5.28 and 1.50; for Torsdag, 5.48 and 1.48; for line 888, 6.65 and 1.31; and so on). As the results obtained show, the process of hybridization acts ambiguously on the activity of the inhibitor proteins of the hybrids. In some combinations, the hybrids obtained were close with respect to the inhibition of the amidase activity of trypsin to one of the parents (line 419 — to Torsdag; lines 940 and 828 — to Falenskii 42) or occupied an intermediate position (lines 900 and 990). In a number of plant combinations, particularly with respect to the inhibition of the protein activity of chymotrypsin, a lower level of inhibitor activity was observed in the hybrids as compared with the parental forms.

For the pea varieties used as the male parent plant in hybridization (Torsdag, Falenskii 42, and Ramonskii 77) no appreciable dependence of inhibitor activity on the protein content of the seeds was observed.

For peas of the Torsdag variety and mutants (K-2, K-5, K-2004), negative correlation was traced between the protein content in the seed, the total activity of the inhibitors, and the inhibition of the amidase activity of trypsin, while for the hybrids a positive correlation was observed (Table 2). The activities of the inhibitors with respect to chymotrypsin were close for all the mutants and hybrids studied.

In a number of cases, the comparative amino acid analysis of the inhibitor proteins of pea seeds of the parental form and their hybrids showed an intermediate position with respect to the amounts of certain amino acids (Table 1). This relates to the amino acids aspartic acid, threonine, cysteine, valine, methionine, and isoleucine. So far as concerns lysine, serine, glutamic acid, glycine, and alanine, for the majority of hybrids studied their levels were higher, while the amounts of histidine, arginine, and proline were smaller than in the parental forms.

The paternal varieties Ramonskii 77, Falenskii 42, and Torsdag differed considerably from one another with respect to their serine, glutamic acid, proline, glycine, alanine, and valine, contents (Table 2). This is apparently explained by the fact that these varieties differ in their genotypic features. For example, the variety Ramonskii 77 has recessive and dominant genes, while dominant genes predominate in Falenskii 42 (a fodder variety) [11].

The indices of the serine, glutamic acid, alanine, and valine contents correlate positively with the inhibitor activity and negatively with the protein content of the seeds.

A deeper study of the inhibitor proteins, their isolation in purer form, and their separation into individual components will possibly assist in establishing clearer correlations between the activity of individual inhibitor proteins and their amino acid compositions and in the elucidation of the mechanism of their action.

EXPERIMENTAL

Physiologically ripe pea seeds of the varieties Ramonskii 77 (R-77), Falenskii 42 (F-42) and Torsdag — the paternal forms — and of the pea mutants K-2, K-5, and K-2004 — the maternal plants — and their constant hybrids of 7-8 generations, lines 888, 900, 990 (the combination K-5 × R-77), 940 (K-5 × F-42), 829 (K-2004 × F-42), and 419 (K-2 × Torsdag) from the collection of the Institute of Cytology and Genetics of the Siberian Branch of the USSR Academy of Sciences (Novosibirsk) were investigated.

The protein contents of the seeds of the plants studied were determined by the biuret method [12, 13] after a finely ground flour had been prepared.

The isolation of the protein inhibitors, the determination of the specific inhibition of the amidase activity of trypsin (arbitrary units per 1 mg of protein/min), and the amount of inhibitor protein in a sample were determined by the procedure developed by Gofman and Vaisblai [14].

The inhibition of the proteinase activity of chymotrypsin was determined in accordance with [15] and was expressed in arbitrary units per 1 mg of protein/min. As the arbitrary unit of the activity of an inhibitor we took that activity of it at which the optical density of a solution of chymotrypsin with respect to casein measured spectrophotometrically at 280 nm fell by 0.01. The activities of the trypsin and chymotrypsin inhibitors were studied in parallel.

The amino acid compositions of the inhibitor proteins were determined by the analysis of standard acid hydrolysates of them by the method of Spenser and Wold [16]; the amount of cysteine was found after its oxidation to cysteic acid by low concentrations (2%) of DMSO in 6 N HCl [17]. The analyses were performed with the aid of an ILC-6 AH amino acid analyzer (Japan).

SUMMARY

1. A comparative evaluation has been made of a number of pea varieties and mutants and their hybrids with respect to protein content, and the activity of inhibitory proteins in the seed and their amino acid compositions.

2. An inverse correlation has been shown between the protein content of the seeds and

the inhibitor activity of pea mutants (K-2, K-5, and K-200 — maternal forms). A positive correlation of these indices has been found for hybrid forms.

3. The use of mutants in the hybridization process does not lead to a sharp change in the amino acid composition of the inhibitor proteins and, consequently, it is more difficult to trace correlations.

4. Considerable differences have been found in the amounts of a number of amino acids of inhibitory proteins of parental varieties and pea mutants, the amounts of serine, glutamic acid, and valine correlating positively with inhibitory activity.

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