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INVESTIGATION ON PROTEIN CONTENT AND AMINO ACID COMPOSITION IN THE KERNELS OF SOME SUNFLOWER LINES

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SUMMARY

This study took into account the protein content in the kernel of ten lines derived from interspecific hybrids *Helianthus annuus* (line 2607) × *Helianthus resinosus* and *Helianthus annuus* (line 2607) × *Helianthus salicifolius*. The amino acid composition of storage protein was also studied. The protein in the new lines exceeded the protein in the parental forms with up to 10.6%. The essential amino acids lysine, valine, threonine and phenylalanine had higher amounts. With regard to sulfur-containing amino acids (cystine and methionine), the new lines had lower content than the line 2607. From the non-essential amino acids, mainly asparagine and glycine exceeded the parental lines.

Key words: **sunflower, protein, amino acids, *Helianthus annuus* L.**

INTRODUCTION

Sunflower is the second most important oil seed crop in the world. The relatively high and stable seed yield and the high protein content (over 40% in cake) give sunflower a prominent position among several high-protein crops involved in the protein problem solution in many countries worldwide. From the biological point of view sunflower protein is highly valuable due to the absence of toxic components in it which distinguishes it from all other sources of protein, and also due to the high percent of water-soluble vitamins of group B (Ivanov, 1991). Amino acids are the major structural elements of proteins in the human body. Protein quality is assessed by the amount of amino acids in its composition. There are eight essential amino acids:

- methionine, threonine, tryptophan, leucine, isoleucine, lysine, phenylalanine, and valine.

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They are indispensable because they cannot be synthesized in the human organism and therefore must be supplied in the diet by plants and micro organisms. Other twelve amino acids are not essential and the human organism is able to produce them when needed. The nutrition value of sunflower protein is determined by the good balance of the essential amino acids contained in it. Each amino acid plays a specific biological role (Ribarova, 1987). Lysine is one of the three most important essential amino acids (the other two are tryptophan and methionine). Lysine deficiency in the diet causes a number of metabolic disturbances related to nitrogen balance, blood synthesis, bone calcification, liver and muscle functioning. The daily requirements of the organism for lysine are 3-5 g. Sulfur-containing amino acids are important for the structure and functioning of proteins. Thus, for example, cystine provides the covalent cross-bridges between the polypeptide chains in protein when forming its tertiary structure. Methionine has an important function in the methylation processes as the main provider of methyl groups. It is the sole donor of sulfur atoms in the organism. The methionine daily requirements of the organism are 3 g. The amino acids valine, leucine and isoleucine have branched chains. Valin-deficient diet causes motor incoordination. Isoleucine and leucine are a part of the composition of almost all proteins. The lack or deficiency of branched-chain amino acids leads to expressed malfunctions. The biological role of the aromatic amino acids tyrosine and phenylalanine is determined by the large number of biologically active substances formed in the process of their metabolisation. Phenylalanine is irretrievably converted into tyrosine; when estimating protein quality, the total uptake of tyrosine and phenylalanine in the diet should be considered. In order to meet the requirements of the human organism for essential amino acids, their balanced ratio is very important. According to F.A.O-WHO standards, the model protein should contain the following essential amino acids (g) in 100 g: lysine – 5.5%, methionine + cystine – 3.5%, threonine – 4%, isoleucine – 4%, leucine – 7%, valine – 5%, phenylalanine + tyrosine – 6%, or a total of 35% from protein composition.

The aim of this investigation was to analyze and select lines with enhanced protein content in kernel and balanced amino acid composition, which would be valuable material for solving a number of breeding tasks related to the improvement of sunflower seed indices.

MATERIAL AND METHODS

The investigation involved fertility restorer lines developed through interspecific hybridization in combination with embryo cultivation to accelerate the breeding process. Lines L60, L70, L80, L14 and L15 originated from the interspecific experimental hybrid *Helianthus annuus* × *Helianthus resinosus*, and the other lines included in the investigation (LS18, LS19, LS20, LS30, LS31) originated from hybrid *Helianthus annuus* × *Helianthus salicifolius*. The cultural sunflower in these hybrids was represented by line 2607.

Protein was processed by Kjieldahl's classical method. Amino acid composition of storage protein in kernel was determined by the method of protein hydrolysis with 6n HCl using a high-efficiency amino analyser HITACHI L 8500 in three replications.

Statistical processing of the data was performed by the software BIOSTAT, version 1 (Penchev, 1998).

RESULTS AND DISCUSSION

The data in Table 1 show that all lines had protein content higher than the mother line. Protein content in defatted kernel varied from 53.4% to 61.8%. The wild species (male parent) had significantly lower index: 35.3%. Similar tendency has also been observed by Christov *et al.*, 1993.

Table 1: Protein content in defatted kernel (% of absolute dry matter) and amino acid composition (mol%) in lines derived from interspecific experimental hybrid *Helianthus annuus* × *Helianthus resinosus.*

	Line						
	L60	L70	L80	L14	L15	2607	*H. resinosus*
Protein,%	56.5^{c}	61.8^{c}	53.4	54.6^{b}	58.0^{c}	52,4	35.3
	Amino acid, mol%						
Lysine	3.39	3.34	3.39	3.82^{c}	3.64^{c}	3,39	3.25
Histidine	2.56	2.51	2.61	2.78	2.72	2,47	2.08
Arginine	7.00	7.14	6.89^{a}	7.03	7.11	7,13	8.47
Asparagine	9.81^{a}	9.81^{a}	10.19^{b}	10.33^{c}	10.27^{c}	9,61^{c}	10.15
Threonine	4.63^{a}	4.57	4.63^{a}	4.71^{c}	4.69	4,45^{c}	4.10
Serine	5.57	5.94	5.78	5.78	5.82	5,34	5.66
Glutamine	19.18	19.96	19.49	19.04	19.19	19,37	21.18
Proline	5.62	5.49	5.52	5.52	5.61	5,68	5.96
Glycine	11.11^{c}	10.90^{c}	10.84^{c}	10.75^{c}	10.98^{c}	10,02	9.37
Alanine	9.96^{a}	6.75^{a}	6.98	7.25^{b}	7.08^{b}	6,69	6.44
Cysteine	0.08^{c}	0.07^{c}	-	-	-	0,77	0.29
Valine	5.81^{b}	5.82^{b}	6.14^{b}	6.09^{c}	6.0^{b}	5,27	5.18
Methionine	0.08^{c}	0.79^{c}	0.86^{c}	0.15^{c}	0.16^{c}	1,80	0.83
Isoleucine	4.84^{c}	4.80^{c}	4.77^{c}	4.84	4.83	4,25	4.60
Leucine	7.46	7.32	7.38	7.48	7.40	7,36	6.92
Tyrosine	0.73^{c}	0.60^{c}	0.40^{c}	0.30	0.35	1,86	1.56
Phenylalanine	4.14^{a}	4.15^{a}	4.09	4.13^{a}	4.11	3,98	3.91
$\sum$ of essential amino acids,%	31.16	31.46	31.66	31.52	31.18	33,13	30.64

Shaded cels represents non-essential amino acids

Concerning essential amino acids, lysine content in the defatted kernel of two of the lines exceeded both parents, the variation being from 3.25% in *H. resinosus* to

3.82% in line L14. With regard to other essential amino acids, the lines had higher content of valine and phenylalanine in comparison to the two parental forms. Valine content in the lines was highest (from 5.81% to 6.14%), the respective content of the parental lines being 5.27% for line 2607 and 5.18% for the wild species. The low content of the sulfur-containing acids cystine and methionine is typical for sunflower. Unfortunately all lines had significantly lower content of these amino acids than cultural sunflower.

Since tryptophan, which is an essential amino acid, is completely degradable in acid hydrolysis of protein, we could not obtain any data on it in this investigation. There were no significant variations in leucine content between the wild and the cultural species, nor between the lines derived from the experimental hybrid of the wild and cultivated species.

All lines had lower content of tyrosine than both the wild and cultivated species, but their phenylalanine content was higher. The variations were low but significant.

The total amount of amino acids was the highest in the line 2607 (33.13%), and lowest in *Helianthus resinosus* (30.64%). In the lines derived from this crossbreeding the total amount of essential amino acids was from 31.16% to 31.66%, which was an indication that the inheritance of protein content was most probably intermediary.

With regard to non-essential amino acids, all lines had higher content of asparagine than the cultural species and higher content of glycine than both the cultural and the wild species.

Similar to the experimental hybrid of the cultural species with *Helianthus resinosus*, in the experimental hybrid *Helianthus annuus* × *Helianthus salicifolius* all lines also had significantly lower amount of sulfur-containing amino acids (Table 2).

The inheritance of methionine was closer to the wild species. The amount of isoleucine varied from 4.25% (line 2607) to 4.88% (line LS19) and 4.94% (line LS20). The differences were from 0.46% to 0.92% with high level of significance. Leucine content was averagely 7.4%, equal to that of the cultural parent. Phenylalanine was 4.14% averaged for the 5 tested lines and significantly exceeded both parents (3.98% and 3.95%, respectively).

The total content of essential amino acids in the lines with this species, too, showed that inheritance was intermediary. In the line 2607 the content was 33.13%, and in the wild species *Helianthus salicifolius* – 30.14%. In the newly developed lines it was within 31.06% - 32.33%.

With regard to non-essential amino acids, only asparagine and glycine were higher than the parental forms, the differences being significant.

The data in Table 2 also show that all lines had higher content of protein than the parental lines. Protein content in defatted kernel varied from 51.9% to 63.0%. Only line LS20 had insignificantly lower protein content (0.5%) than the line 2607. The wild (male) parent significantly conceded to the new lines by this index (42.5%).

Table 2: Protein content in defatted kernel (% of absolute dry matter) and amino acid composition (mol%) in lines derived from interspecific experimental hybrid *Helianthus annuus* × *Helianthus salicifolius.*

	Line						
	LS18	LS19	LS20	LS30	LS31	2607	*H. salisifolius*
Protein,%	55.6^{b}	53.8^{a}	51.9	63.0^{c}	55.6^{b}	52,4	42.5
	Amino acid, mol%						
Lysine	3.51	3.50	3.43	3.34	3.54	3,39	3.12
Histidine	2.60	2.63	2.62	2.72	2.75^{a}	2,47	2.22
Arginine	7.04	7.32	7.23	6.96	6.77^{a}	7,13	8.31
Asparagine	10.16^{a}	10.13^{a}	9.98	10.30^{c}	10.11^{b}	9,61	9.62
Threonine	4.73^{b}	4.47	4.60	4.78^{b}	4.95^{c}	4,45	4.11
Serine	5.86	5.77	5.82	5.77	5.87	5,84	5.77
Glutamine	19.35	20.05^{a}	19.98^{a}	19.78	19.79	19,37	22.09
Proline	5.49	5.46^{a}	5.75	5.67	5.41	5,68	6.01
Glycine	11.0	10.35	10.48	10.61	11.04^{c}	10,02	9.56
Alanine	7.10	7.14^{b}	6.82	7.18	7.29^{a}	6,69	6.21
Cysteine	-	-	0.07	-	-	0,77	0.18
Valine	6.07	6.06^{c}	5.86^{b}	6.14^{c}	6.22^{c}	5,27	5.29
Methionine	0.12^{c}	0.21^{c}	0.47^{c}	0.36^{c}	0.21^{c}	1,80	0.55
Isoleucine	4.79^{c}	4.88^{c}	4.94^{c}	4.68^{b}	4.72^{b}	4,25	4.62
Leucine	7.30	7.39	7.41	7.37	7.51	7,36	6.94
Tyrosine	1.63^{c}	0.46^{c}	0.35^{c}	0.24^{c}	0.69^{c}	1,86	1.38
Phenylalanine	4.18^{a}	4.17^{a}	4.15^{a}	4.15^{a}	4.09	3,98	3.95
$\sum$ of essential amino acids,%	32.33	31.14	31.28	31.06	31.94	33,13	30.14

Shaded cels represents non-essential amino acids

CONCLUSION

The development of more precise indices and criteria for characterization of the protein component in conventional or new protein foods is being intensely debated in the research on nutrition. The most important criterion for the biological value of protein is its amino acid composition. In this investigation we showed the potential of deriving lines from interspecific hybrids which have significantly higher protein content in kernel and variable amino acid composition. A number of international organizations are executing special programs for the development of new protein sources of plant or animal origin. Protein content was with 10.6 % higher in most of our new lines, being most significant in the lines L70 and LS30. Increased content of essential amino acids in the protein was foud in most of them.

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