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STUDY OF SEED OIL AND PROTEIN QUALITY OF SOME *Bidens tripartita* ACCESSIONS

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SUMMARY

Lysine content in sunflower seed storage protein is not sufficient to meet the requirements of FAO. As far as storage protein amino acid composition of *Helianthus annuus* is concerned, lysine content of the species from the IWS "Dobroudja" collection investigated in our laboratory is not substantially different from that of cultivated *Helianthus annuus* varieties and hybrids. In a previous study we have reported data about *Bidens tripartita* high lysine content in storage protein of seeds (3.4% for *Helianthus annuus* and 5.8% for *Bidens tripartita*). In 1994, we collected 14 new *Bidens tripartita* accessions from different parts of Bulgaria. A biochemical characterization of these genotypes including fatty acid composition, storage protein electrophoretic subunits and amino acid composition is presented in this study. It was established that linoleic acid varied from 64.0% to 73.8% and oleic acid from 18.3% to 23.4%. Seven different storage protein electrophoretic patterns were found in the 14 investigated genotypes. Lysine content varied from 4.83% to 5.45%

Key words: *Bidens tripartita*, storage proteins, amino acid composition, fatty acid composition, lysine content.

INTRODUCTION

Sunflower storage protein and oil-cake obtained after seed processing are valuable products for animal feed. According to Earle et al., (1968), the content of nutritionally essential amino acids in sunflower meals is comparable to the estimated nutritional requirements for human, pigs and chicken.

Regarding human food, the chemical analyses indicate adequacy of essential amino acids, exept for lysine and isoleucine. The phenylalanine content is sufficient to meet the nutritional requirement of growing pigs, when added to available tyrosine. For young chickens, the feed lacks in lysine, leucine and the values for sulfur-containing amino acids, isoleucine and glycine are barely sufficient to meet the limit requirements. Therefore, improvement of sunflower stor-

Figure 1. Bidens tripartita seeds

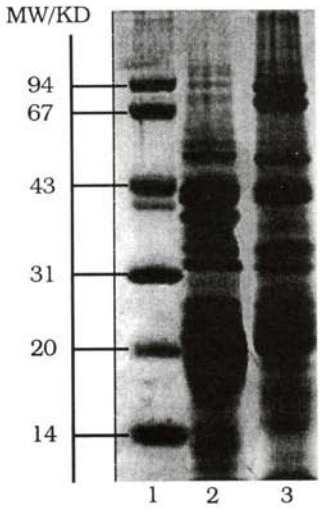

Figure 2. Electrophoretic patterns of Helianthus annuus (2) and Bidens tripartita (3)

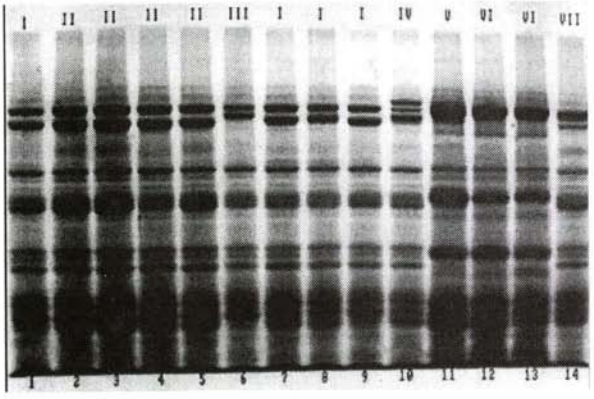

Figure 3. Electrophoretic patterns of 14 Bidens tripartita accessions

age protein amino acid composition will increase the storage protein importance for monogastric animal nutrition.

A study on amino acid composition of storage protein in some *Helianthus* species has shown that none of them is substantially different from cultivated sunflower in lysine content (Ivanov et al., 1984; Christov et al., 1993). In a previous communication (Ivanov et al., 1994) we have reported data for storage protein amino acid composition of some species systematically related to the *Helianthus* genus. Three of the investigated species, *Bidens tripartita*, *Arctium lappa* and *Cirsium vulgare* contain 5.8%, 5.1% and 4.9% lysine, respectively, which is higher in comparison with *Helianthus annuus* (3.4%). Storage protein subunits pattern for *Bidens tripartita* is characterized by several unique sharp bands with approximate molecular weight 80,67 and 50 KD. The conclusion was drawn that *Bidens tripartita* can be used as a promising donor for lysine content improvement in cultivated *Helianthus annuus* varieties and hybrids.

The second stage in characterizing the genetic potential of *Bidens tripartita* was to collect 14 new accessions from different parts of Buglaria. The data for their biochemical characteristics are presented in this study.

MATERIAL AND METHODS

In 1994, 14 *Bidens tripartita* (2n=48) accessions were collected from different parts of Bulgaria. Kernel oil fatty acid composition was determined by gas chromatography. Amino acid composition of the storage protein from defatted kernel was determined by a high efficient Hitachi amino acid analyzer L-8500.

Storage proteins of the defatted kernel meal were extracted and separated electrophoretically according to the method of Laemmli (1970) modified by adding 4M urea in the sample and tank buffers.

RESULTS AND DISCUSSION

The *Bidens* genus belongs to the *Compositae* family. *Bidens tripartita* (2n=48) is an annual plant with upright and branched stem. The form of the leaves varies from ovoid-rhomboid to lanceolate (consisting of three parts), the peduncle is short and wing shaped.

Inflorescenes are 15-20 mm wide. Fruits (seeds) have 2, rarely 3 awns (Figure 1). The species is widely distributed in humid areas. It is used as a herb in Bulgaria and other European countries. The crossing of *B. tripartita* with *Helianthus annuus* is accompained with several difficulties, exept for cases when it is used as a male parent (Christov et al., 1994).

Most of the obtained F_2, F_3 and F_4 plants were without top parts and inflorescenes. A large number of normal plants was obtained in F_5. This result proves

Table 1: Fatty acid composition of 14 *Bidens tripartita* accessions (% in oil from the kernel).

Accession	Fatty Acid				
	Linoleic	Oleic	Stearic	Palmitoleic	Palmitic
1	72.4	17.4	0.4	0.9	8.9
2	70.9	20.9	0.9	1.0	6.3
3	71.0	22.1	1.4	traces	5.5
4	70.3	22.9	1.0	-	5.8
5	71.3	20.2	0.8	-	7.7
6	73.1	18.6	0.7	1.1	6.5
7	73.7	18.4	0.9	1.1	5.9
8	68.0	19.9	3.1	1.2	7.8
9	71.5	17.9	0.6	1.2	8.8
10	73.7	20.8	1.2	-	4.3
11	68.0	17.0	0.8	1.7	12.5
12	64.2	21.7	0.7	1.3	12.1
13	65.6	20.2	0.7	1.5	12.0
14	65.1	22.4	1.6	1.6	9.3

Table 2: Storage protein amino acid composition of 14 Bidens tripartita accessions (Harvest 1994, in mol%).

Amino acid	st	1	2	3	4	5	6	7	8	9	10	11	12	13	14
lysine	3.12	5.08	5.18	5.03	5.45	5.11	5.41	5.15	5.16	5.04	5.11	4.84	4.83	4.85	5.07
histidine	2.36	2.39	2.58	2.62	2.51	2.51	2.51	2.36	2.51	2.53	2.46	2.71	2.67	2.75	2.85
arginine	7.21	5.61	5.86	5.89	5.51	5.74	5.18	5.38	5.51	5.3	5.38	6.29	5.63	5.68	5.67
aspartic acid	9.31	9.14	9.71	9.64	9.31	9.49	9.67	9.03	9.41	9.21	9.23	9.44	8.83	8.66	9.66
threonine	4.16	4.31	4.41	4.29	4.57	4.31	4.68	4.38	4.57	4.42	4.48	4.21	4.23	4.39	4.43
serine	5.54	5.75	6.32	5.91	5.92	5.86	6.14	5.65	5.96	5.69	5.96	5.94	5.69	5.76	5.92
glutamic acid	20.29	19.27	19.52	19.57	19.44	19.78	18.83	19.71	19.21	19.57	19.71	21.07	20.43	20.51	19.24
proline	5.86	6.91	6.78	6.66	7.01	6.82	6.43	7.11	6.53	7.18	7.21	6.11	7.21	7.17	6.83
glycine	10.07	10.03	9.99	10.01	10.24	9.88	10.53	9.81	10.05	9.85	9.87	9.91	9.58	9.98	9.97
alanine	6.61	5.91	5.86	5.94	5.94	5.93	6.15	5.82	6.01	5.89	5.92	5.81	5.68	5.76	6.01
cystine	0.98	1.01	-	-	-	0.21	0.21	0.49	0.44	0.23	0.26	0.31	0.65	0.55	0.43
valine	5.53	5.74	5.21	5.99	6.31	5.69	5.69	5.69	5.73	5.74	5.61	5.64	5.41	5.34	5.68
methionine	2.08	1.03	1.03	0.93	0.57	1.15	1.24	1.21	1.41	1.23	0.94	0.59	1.32	0.89	0.62
isoleucine	4.11	3.71	3.77	3.81	3.79	3.72	3.71	3.89	3.71	3.99	3.88	3.67	3.82	3.89	3.72
leucine	6.87	6.82	7.01	7.01	7.09	6.96	6.97	7.01	6.91	7.09	6.93	6.73	6.82	6.71	6.97
tyrosine	2.04	2.11	1.48	1.43	1.64	1.59	1.61	2.24	1.84	1.94	2.14	1.56	2.25	2.24	1.81
phenylalanine	3.88	5.19	5.26	5.31	5.21	5.31	5.49	5.06	5.02	5.08	5.04	5.14	4.89	4.84	5.05

Note: st - Hybrid Albena

that hybridization between these two spicies gives a good opportunity to transfer desirable traits from *B. tripartita* to *H. annuus* which is a major challenge in investigating the qualitive composition of *B. tripartita* seed.

In this study we investigated whole seed meal because the seeds were very small and difficult to husk.

Oil fatty acid composition of the tested *Bidens tripartita* accessions (Table 1) was similar to that of cultivated sunflower oil. Only palmitoleic acid is not typical for sunflower oil.

The total storage protein amino acid composition (Table 2) of the *Bidens* accessions was quite different from that of sunflower seed protein. Lysine content varied from 4.83% to 5.46% and was substantially different from that of sunflower (3.12%). Accessions 4 and 6 were most interesting.

Electrophoretic patterns of the storage protein extracted according to the Laemmli (1970) method (Figure 2) were different from the patterns of *H. annuus* (hybrid Albena). Several sharp bands with approximate molecular weights of 80, 67 and 50 KD were unique for the previously investigated *Bidens tripartita* accessions (Ivanov et al., 1994). Similar characteristics were observed for the newly studied accessions (Figure 3). These new genotypes can be classified into 7 groups according to these unique high molecular weight subunits. The first one includes numbers 1, 7, 8 and 9; the second - numbers 2, 3, 4 and 5; the third - number 6. Group four includes number 10, group five - number 11, group six - numbers 12 and 13, and group seven - number 14. The two accessions highest in lysine content (4 and 6) belonged to the second and third group. Amino acid composition of storage protein extracted according to Laemmli method was determined on the basis of the same procedure used for establishing total storage protein. Lysine content (Table 3) average 4.8% was also higher than that of *H. annuus* but lower than the man value of the total storage protein (5.09%). In Table 3 the value of lysine content of accession 6 is highest, too.

Amino acid composition of the residual protein after applying the Laemmli method of extraction of soluble proteins from the total storage proteins was also studied (Table 4). In this case, the average lysine content determined for the *Bidens* accessions was 5.25%, which is higher in comparison with the lysine content in the total storage protein - 5.09%. The lysine content values for residual protein in accessions 4 and 6 were highest in Table 4, too.

These new data on *Bidens tripartita* storage protein amino acid composition give us reasons to consider it a promissing partner in crossing programs for improving the lysine content of *H. annuus* cultivated lines and hybrids.

Table 3: Amino acid composition of storage protein extracted according to Laemmli method in 13 *Bidens tripartita* accessions (Harvest 1994, in mol %).

Amino acid	st	1	2	3	5	6	7	8	9	10	11	12	13	14
lysine	3.20	4.93	4.76	4.78	4.73	5.04	4.91	4.94	4.95	4.66	4.50	4.73	4.78	4.75
histidine	2.53	2.80	2.43	2.38	2.35	2.33	2.34	2.31	2.28	2.29	2.44	2.44	2.40	2.35
arginine	7.66	6.04	6.18	6.07	6.12	5.85	5.87	5.88	5.90	5.98	6.68	6.26	6.23	6.04
aspartic acid	9.28	8.76	9.01	9.04	8.9	8.59	8.65	8.67	8.70	8.68	8.65	8.53	8.01	8.84
threonine	4.19	4.14	3.99	4.21	4.02	4.25	4.15	4.16	4.31	4.25	3.83	4.22	4.23	4.14
serine	5.39	4.98	5.01	5.62	5.01	4.99	4.96	5.01	5.42	5.41	5.25	5.57	5.21	5.31
glutamic acid	21.13	19.76	19.72	19.94	19.79	19.31	19.46	19.55	19.58	19.76	21.11	20.68	20.27	19.18
proline	6.84	9.58	9.24	9.15	9.22	9.21	9.11	9.22	9.26	9.54	9.15	9.05	9.88	9.56
glycine	8.39	8.65	8.41	8.65	8.64	8.82	8.46	8.56	8.67	8.59	8.20	8.52	8.28	8.37
alanine	6.51	5.83	5.73	5.91	5.76	6.01	5.78	5.89	5.86	5.82	5.48	5.87	5.68	5.77
cystine	0.23	0.48	0.43	0.56	0.51	0.45	0.64	-	0.55	-	0.64	0.38	0.53	0.59
valine	5.16	6.05	5.92	5.08	5.89	6.06	6.07	6.13	5.43	5.66	5.22	5.06	5.43	5.52
methionine	1.99	1.53	1.57	1.46	1.52	1.58	1.82	1.79	1.88	1.91	1.81	1.62	2.12	1.86
isoleucine	4.03	3.82	3.79	3.24	3.79	3.89	3.91	3.88	3.46	3.72	3.52	3.46	3.61	3.61
leucine	7.08	6.63	6.61	6.61	6.57	6.67	6.66	6.72	6.55	6.55	6.34	6.58	6.41	6.63
thyrosine	2.28	1.72	2.04	2.19	1.96	1.81	2.22	2.07	2.22	2.31	2.21	2.02	2.23	2.41
phenylalanine	4.10	5.27	5.27	5.18	5.21	5.19	5.05	5.23	5.01	4.93	4.99	5.04	4.84	5.12

Note: st - Hybrid Albena

Table 4: Residual protein amino acid composition of seed defatted meal after extraction according to Laemmli method in soluble proteins of 12 *Bidens tripartita* accessions (Harvest 1994, in mol %).

Amino acid	st	1	2	3	4	6	7	8	10	11	12	13	14
lysine	3.06	5.32	5.23	5.26	5.52	5.53	5.36	5.34	5.24	4.85	5.11	5.01	5.17
histidine	2.53	2.61	2.61	2.71	2.65	2.79	2.64	2.61	2.52	2.68	2.67	2.53	2.53
arginine	6.54	4.71	4.99	4.76	4.57	4.67	4.89	4.87	4.64	5.35	4.16	4.99	5.04
aspartic acid	8.94	9.09	9.39	9.35	9.12	9.21	9.02	9.61	8.84	8.91	9.05	8.65	9.35
threonine	4.09	4.44	4.54	4.35	4.53	4.65	4.63	4.55	4.51	4.13	4.37	4.38	4.48
serine	5.61	6.01	6.86	6.09	6.08	6.38	6.39	6.24	6.14	5.93	6.14	6.11	6.17
glutamic acid	20.01	17.51	18.52	18.43	18.41	17.76	17.93	17.37	17.71	19.41	18.66	18.32	18.03
proline	8.09	7.79	7.92	7.81	8.08	7.91	8.18	7.79	9.04	9.07	9.05	8.88	8.55
glycine	10.32	11.31	11.74	11.01	11.08	11.61	11.32	11.03	10.93	10.74	10.93	11.32	10.69
alanine	6.63	6.01	6.25	6.01	6.15	6.41	6.11	6.21	6.21	5.84	6.01	6.13	6.18
cystine	-	-	-	-	-	-	0.48	-	-	-	-	-	-
valine	5.17	5.81	4.81	5.78	5.61	5.29	5.27	5.41	5.46	5.12	5.22	5.15	5.21
methionine	1.91	1.46	1.72	1.21	1.31	1.41	1.41	1.64	1.53	1.48	1.67	1.81	1.69
isoleucine	4.11	3.73	3.06	3.65	3.51	3.41	3.37	3.48	3.52	3.39	3.54	3.45	3.37
leucine	6.81	6.77	6.41	6.66	6.57	6.57	6.48	6.81	6.71	6.39	6.46	6.39	6.48
tyrosine	2.29	2.01	1.49	1.96	1.98	1.91	2.01	2.22	2.27	1.98	2.28	2.30	2.41
phenylalanine	3.86	5.12	4.44	4.95	4.79	4.51	4.67	4.68	4.71	4.72	4.65	4.52	4.66

Note: st - Hybrid Albena

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ALTOS NIVELES DE LISINA EN LA PROTEÍNA DE ALGUNAS ENTRADAS DE *Bidens tripartita* Y ESPECIES DE *Helianthus*

RESUMEN

La proteina de reserva del girasol es deficiente en contenido de lisina de acuerdo con los requerimientos de la FAO. La composición de aminoácidos de la proteina de reserva de especies de *Helianthus* de la colección del IWS "Dobroudja" investigadas en nuestro laboratio ha mostrado que ninguna de ellas es sustancialmente diferente de las variedades e híbridos cultivados de *Helianthus annuus* en relación con el contenido de lisina. En un estudio previo se publicaron datos en *Bidens tripartita* de contenidos má altos de contenido de lisina (3.4% para *Helianthus annuus* y 5.8% para *Bidens tripartita*). Durante 1994 se hizo una colección de 14 muevas entradas de Bidens tripartita en diferentes partes de Bulgaria. En este estudio se presenta una caracteización bioquímica de estos genotipos incluyendo composición de ácidos grasos, subunidades electroforéticas de proteina de reserva y composición de aminoácidos. El contenido de ácido linoleico varia de 64.0 al 73.8% y el oleico desde 18.3 a 23.4%. Se encontraron siete diferentes formas electroforéticas de proteina de reserva dentro de los genotipos investigados. El contenido de lisina varia de 4.83 al 5.43.

AUGMENTATION DE LA TENEUR EN LYSINE DES PROTÉINES DE RÉSERVE CHEZ CERTAINES ACCESSIONS DE *Bidens tripartita*

RÉSUMÉ

Selon les spécifications de la F.A.O., les protéines de réserve du tournesol sont déficitaires pour la teneur en lysine. La composition en acides aminés des protéines de réserve des espèces d'*Helianthus annuus* de la collection de l'IWS "Dobroudja" étudiée dans notre laboratoire a montré que l'une d'entre elles est très différente des variétés et hybrides cultivés d'*Helianthus annuus* pour la teneur en lysine. Dans une étude préalable réalisée sur *Bidens tripartita* on a souligné la teneur plus élevée en lysine des protéines de réserve (3.4% pour *Helianthus annuus* contre 5.8% pour *Bidens tripartita*). En 1994, on a collecté 14 nouvelles accessions de *Bidens tripartita* dans différentes parties de la Bulgarie. Une caractérisation biochimique de la composition en acides gras des sous-unités des protéines de réserve par électrophorèse et de la composition en acides aminés est rapportée dans cette étude. Le teneur en acide linoléique varie de 64% à 73.8% et celle de l'acide oléique de 18.3% à 23.4%. Sept profils différents ont été trouvés dans les électrophorèses de protéines de réserve des 14 génotypes étudiés. La teneur en lysine varie de 4.83 à 5.45%.