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Identification and Differences of Total Proteins and Their Soluble Fractions in Some Pseudocereals Based on Electrophoretic Patterns

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Genetic diversity and relationships of 11 species and cultivars belonging to different *Angiosperms* families were examined using sodium dodecyl sulfate seed protein markers. The protein was resolved into 36 bands (for soybean), 41 (for quinoa), 35 (for buckwheat), and 28 to 39 bands of Amaranth species, respectively. All species and cultivars can be distinguished from each other. Soybean, quinoa, and buckwheat species had a characteristic protein pattern showing a high degree of polymorphism. The protein patterns of soybean were considerably different from other species. Amaranth species had similar seed protein electrophoretic profile. The similarity coefficients calculated on the basis of presence and absence of bands ranged from 0.08 to 0.97. Following the UPGMA algorithm of similarity coefficients, the examined species and varieties could be clustered into two similarity groups. Our results did not confirm the Tachtadzjan hypothesis that *Polygonales* (e.g., buckwheat) and *Caryophyllales* (e.g., quinoa and amaranth) are closely related. Our data rather indicate occurrence of significant genetic distance (similarity coefficients 0.05−0.10). Also, it is doubtful that amaranth and quinoa species are also closely related (similarity coefficients varied from 0.16 to 0.25). It seems that soybean, quinoa, buckwheat, and amaranth (as a genus) can be considered as phylogenetic distant taxa. Differences and similarities in the secondary structure were observed by circular dichroism spectra. Some similarity was found between these plants in their soluble protein fractions and amino acid composition. These plants can be a substitution of each other as well as for cereals.

KEYWORDS: Plants; proteins; properties; identification; differences; spectroscopy

INTRODUCTION

The world protein demand has increased in the past decades, especially due to a population increase (*1*−*3*). Protein digestibility, available lysine, protein utilization and protein efficiency ratio have been widely used as indicators of protein nutritional quality. On the basis of these indices, the values for amaranth, soybean, quinoa, and buckwheat proteins are definitively higher as compared to cereal grains, and are close to those of casein (*2*, *4*−*6*). All these plants are promising food ingredients that can substitute cereal proteins. Cereals in comparison with the pseudocereals very often cause allergy symptoms by gluten or prolamin proteins (*7*). It was also shown that the main storage protein of some pseudocereals prevents coronary artery disease (*8*, *9*).

Amaranth, soybean, buckwheat, and quinoa proteins are highly soluble and applicable in functional foods (*2*−*4*, *10*−*12*).

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Information on genetic diversity and relationships among crop species is essential for the efficient explanation of taxonomic relationships. There are several groups of chemical compounds, such as primary and secondary metabolites (as phenols) and sequencing of amino acids (*13*). Lately, several molecular approaches have been employed to assess genetic diversity and taxonomic relationships. Among them are isozymes or random amplified polymorphic DNA (RAPD), which generates data faster than restriction fragment length polymorphism (RFLP) or the use of microsatellites (*14*). The use of proteins is limited. There are few publications connected with the taxonomy of plants from sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis (*15, 16*).

There are two types of protein markers: the immunochemical, or serological, and the electrophoretical. Serological markers well indicate rather biological properties of the protein, its belonging to species, genus, division, family up to class. This allows utilization of protein-antigens as biological or phyllogenetic markers. For definition of belonging and revealing of phylogenetic relationships in plants at the level of highest taxa, only the evolutionarily conservative proteins are suitable as histones (*17*). On the other hand, it is considered that electrophoretic protein markers can be suitable for solving taxonomy problems only to the genus level (*13*).

Electrophoretical protein markers are used at all stages of breeding process, starting from studying the initial material and searching the sources to variety trial and seed production (*13*−*17*).

The most commonly used proteins are seed storage proteins, which are known to be polymorphic with respect to size, charge, or both these parameters (*18*−*20*).

Storage proteins are predominant in seeds of dicotyledon plants as well as in cereal grains. They are synthesized as secretory proteins (i.e., in the endoplasmatic reticulum of storage cells and are accumulated in the form of protein bodies). They differ from prolamins in respect to morphogenesis because they are formed in cotyledons and controlled by genetic systems of diploid nucleus (cereal grain endosperm is triploid). The storage proteins of dicotyledon plants represent mainly globulins and albumins (*2, 4*−*6, 19*−*21*).

No works have been reported based on comparison of seed proteins of dicotyledon plants belonging to different groups of the higher taxon than genus. Protein is a primary product of the genetic system. On the basis of this, a protein can serve as a marker of genome, and protein markers as protein phenotype indicate the genome structure and genotype specificity as a whole. According to several phylogenetic classifications (*22*−*25*) *Amaranthus* and *Chenopodium* genus belong together to *Caryophyllales*, and separately, Glycine *max* belongs to *Fabales* or *Rosales*, and Fagopyrum *tatricum* belongs to *Polygonales*. According to Tachtadzjan, (*22*) *Polygonales* and *Caryophyllales*, as they are closely related, are combined together in *Caryophyllidae*. Usually, *Fabales* and *Rosales* are classified as distinct phylogenetic units. As it was mentioned above, the main storage protein of amaranth, quinoa, buckwheat, and soybean is the oligomeric salt-soluble globulin, as well as the albumin-2 fraction (*19, 21, 26, 27*). Thus, we would like to compare electrophoretic data obtained from total proteins for estimation of genetic similarity between species and varieties.

Another question we hoped to answer was whether the SDS-PAGE electrophoresis of seed proteins is suitable for verification of taxonomic data based on the morphological ones.

Therefore, the main objective of the present study was to evaluate genetic diversity and relationships in eight species belonging to different orders and families of dicotyledon based on the extraction of total proteins and amino acid analysis. This work also reports an application of circular dichroism spectroscopy (CD) to compare the properties of soluble protein fractions in the studied plants.

MATERIALS AND METHODS

Materials. Sodium dodecyl sulfate (SDS), β-mercaptoethanol (β-ME), acrylamide, polyacrylamide, Coomassie Brilliant Blue R, and molecular weight marker (20−205 kDa) were of analytical grade and were purchased from Sigma Chemical Co, St Louis, MO. The protein standard IV (30−78 kDa) was purchased from Merck, Darmstadt, Germany. Deionized water was used throughout.

Samples. Whole mature seeds of plants such as soybean (*Glycine max L. Merr*. Fabaceae (Brazil)), quinoa (*Chenopodium quinoa Wild*., Chenopodiaceae (Peru)), buckwheat (*Fagopyrum esculentum Mnch*., Polygonaceae (Peru)), and eight species of amaranth (*Amaranthus*, Amaranthaceae): *hybridum v.*1004, (Pakistan); *flavus, cruentus v.* Rose and *caudatus* (Brazil); *cruentus*; *hypochondriacus*; and *hypochondriacus v.*1023 (Mexico) and *cruentus v.* R104 (USA) were investigated.

Species of A. *hypochondriacus* 1023; A. *cruentus* R104 and A. *hybridum* 1004 were the results of hybridization and crossing of original *A. hypochondriacus* and *A. cruentus*, Mexico, and were genetically different from other amaranth lines.

Jasmin rice (*Oryzea sativa*, Khao Dawk Mali) and defatted rice bran and potato tuber Satina (*Solanum tuberosum*, Germany) were used for comparison of electrophoretic patterns of extracted protein soluble fractions.

Seeds were ground on a mill (Janke & Kunkel GmbH & Co. KG −IKA, Labortechnik, Staufen, Germany) through a 60-mesh screen. The meal was defatted in a Soxhlet extractor with *n*-hexane for 10 h and then was stored at 5 °C after removal of hexane.

Protein Extraction. Total proteins from defatted meals of: *Glycine max*. single seeds and 40 mg; *Chenopodium*, 20 seeds and 40 mg; *Fagopyrum* 40 seeds and 60 mg, and for *Amaranthus* 60 seeds and 40 mg, respectively, were extracted with 1 mL of 1 M Tris buffer pH 6.8, containing sodium dodecyl sulfate (SDS), bromophenol blue, glycerol, and mercaptoethanol. The extracts were allowed to stand overnight at room temperature (*27, 28*). Samples were boiled for 5 min, and then centrifuged at 18 000*g* for 15 min at 15 °C. Each step was repeated twice.

Albumins-1 (Alb-1) and globulins (Glo) were extracted from defatted meal with 0.5 M NaCl (1:10) at 4 °C. The supernatants were dialyzed (M_W cutoff 6000) for 3 days against deionized water at 4 °C. The content of dialysis tubes was centrifuged at 9000*g* for 20 min. The supernatant was the albumin-1 (Alb-1) and the pellet−globulin fraction (Glo). Alb-1 and Glo were lyophilized. The extraction residue was washed with deionized water and centrifuged. Albumin-2 (Alb-2) was extracted from the pellet with deionized water (1:10) at 4 °C and centrifuged at 9000*g* for 20 min. The extracted samples were lyophilized. The residue was suspended in 0.125 M (1:10) sodium borate buffer (pH 10), containing 3% (v/v) 2-ME plus 0.5% (w/v) SDS.

The nitrogen content in each fraction was determined by micro-Kjeldahl method combined with a colorimetric determination (*29*).

Amino Acid Analysis. Samples of extracted proteins were hydrolyzed with 6 M HCl and 3% phenol solution in a MLSMEGA-Microwave system for 20 min at 160 °C. The energy was 1000 W for the first 5 min and then 500 W for 15 min. The samples were then dissolved in 100 μL of HCl (20 mM) and filtered through a 0.45-μm filter. Derivatization was done with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (*30*). The sample was injected into a Multi-Pump Gradient Water HPLC system with a vertex knauer column B1184742 (Knauer, Berlin), length XID 150 × 4.6 mm ID, spherimage-80 = DS2-5 μm. The Millenium chromatography manager system from Waters (Waters, Milford, MA) was used to evaluate the amino acids. Scanning fluorescence detector was used at an excitation of 250 nm and emission of 395 nm. Gradient program consisted of 40% acetate phosphate buffer and 60% acetonitrile.

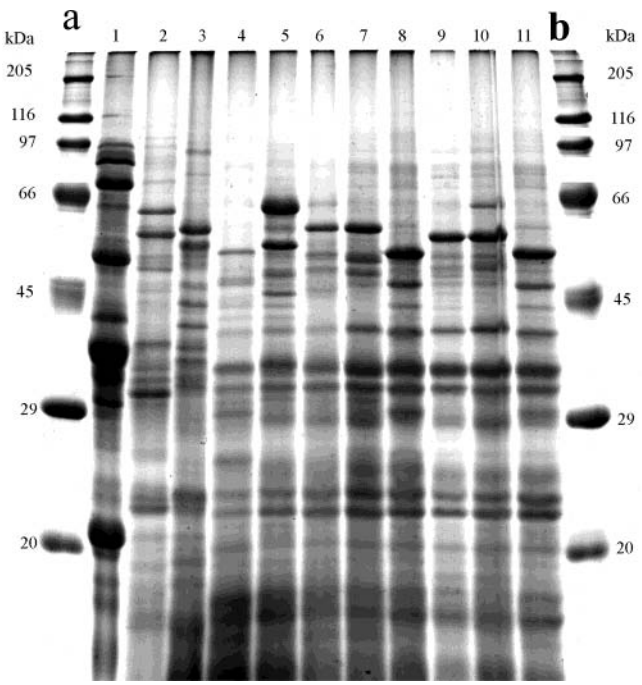

Figure 1. SDS-PAGE electrophoregram of seed proteins extracted with tris-buffer, pH 6.8. **a.** Molecular weight marker (20–205 kDa); 1, soybean; 2, quinoa; 3, buckwheat; 4, *Amaranthus* (*A.*) *hybridum* v. 1004; 5, *A. flavus*; 6, *A. caudatus*; 7, *A. cruentus* v. Rose; 8, *A. cruentus*, Mexico; 9, *A. cruentus* v. R104; 10, *A. hypochondriacus*, Mexico; 11, *A. hypochondriacus* v. 1023. **b.** Molecular weight marker (20–205 kDa).

The values of leucine and lysine were added and presented as a total of both. TYR and CYS eluted together and are presented as TYR. The results are given as g/100 g of protein.

SDS-PAGE. SDS-PAGE was performed with the Hoeffer SE 600 vertical unit (Hoeffer Scientific Instruments San Francisco, CA) according to Laemmli (*31*), our modifications (*32*−*34*), and to ISTA Standard Reference Method for verification of *Pisum* and *Lolium* (*35*), using (12.5% polyacrylamide, resolving gel, 5% polyacrylamide, stacking gel, 180 × 160 × 1.5 mm in size) gels. β-ME was used in the SDS-PAGE. The run was carried out at 25 mA per gel at the beginning and then 45 mA per gel until the end of electrophoresis. Gels were stained with 0.25% Coomassie Brilliant Blue R in methanol/water/acetic acid (5:5:1 v/v) and destained in the same solvent. The following molecular weight markers (kDa) were used: 116-β-galactosidase, 66-bovine albumin, 45-ovalalbumin, 29-carbonic anhydrase, 24-trypsinogen, 20-trypsin inhibitor.

The protein standard IV of 30.0, 42.7, 66.2, and 78.0 was injected in each gel in the separation of extracted protein fractions.

Analysis of Similarity Between Species and Varieties. The banding pattern of each species and variety profile from the electrophoregrams were scored as bands for presence or absence of the band. The computer software BIO-GENE (Vilber Lourmat, France, version 1999.03) was used to calculate the Nei and Li (*36*) genetic similarity coefficients and for generating the similarity matrix for all possible pairs of species and varieties. Similarity coefficient (also called Dice) = $2nxy/(nx + ny)$, where nx and ny are the number of bands in the lane x and in the lane y, respectively, and nxy is the number of shared bands between the two lanes. The dendrogram was calculated (*37*) from the similarity values in the matrix and using the UPGMA algorithm (Unweighted Pair-Group Method Analysis).

CD Spectra. CD spectra were measured over the wavelength range 180−250 nm of far-UV (FUV) with a Jasco J-600 spectropolarimeter (Japan Spectroscopic Co., Ltd., Japan) at room temperature under constant nitrogen purge. Solutions (0.03 mg/mL) of proteins were prepared in 0.01 M phosphate buffer, pH 7.2. The absorbancies of all solutions were kept below 1.0. Secondary structure content was calculated using a Provencher nonliner least-squares curve-fitting program and the results of CD measurements (*38*).

RESULTS AND DISCUSSION

It was established that seed proteins of all examined species are heterogeneous and have revealed the extensive polymorphism of total seed proteins. Using SDS-PAGE electrophoresis of total proteins, 36 bands of soybean seeds (**Figure 1**, lane 1), 41 of quinoa (**Figure 1**, lane 2), 35 of buckwheat (**Figure 1**, lane 3) and from 28 to 39 bands of amaranth species (**Figure 1**, lanes 4−11), respectively, were detected. Banding patterns of quinoa, buckwheat, and amaranth, obtained from seed bulks, were exceptionally reproducible. All the species examined can be distinguished from each other (**Figure 1**, lanes 1−11). The banding patterns of soybean, buckwheat, and quinoa are very distinct. It indicates a high level of polymorphism of protein patterns between these species; similarity coefficients calculated from electrophoretic data were from 0.08 to 0.26 (**Figure 2**). These three species were combined in dendrogram in separate, defined cluster (**Figure 3**).

Also the differences in protein pattern between amaranth and other species are significant; similarity coefficients were from 0.05 to 0.25 (**Figure 2**).

The soybean protein pattern is very characteristic; there is a specific group of polypeptides, more than 66 kDa, revealed as three thick and two thin bands (**Figure 1**, lane 1). Differences observed between amaranth cultivars were smaller (**Figure 1**, lanes 7−9 and 10−11) than those between their species (**Figure 1**, lanes 4−7 and 10). Nevertheless, all amaranth varieties were distinguishable, especially in zone of 45−116 kDa (**Figure 1**). The protein pattern of low molecular weight polypeptides (less than 45 kDa) was almost the same for all examined amaranth samples (**Figure 1**). As a result, similarity coefficients for amaranth varied from 0.59 to 0.97 (**Figure 2**), and these species were grouped together in dendrogram (**Figure 3**).

Our results were not confirmed by Tachtadzjan hypothesis (*22*) that plant species of Polygonales (e.g., buckwheat) and Caryophyllales (e.g., quinoa and amaranth) are closely related. Our data rather indicate the occurrence of significant genetic

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11
L1	1.00										
L2	0.26	1.00									
L3	0.14	0.08	1.00								
L4	0.05	0.16	0.05	1.00							
L5	0.05	0.21	0.05	0.68	1.00						
L6	0.05	0.21	0.05	0.70	0.80	1.00					
L7	0.10	0.20	0.10	0.72	0.76	0.83	1.00				
L8	0.05	0.21	0.05	0.59	0.63	0.71	0.67	1.00			
L9	0.15	0.21	0.05	0.70	0.74	0.82	0.78	0.76	1.00		
L10	0.15	0.25	0.05	0.68	0.78	0.80	0.76	0.74	0.97	1.00	
L11	0.05	0.20	0.05	0.75	0.68	0.76	0.72	0.65	0.76	0.74	1.00

Figure 2. The similarity matrix of soybean, quinoa, buckwheat, and eight samples of Amaranth based on protein data using Nei (*36*) genetic similarity coefficient. L1, soybean; L2, quinoa; L3, buckwheat; L4, *Amaranthus* (*A.*) *hybridum* v. 1004; L5, *A. flavus*; L6, *A. caudatus*; L7, *A. cruentus* v. Rose; L8, *A. cruentus*, Mexico; L9, *A. cruentus* v. R104; L10, *A. hypochondriacus*, Mexico; L11, *A. hypochondriacus* v. 1023.

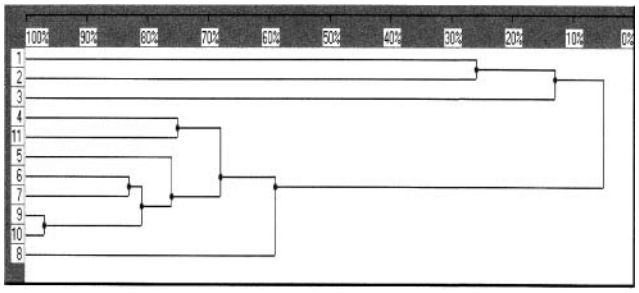

Figure 3. UPGMA dendrogram of soybean, quinoa, buckwheat, and eight samples of Amaranth based on protein data using Nei (*36*) genetic similarity coefficient matrix. 1, soybean; 2, quinoa; 3, buckwheat; 4, *Amaranthus* (*A.*) *hybridum* v.1004; 5, *A. flavus*; 6, *A. caudatus*; 7, *A. cruentus* v. Rose; 8, *A. cruentus*, Mexico; 9-*A. cruentus* v. R104; 10-*A. hypochondriacus*, Mexico; 11- *A. hypochondriacus* v.1023.

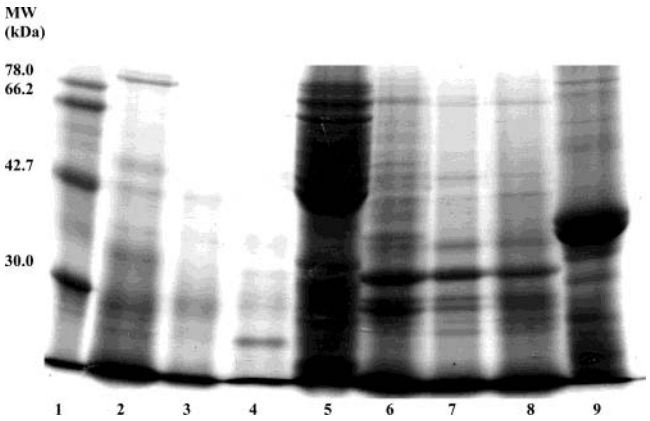

Figure 4. SDS-PAGE electrophoregram of albumin-1 (Alb-1) protein soluble fractions from pseudocereal plants. 1, protein standard IV of 30.0; 42.7, 66.2 and 78.0 kDa; 2, buckwheat; 3, soybean; 4, Jasmin rice; 5, soybean; 6, *A. cruentus* v. R104; 7, *A. hybridum* v. 1004; 8, *A. hypochondriacus* v. 1023; 9, potato.

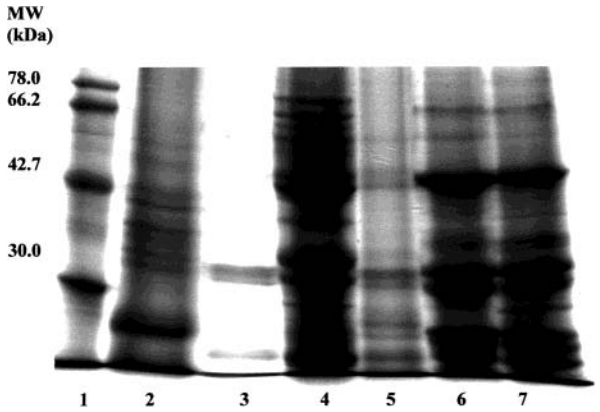

Figure 5. SDS-PAGE electrophoregram of albumin-2 (Alb-2) protein soluble fractions from pseudocereal plants. 1, protein standard IV of 30.0, 42.7, 66.2 and 78.0 kDa; 2, buckwheat; 3, Jasmin rice; 4, soybean; 5, *A. cruentus* v. R104; 6, *A. hybridum* v. 1004, 7, *A. hypochondriacus* v.1023.

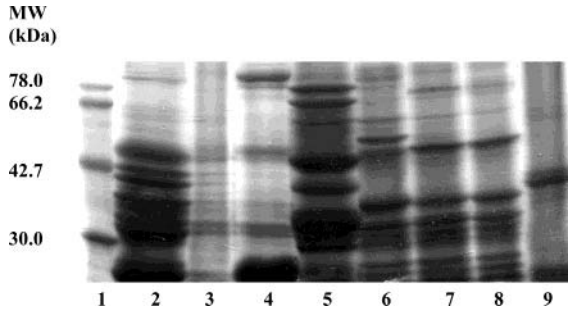

Figure 6. SDS-PAGE electrophoregram of globulin (Glo) protein soluble fractions from pseudocereal plants. 1, protein standard IV of 30.0, 42.7, 66.2, and 78.0 kDa; 2, buckwheat; 3, rice bran; 4, Jasmin rice; 5, soybean; 6, *A. cruentus* v. R104; 7, *A. hybridum* v. 1004; 8, *A. hypochondriacus* v. 1023; 9, potato.

distance (similarity coefficients were very low, 0.05−0.10, **Figure 2**). Also, it is doubtful that amaranth and quinoa species are closely related. In this case, similarity coefficients varied from 0.16 to 0.25, but they were still low. On the other hand, our results confirm the phylogenetic distinction of soybean from other examined species. It seems that also quinoa, amaranths and buckwheat can be considered as phylogenetic distant species.

As SDS-PAGE showed that soybean (**Figure 4**, lane 3) and potato (**Figure 4**, lane 9) had Alb-1 bands with higher molecular weights than buckwheat and amaranth (**Figure 4**, lanes 2, 7, and 8). It is also well known that this protein band is responsible for the high quality of potato tubers (*39*) as well as for other plants. Apparently, there is a relationship between albumin quality and molecular weight of Alb-1 fraction. Amaranth showed a major Alb-1 band at 34 kDa (**Figure 4**, lanes 6−8) and minor bands were observed with a molecular weight under 30 kDa (**Figure 4**, lanes 6−8). Major and minor bands were observed in buckwheat (**Figure 4**, lane 2) and only a low molecular weight band was observed in Alb-1 rice sample (**Figure 4**, lane 4).

Amaranth had a main Alb-2 band with a molecular weight higher than 42.7 kDa (**Figure 5**, lanes 5−7). More protein bands were observed for buckwheat, rice, and amaranth in Alb-2 than in Alb-1 fractions (**Figures 4** and **5**). The water solubility of buckwheat, soybean, and amaranth increased after globulin extraction, and their solubility may depend on the ionic strength of the extracting agents (*40*). Rice bran had no Alb-2, while a major band of molecular weight over 30.0 kDa was observed in Jasmin rice Alb-2 (**Figure 5**, lane 4). Alb-2 was not extracted from potato. This was the main difference between the pseudocereals and other plants such as potato and rice bran, which have been used for the comparison of soluble protein fractions.

Our results are in accordance with others that Alb-2 protein fraction of amaranth was formed by several major polypeptide subunits of molecular masses of 52.3, 54, and 56 kDa which were composed of a peptide of 31 and 38 kDa linked by S−S bonds, with another peptide of 19 and 23 kDa. The 54 kDa subunit together with the 31−38 and 19−23 kDa subunits formed S−S-linked aggregated polypeptides (*4*, *20*, *21*, *32*, *41*). Soybean showed a major globulin band at 30 kDa and a low molecular weight band confirming findings of (*4*, *5*, *32*, *42*). Major differences were observed between albumins (Alb-1 and Alb-2) and globulins of buckwheat.

While A. *hybridum* 1004 and *hypochondriacus v.*1023 had main bands of Alb-2, most bands were observed in globulins for A. *cruentus* R104 (**Figure 6**). Soybean showed a major globulin band at 30 kDa and a low molecular weight band confirming findings of (*41*, *42*). Differences were observed between albumins (Alb-1 and Alb-2) and globulins of buckwheat and rice. Major globulin bands were found for buckwheat and rice. *A. cruentus v.* R104 also had a globulin band at about 50 kDa, which was not present in amaranth 1004 and 1023. These results have shown that the Alb-2 fraction is similar to the globulin one. In other reports, it was shown that Alb-2 was very

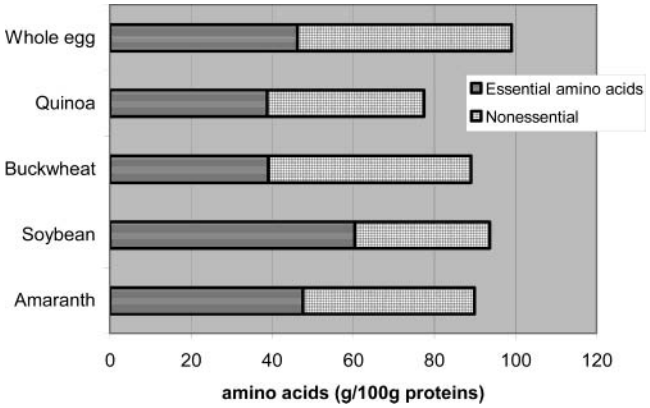

Figure 7. Essential and nonessential amino acids of whole egg, quinoa, buckwheat, soybean, and amaranth.

similar to amarantin except for the presence of the 54 kDa subunit and its tendency to polymerize. The major peptides had molecular masses of 78, 72, 39, 30, and 20 kDa, similar to the 7S type globulin (*19*, *20*, *32*, *41*).

Our results are in accordance with others relating to the percentages of the nutritionally important protein fractions (i.e., albumins + globulins + insoluble remnant) to the nutritionally least important prolamin fraction. The nutritional value of the studied species can be lined up in the following way: *A. paniculatus*, *A. caudatus*, *A. cruentus*, *A. hypochondriacus* (*6*).

Amino Acids of Total Proteins. Amaranth proteins show a significantly higher ($p < 0.05$) concentration of GLU, GLY, and MET than soybean, while TYR + CYS and the essential amino acids such as ILE, LEU, and PHE are significantly higher ($p < 0.05$) in soybean than in amaranth (**Figure 7**). The sum of essential amino acids was 47.59 and 60.29 g/100 g of protein in amaranth and soybean, respectively. The results of some amino acids in soybean (ASP, GLU, ARG, PRO, and LYS) were lower than those in the literature (*42*). It can be explained by the biological variation of low-fat soybean as well as by fluorescence measurements used for amino acid determinations (*30*). Consequently, the essential amino acid concentration was higher in soybean than in amaranth. Amaranth and soybean have comparable or higher amounts of essential amino acids as whole egg protein. Quinoa (38.710 g/100 g) and buckwheat (39.011 g/100 g) proteins have a similar concentration of essential amino acids, but 16% less than whole egg protein (**Figure 7**). On the other hand, soybean and the pseudocereal proteins of amaranth have a higher concentration of essential amino acids in relation to the total amino acid concentration, compared to whole egg protein. Amaranth, soybean, quinoa, and buckwheat can cover preschool child and adult requirements of ILE, LEU, PHE, VAL, and HIS, while MET and THR requirements are met from amaranth and soybean, respectively. Requirements of LYS for adults (not for preschool children) can be covered with amaranth and soybean proteins. The protein composition in these plants is relatively high and digestible (*42*). Our results are in accordance with others (*2*−*6*, *10*, *41*−*44*). The lysine level in quinoa protein (5.6%) was comparable to that of soybean and was typical of dicotyledonous seed protein in being deficient in methionine. On the basis of animal studies (protein efficiency ratio, protein digestibility, and nitrogen balance), the quality of protein in quinoa matched that of the milk protein casein (*2*, *45*). Protein fraction of quinoa, nearest to the FAO-recommended pattern in essential amino acids, was identified (*46*). According to the amino acid composition (**Figure 7**) amaranth, buckwheat, and quinoa differ from soybean.

Table 1. Secondary Structure Composition (%)

plants	α-helix	β-sheet	aperiodic structure
		Amaranth	
Glo	31	27	42
Alb-1	4	37	55
Alb-2	16	41	53
Soybean			
Glo	33	24	43
Alb-1	6	42	52
Alb-2	19	28	53
		Buckwheat	
Glo	25	30	45
Alb-1	2	46	52
Alb-2	14	29	57
		Quinoa	
Glo	20	35	45
Alb-1	4	50	46
Alb-2	10	37	53

CD Spectra. Results of secondary structure composition are shown in **Table 1**.

Our results are in correspondence with others, where it was shown that conglutin δ_1 and δ_2 of lupins are rich in α-helix, consisting of about 38% and in amaranth of 31.4% (*47*). Basic subunit (26 kDa) of 280 kDa buckwheat grain legumin was characterized by 22% α-helix, 36% β-sheet, 12% β-turn, and 30% random coil secondary structure (*48*). Probably crude globulins, which are a mixture of 7S and 11S globulins, represent α-helix and β-sheet as a total amount of both oligomers. Soybean globulins, which have mostly β-structure showed slightly higher amount of α-helix than amaranth. It can also be explained by the purity of soybean sample, which contained its 7S and 11S oligomers.

Comparison of electrophoretic patterns of total and soluble fractions, and amino acid spectra revealed that some heterogeneity existed with respect to the overall molecular weight of the plants tested, as did differences in their constituent (individual) subunits.

Comparison of relative structural stability of native albumins and globulins by used measurements showed that globulins are relatively more stable in comparison with Alb-1 and Alb-2. Our results are not in full correspondence with others, who have shown that the amount of Alb-2 comprised about 30% of the total albumin and globulin content. Little or no measurable protein corresponding to the amaranth Alb-2 was extracted from seeds of buckwheat and quinoa (*21*, *41*).

Our results are also in correspondence with others (*38*) that circular dichroic studies of total proteins shared similar secondary structural conformations characterized by low alpha-helical and high beta-sheet contents. Alb-2, a specific protein, was found in amaranth seeds, soybean, buckwheat, and quinoa and probably associated with protein bodies (*21*). In summary, Glo and Alb-2 have similar properties in investigated samples, suggesting that Alb-2 is also a storage protein like Glo. The relatively high content of essential amino acids shows that pseudocereals could be used as a nutrient substitute for cereals (*4*, *32*, *34*, *48*−*53*).

SDS-PAGE and analysis of similarity were performed. It is routinely used to solve or verify taxonomic data based usually on the morphological observations. Molecular markers usually are used for these purposes, but we have also proposed protein markers. This research has shown the following:

1. Phylogenetic distinction of soybean from other examined genus (quinoa, buckwheat, and amaranth) is confirmed.

2. Tachtadzjan hypothesis that buckwheat (Polygonales) and Caryophyllales (quinoa, amaranth) are closely related is not confirmed.

3. Quinoa and amaranth (according to Tachtadzjan, both genus belong to Caryophyllales) are also not related.

4. Verification of taxonomy relations based on the protein markers data is possible.

5. The studied plants can substitute each other and cereals based on amino acid composition and their electrophoretic similarity.

There are interspecies differences of electrophoretic patterns of seed total proteins extracted by tris-buffer pH 6.8. The differences are bigger between soybean, quinoa, and buckwheat than between amaranth species. On the basis of protein pattern, these species were clustered. Some phylogenetic relations between *Chenopodiaceae* and *Amaranthaceae* were found. Electrophoretic analysis of seed proteins proved to be useful for distinguishing species and cultivars of the plants, for describing their similarity. Similarities were found in soluble fractions of these plants, showing that Alb-2 is an additional storage protein.

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