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ORIGINAL PAPER

Shela Gorinstein · Jerzy Drzewiecki · Efren Delgado-Licon · Elke Pawelzik · Alma Leticia Martinez Ayala · Oscar J. Medina · Ratiporn Haruenkit · Simon Trakhtenberg

Relationship between dicotyledone-amaranth, quinoa, fagopyrum, soybean and monocots- sorghum and rice based on protein analyses and their use as substitution of each other

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Author, S. Gorinstein is affiliated with the David R. Bloom Center for Pharmacy.

S. Gorinstein (✉)
Department of Medicinal Chemistry and Natural Products,
School of Pharmacy,
The Hebrew University-Hadassah Medical School,
P. O. B. 12065, Jerusalem, 91120, Israel
e-mail: gorin@cc.huji.ac.il
Tel.: +972-2-6758690
Fax: +972-2-6757076

J. Drzewiecki
Plant Breeding and Acclimatization Institute,
Radzikow, Poland

E. Delgado-Licon
Department of Chemistry and Biochemistry,
Technological Institute of Durango,
Durango, Mexico

E. Pawelzik
Institute of Agricultural Chemistry,
Georg-August-University,
Goettingen, Germany

A. L. M. Ayala
Departamento de Biotecnologia,
Centro de Desarrollo de Productos Bioticos,
Instituto Politecnico Nacional,
Morelos, Mexico

O. J. Medina
Food Chemistry Department,
UPTC,
Tunja, Colombia

R. Haruenkit
Department of Agricultural Industry,
Faculty of Agricultural Technology,
King Mondkut Institute of Technology,
Ladkrabang, Bangkok, Thailand

S. Trakhtenberg
Kaplan Medical Center,
Rehovot, Israel

Abstract Comparative protein studies of cereals and pseudocereals are important, especially in cases of cereal protein allergy when pseudocereal substitution is unavoidable. Therefore, ten species and cultivars belonging to different *Angiosperms* families (*Oryza sativa* normal Poaceae, bran Poaceae and Jasmin Hom Dokmali Poaceae, *Sorghum technicum* Battand et Traubt Poaceae, *Glycine max L.* Merr. Fabaceae, *Fagopyrum esculentum* Mnch. Polygonaceae, *Chenopodium quinoa* Wild *Chenopodiaceae, Amaranthus hybridum v.1004* Amaranthaceae*, cruentus v*. R104 *Amaranthaceae and hypochondriacus v*.1023 Amaranthaceae) were examined by sodium dodecyl sulphate (SDS-PAGE) seed protein markers, fluorescence, circular dichroism (CD) spectra and Fourier transform infrared (FT-IR) measurements. A high degree of polymorphism of all species and cultivars was found. Amaranth species have very similar seed protein electrophoretic profiles. According to UPGMA algorithm the examined species and varieties could be clustered into two similarity groups. Soybean, quinoa, buckwheat and Amaranth (as a genus) can be considered as phylogenic distant taxa. The fluorescence properties of amaranth, soybean, quinoa, rice and buckwheat soluble protein fractions were measured by fluorescence of tryptophan at 295 nm, light intensity, peak response and shift in the maximum of emission. Relative structural stabilities of native proteins were estimated by CD and FTIR. Similarities were found between these plants, which could make them a substitution of each other as well as for cereals.

Keywords Cereal and pseudocereals · Proteins · Diversity · Similarity

Introduction

Cereals and to a less degree pseudocereals meet the major dietary requirements in proteins of the world population

Table 1 Species and cultivars of investigated plant samples

Sample number	Species and cultivar, family	The plant material of extraction	Origin
1	Oryza sativa *normal Poaceae*	meal	Thailand
2	Oryza sativa bran *Poaceae*	meal, brown in colour	Thailand
3	Oryza sativa Jasmin Hom Dokmali *Poaceae*	meal	Thailand
4	Sorghum technicum *Battand et Traubt Poaceae*	spikelet	Poland
5	*Glycine max* L. Merr. *Fabaceae*	individual seed	Brazil
6	*Fagopyrum esculentum* Mnch. *Polygonaceae*	One seeded nutlet	Peru
7	*Chenopodium quinoa* Wild *Chenopodiaceae*	individual seed	Peru
8	*Amaranthus hybridum v.* 1004 *Amaranthaceae*	individual seed	Pakistan
9	*Amaranthus cruentus v.* R104 *Amaranthaceae*	individual seed	USA
10	*Amaranthus hypochondriacus v.* 1023 *Amaranthaceae*	individual seed	Mexico

[1–3]. However, more than 2% of the adults of the developed countries suffer from IgE-mediated hypersensitivity reactions after ingestion of foods, including cereal products [4–7]. Therefore, an increased interest in allergy-free pseudocereals and first of all of amaranth and quinoa as substitute is understandable [8–10]. The above-mentioned was the basis of our decision to investigate ten species and cultivars belonging to different *Angiosperms* families (Oryza sativa normal Poaceae, bran Poaceae and Jasmin Hom Dokmali Poaceae, Sorghum technicum Battand et Traubt Poaceae, Glycine max L. Merr. Fabaceae, Fagopyrum esculentum Mnch. Polygonaceae, Chenopodium quinoa Wild Chenopodiaceae*, Amaranthus hybridum v*.1004 *Amaranthaceae, cruentus v*. R104 *Amaranthaceae and hypochondriacus v*.1023 *Amaranthaceae*). The aim of our investigation was to study the diversabilities and similarities of the proteins of the above-mentioned samples in order to find proper substitutes for each other.

Rice was used as a model in order to establish the indel polymorphism [11–13]. It was shown that alcohol soluble proteins could be used to distinguish rice subspecies and cultivars. The differences were more obvious in varieties of different genetic background than in closely related varieties. A comparison of PAG electrophoresis and isoelectrofocusing of alcohol soluble proteins showed isoelectrophoresis to be the most discriminatory. SDS-PAGE, as the most common method of electrophoresis in practice suitable for distinguishing closely related rice varieties as well as the other investigated plants, was used.

Application of fluorescence, Fourier transform infrared (FT-IR) and circular dichroism spectroscopy (CD) are necessary to compare the properties of soluble protein fractions. Therefore, also these methods as an addition to SDS-PAGE were used.

Material and methods

Chemicals

Sodium dodecyl sulphate (SDS), one set of molecular weight markers (kDa), Tris buffer and Coomassie Brillant Blue R were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

Samples

Species and cultivars used are listed in Table 1.

The extraction of proteins was performed as previously described [14]. 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. Total proteins from defatted meals of *Oryza sativa* var. normal 45 mg, *Oryza sativa* var. bran 55 mg, *Oryza sativa* var. Jasmin 45 mg; *Sorghum technicum*, 40 spikelets and 65 mg; *Glycine max* single seeds and 40 mg; *Fagopyrum esculentum* 40 seeds and 60 mg; *Chenopodium quinoa* 20 seeds and 40 mg, and for *Amaranthus* 160 seeds and 40 mg, respectively, were extracted with 1 ml of 1 M Tris buffer pH 6.8, containing SDS, glycerol, mercaptoethanol (2-ME) and bromophenol blue. The extracts were allowed to stand overnight at room temperature. Samples were boiled for 5 min, and then centrifuged 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 6, 000) for 3 days against deionized water at 4°C. The content of dialysis tubes was centrifuged at 9,000 *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 9,000×*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 [15].

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis was performed with the Hoeffer SE 600 vertical unit (Hoeffer Scientific Instruments San Francisco, CA, USA) according to Laemmli [16], and to ISTA Standard Reference Method for Verification of Varieties of Pisum and Lolium by PAGE using (12.5% polyacrylamide, resolving gel; 5% polyacrylamide, stacking gel, 180×160×1.5 mm in size) gels. 2-ME was used in SDS-PAGE. The run was carried out at 25 mA per gel and then 45 mA per gel until the end of electrophoresis. The gels were stained with 0.25% Coomassie Brillant Blue R in methanol/water/acetic acid (5:5:1 v/v) and destained in water. The following molecular weight markers (kDa) were used: 205- myosin, 116- β-galactosidase, 97-β-phosphorylase, 66-bovine albumin, 45-ovalbumin, 29-carbonic anhydrase, 20-trypsin inhibitor.

The protein standard IV of 30.0; 42.7; 66.2 and 78.0 (Merck, Darmstadt, Germany) was injected in each gel in the separation of extracted protein fractions.

Analysis of similarity between species and varieties

The banding patterns of each species and variety profiles from the electrophoregram were scored as bands for presence or absence of the band. The computer software BIO-GENE (Vilber Lourmat, France ver. 99) was used to calculate the genetic similarity coefficients and for generating the similarity matrix for all possible pairs of species and varieties [17]. Similarity coefficient (also called Dice = $2nxy/nx$ + ny, where nx and ny are the number of bands in lane x and in lane y, respectively, and nxy is the number of shared bands between the two lanes) was determined. The dendrogram was calculated from the similarity values in the matrix and using the UPGMA algorithm (Unweighted Pair-Group Method) [18].

Fluorescence spectra

Fluorescence measurements were done using a model FP-770 Jasco spectrofluorometer. Fluorescence emission spectra measurements for all native Glo, Alb-1 and Alb-2 samples were taken at excitation wavelengths (nm) of 274 and 295 and recorded over the frequency range from the excitation wavelength to a wavelength of 500 nm [19–21].

Fourier transform infrared (FT-IR) measurements

Perkin Elmer 2000 FTIR spectrometer was used to record IR spectra. The samples for measurements were prepared from granulated protein, and the pellets were pressed applying 10000 kg/cm^2 for 15 s [22, 23].

Circular Dichroism (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 [24].

Results

It was confirmed that total seed proteins of all examined dicotyledon species are heterogenous. Two species belonging to monocotyledones, grasses- sorghum and rice are less heterogenous. Using SDS-PAGE electrophoresis, 20 to 22 bands of rice (Fig. 1, lanes 1–3), 29 of sorghum (Fig. 1, lane 4), 36 bands of soybean seeds (Figs. 1 and 5), 35 of buckwheat (Fig. 1, lane 6), 41 of quinoa (Fig. 1, lane 7) and from 28 to 39 bands of Amaranth species (Fig. 1, lanes 8–10), respectively, were detected.

The banding patterns of soybean, buckwheat, quinoa and amaranths are very distinct. The group of total proteins found in the 29 kDa–97 kDa range is resolved into distinguishable patterns that allow species to be differentiated from one another (Fig. 1, lanes 4–10).

It indicates a high level of polymorphism of protein patterns between these species. Similarity coefficients calculated from electrophoretic data were from 0.05 to 0.22 (Fig. 2). Resolution in the low molecular weight region (less than 20 kDa) has been poor in all examined

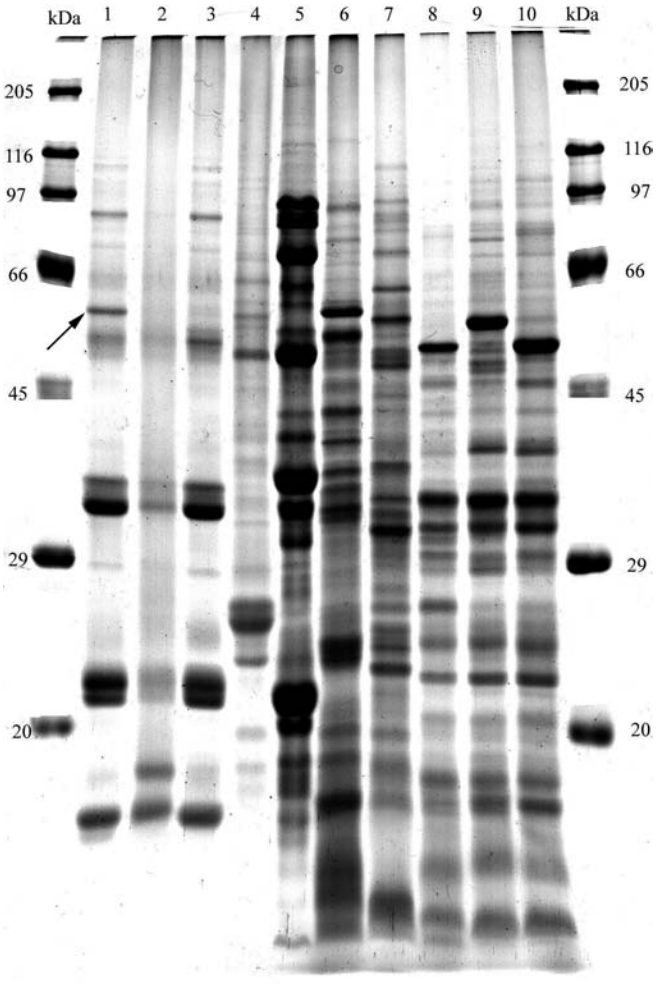


Fig. 1 SDS-PAGE electrophoregram of seed proteins extracted with tris-buffer, pH 6.8. Line 1- rice normal, line 2- rice bran, 3-rice Jasmin, 4-sorghum technicum, 5- soybean, 6- *Fagopyrum esculentum,* 7- quinoa, 8- *A. hybridum v*.1004; 9- *A. cruentus* v. R104, 10- *A. hypochondriacus* v.1023. Molecular weight markers (kDa) were used: 205- myosin, 116- β-galactosidase, 97-β-phosphorylase, 66-bovine albumin, 45-ovalbumin, 29-carbonic anhydrase, 20-trypsin inhibitor

species. Our data are in accordance with opinion of Ng and Bushuk, 1987, [25] that the SDS-PAGE banding patterns in this region are diffused and not clearly discernable for proteins generally.

Coefficient of similarity based on seed total protein pattern for dicots and monocots (rice + sorghum) is low and is less than 0.30 (Fig. 2).

The average genetic diversity between sorghum varieties was also comparable for the three subsets (0.81, 0.77 and 0.80 for the subsets of polymerase chain reactions, L, and T, respectively) [26]. The similarity between cultivated tartary buckwheat accessions ranged from 0.61 to 1.00. Four distinct clusters were formed which corresponded well with the geographic distribution of the tartary buckwheat [27].

It would be additional evidence of significant phylogenetic distance between dicots and monocots plants. On

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
L1	1.00									
L2	0.69	1.00								
L3	0.93	0.75	1.00							
L4	0.18	0.07	0.19	1.00						
L5	0.11	0.06	0.11	0.19	1.00					
L6	0.06	0.07	0.07	0.29	0.10	1.00				
L7	0.06	0.07	0.06	0.11	0.19	0.06	1.00			
L8	0.16	0.12	0.17	0.15	0.09	0.05	0.20	1.00		
L9	0.19	0.16	0.20	0.22	0.12	0.05	0.22	0.57	1.00	
L10	0.20	0.17	0.21	0.18	0.08	0.05	0.18	0.64	0.77	1.00

Fig. 2 UPGMA dendrogram of rice, sorghum, soybean, buckwheat, quinoa and 3 samples of Amaranth based on protein data using Nei's genetic similarity coefficient matrix. 1- 3- rice, 4- sorghum, 5- soybean, 6- buckwheat, 7- quinoa, 8- *A. hybridum v*.1004, 9- *A..cruentus* v. R104, 10-*A. hypochondriacus* v.1023

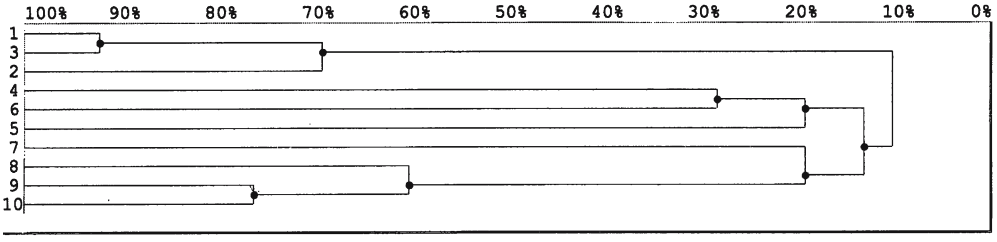


Fig. 3 The similarity matrix of rice, sorghum, soybean, buckwheat, quinoa and 3 samples of amaranth based on protein data using Nei's genetic similarity coefficient. L1- rice "normal", L2- rice bran, L3- rice Jasmin, L4- sorghum, L5- soybean, L6- buckwheat, L7- quinoa, L8- A. hybridum v.1004, L9- *A..cruentus* v. R104, L10-*A. hypochondriacus* v.1023

the other hand similarity between examined dicots species is relatively low, too. It might be evidence of significant phylogenetic distance between examined dicots families, too. Our results are only partially in accordance with Tachtadzjan classification of Magnoliophytov [28] that monocots are separate branch of phylogenetic tree. Some families of dicots (*Polygonaceae*, *Chenopodiaceae* and *Amaranthaceae*) were clustered together in one branch.

The protein pattern of rice differs significantly from other examined species, also from the second member of *Poaceae*-sorghum. The bands in area of 20 to 45 kDa were thick and strongly stained. The protein bands of high molecular weight region (more than 66 kDa) were thin and lightly-coloured (Fig. 1, lanes 1–3). Variety ''normal" differs from Bran and Jasmin varieties by occurrence of 60 kDa (Fig. 1, lane 1, see arrow), variety bran differs by absence of bands 90 and 110 kDa (Fig. 1, lane 2).

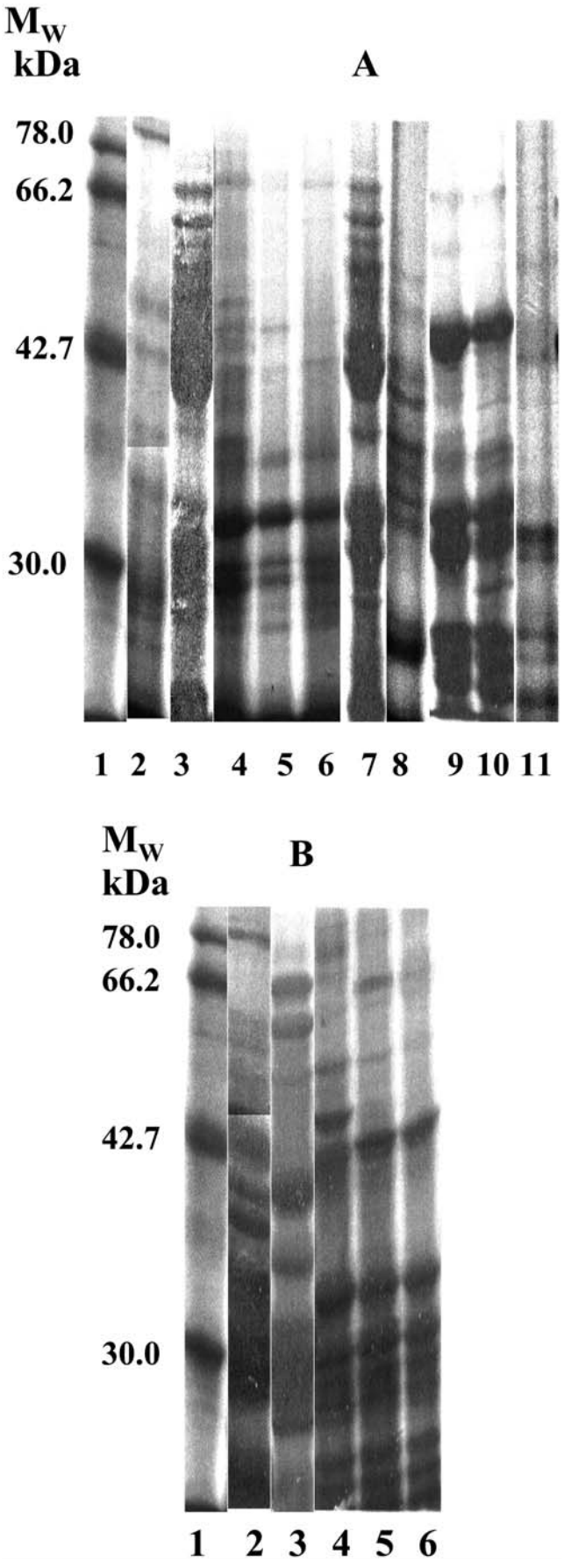
Rice glutelins account for 80% of the total proteins. In sorghum glutelins account for 32% and prolamins for 52%. Glutelins (aggregated prolamins + protoplasmic proteins) are more balanced for essential amino acids. In total proteins of rice the prolamins account for only 5% that is why lysine in protein constitutes from 3 to 7%. It is known that alcohol soluble prolamins predominate in cereals. Globulins predominant in legumes and other dicotyledones [29]. We suppose that specific character of rice protein pattern is determined by higher glutenin content.

On the basis of total protein pattern three varieties of rice were grouped together in one distinct cluster (Fig. 3). Examined varieties possess very similar, but distinguishable protein pattern. As a rule it indicates their common origin or their belonging to the same group of breeding.

The results of the investigation allow us to recommend SDS-PAGE of total proteins for rice varieties verification. However there is much further research which is requested. Firstly, the use of total protein electrophoregrams for accurate verification and identification of rice varieties requires a systematic nomenclature bands. Secondly, testing of wider range of genetic diversity of rice varieties should be performed.

Total protein pattern of sorghum was different in structure and band composition from rice (Fig. 1, lane 4). This pattern is less distinct in comparison with those of soybean, buckwheat, quinoa and amaranths.

As SDS - PAGE showed (Fig. 4a, lane 3), soybean had Alb-1 bands with higher molecular weights than buckwheat and amaranth. Amaranth showed a major Alb-1 band at 34 kDa (Fig. 4a, lanes 4–6) and minor bands were observed with a molecular weight under 30 kDa (Fig. 4). Major and minor bands were observed in buckwheat (Fig. 4a, lane 2). Amaranth had a main Alb-2 band with a molecular weight higher than 42.7 kDa (Fig. 4a, lanes 9–11). More protein bands were observed for buckwheat and amaranth in Alb– 2 than in Alb -1 (Fig. 4a, lanes 7 and 9–11). 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 [3].

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 [3, 19–23].

Major globulin bands were observed for buckwheat (Fig. 4b, lane 2). Soybean (Fig. 4b, lane 3) showed a major globulin band at 30 kDa and a low molecular weight band confirming findings of Arntfield et al. [19], Gorinstein et al. [21] and Drzewiecki et al. [14]. Major differences were observed between albumins (Alb-1 and Alb-2) and globulins of buckwheat (Fig. 4a, lanes 2 and 7). Whilst A. *hybridum* 1004 and hypochondriacus v.1023 had main bands of Alb – 2 (Fig. 4b, lanes 5–6), most bands were observed in globulins for A. *cruentus* R104 (Fig. 4).

A. *cruentus* R104 also had a globulin band at about 50 kDa which was not present in amaranth 1004 and 1023 (Fig. 4b, lane 4). These results have shown that the Alb-2 fraction is similar to globulin one. In other reports it was shown that albumin 2 was very similar to amarantin except for the presence of the 54 kDa subunit and its tendency to polymerize. Their major peptides had molecular masses of 78, 72, 39, 30, and 20 kDa similar to the 7 S type globulin.

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 prolamins fraction, the nutritional value of the studied species can be line up in the following way: *A. paniculatus*, *A. caudatus, A. cruentus, A. hypochondriacus* [21].

At the excitation wavelength of 295 nm (Fig. 5I, A) all spectra were typical for tryptophan content, but the peak of amaranth Alb-2 (Fig. 5I, Aa) was displaced to a longer wavelength (342 nm) and demonstrated more polar environment of the tryptophan residues (Fig. 5I, Aa and Ad). The fluorescence intensity of amaranth is much higher than for quinoa (Fig. 5I, Ad and Ad) and nearly the same for soybean and buckwheat.

The fluorescence intensity at 274 nm of amaranth, buckwheat, soybean and quinoa was 0.92, 0.86, 0.67 and 0.37, respectively (Fig. 5I, Ba-Bd). Oppositely, the fluo-

Fig. 4 SDS-PAGE electrophoregram of albumin-1 (Alb-1), albumin-2 (Alb-2) and globulin (Glo) protein soluble fractions from pseudocereal plants. **A** 1- protein standard IV of 30.0; 42.7; 66.2 and 78.0 kDa; (2-6- Alb-1): 2- buckwheat; 3- soybean; 4- *Amaranthus (A.) cruentus* v. R104; 5-*A. hybridum* v.1004; 6- *A. hypochondriacus* v.1023; (7-11- Alb-2): 7- buckwheat; 8- soybean; 9- *Amaranthus (A.) cruentus* v. R104; 10- *A. hybridum* v.1004; 11- *A. hypochondriacus* v.1023. **B** 1- protein standard IV of 30.0; 42.7; 66.2 and 78.0 kDa; (2-6- Glo): 2- buckwheat; 3- soybean; 4- *Amaranthus (A.) cruentus* v. R104; 5-*A. hybridum* v.1004; 6- *A. hypochondriacus* v.1023

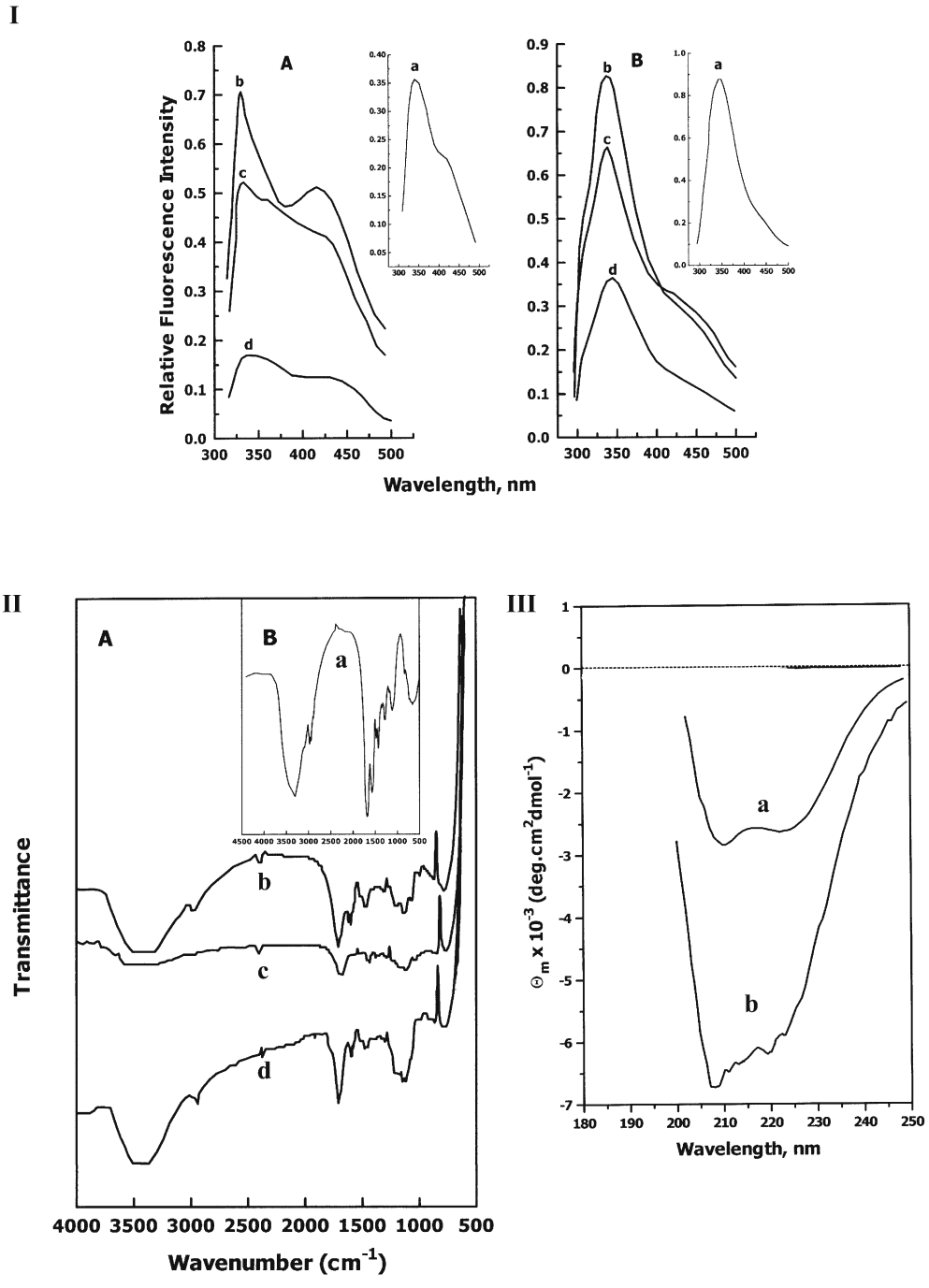


Fig. 5 Intrinsic fluorometry (IF), Fourier transform infrared (FT-IR) and circular dichroism (CD) measurements of plant proteins. **I**: Fluorescence emission spectra of proteins from a- *A. hypochondriacus* v.1023; b- buckwheat; c- soybean; d- quinoa. Excitations (nm) at 295 and 274 on A and B, respectively. **II:** FTIR spectra of a- *A. hypochondriacus* v.1023; b- soybean; c-quinoa, d-buckwheat. **III:** Circular dichroism spectra of proteins. Far-UV from a- *A. hypochondriacus* v.1023; b-buckwheat

rescence intensity at 295 nm was different in comparison with the previous one, showing for amaranth, buckwheat, soybean and quinoa the values of 0.38, 0.73, 0.52 and 0.24, respectively, with the highest one for buckwheat. Amaranth proteins may show remarkably high contents of phenylalanine plus tyrosine (Fig. 5I, Ba), but buckwheat proteins appear to contain high levels of tryptophan (Fig. 5I, Ab). Fluorescence spectra of Glo, Alb-1 and Alb-2 demonstrated peaks (nm) in the range of 338, 346 and 351, respectively. It means that tryptophan residues are situated closer to the surface of the molecule in the case of albumins (Alb-1 and Alb-2) and consistent with the less compact and more hydrophobic structure in comparison with Glo. At λ excitation 274 nm, very slight shoulders were seen only in amaranth Glo (λ emission = 308 nm; I= 0.19) and in Alb-2 (λ emission =308.5 nm; I= 0.37), which was evidence of tyrosine. At λ excitation 295 nm, tyrosine was not shown [20, 21].

The changes that arise from the nature of proteins from cereals and other plants were observed (Fig. 5II, a–d) by the changes in the amide I, II and III bands. We assigned that the broad band in the 1300–1250 cm^{-1} region belongs to α-helix, the relatively sharp band in 1240–1230 cm^{-1} region to β-sheet and a broad, medium intensity band at

1270–1240 cm^{-1} regions to a disordered structure. All plant proteins showed similar bands at 3300 cm^{-1} (amino acid peak) and at 2900 cm^{-1} (-CH2-stretching). Amide I, II and III bands were in the range of 1630–1660 cm^{-1}, 1510–1540 cm^{-1}, and 1310–1235 cm^{-1}). Displacement of bands position and absence of amide III bands can be seen in investigated samples. The intensities of amide I and II bands were nearly similar in quinoa, indicating that the α-helix content of this protein is lower in comparison with amaranth, buckwheat and soybean where the intensities of amides I and II bands were as much as twice higher. In spectra of these proteins is not shown the band at 1515 cm^{-1}, which is associated with β-sheet or random structure. The absence of amide III band in amaranth, soybean, quinoa and buckwheat showed that these proteins are mostly composed of α-helix as the main ordered structure. Our results are in agreement with others, showing in buckwheat bands at 1660 cm^{-1}, 1530 cm^{-1} and 2950 cm^{-1} [22].

Proteins from amaranth and buckwheat (Fig. 3III, A and B) showed a CD spectra in the far ultraviolet (FUV) region. Results of secondary structure composition: amaranth: α-helix,% in native protein: Glo =31; Alb-1 =4 and Alb-2 =16; Soybean: α-helix,% in native protein: Glo =33; Alb-1 =6 and Alb-2 =19; Buckwheat: α-helix,% in native protein: Glo =25; Alb-1 =2 and Alb-2 =14; Quinoa: α-helix,% in native protein: Glo =20; Alb-1 =4 and Alb-2 =10; Rice: α-helix,% in native protein: Glo =22; Alb-1 =6 and Alb-2 =2. Soybean globulins, which have mostly β-structure, showed slightly higher amount of α-helix than amaranth. It can be explained also by the purity of soybean sample, which contained its 7 S and 11 S oligomers.

Discussion

Comparison of electrophoretic patterns of total and soluble fractions, and fluorescent 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 intrinsic fluorescence and circular dichroism measurements showed that globulins are relatively more stable in comparison with Alb-1 and Alb-2. The relatively high content of essential amino acids shows that pseudocereals could be used as a nutrient substitute for cereals [21].

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.

Our results are also in correspondence with others [22] 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, rice and quinoa and probably associated with protein bodies. In summary, Glo and Alb-2 have similar properties in investigated samples, suggesting that Alb-2 is also a storage protein like Glo.

Our results confirm morphological and molecular data [30] that rice and sorghum belong to clearly different subfamilies of *Poaceae- Oryzeae* and *Andropogoneae*, respectively. Konarev et al. [29] established that the composition of components in alcohol soluble prolamin fraction is associated with belonging to different subfamilies of *Poaceae*. The members of subfamilies and tribes related to wheats have "wheat type" of prolamin banding pattern.

Analysis of the character of protein patterns of sorghum and buckwheat represents an interesting problem. Buckwheat is called "pseudocereal" and belongs to family *Polygonaceae*. Both species were combined in one cluster (Fig. 3), though their coefficient of similarity is relatively low- 0.29. Rice and sorghum are a little less related (similarity coefficient of rice and sorghum varies form 0.13 to 0.18). These data can be explained rather by high level of genetic diversity of *Poaceae* than closer relationship buckwheat/sorghum. The diversity of Amaranths was similar to those shown by other researchers. Out of 86 amplified fragments, 66 fragments showed polymorphism among seven genotypes of amaranth (*Amaranthus cruentus L.*), which represents 76.7% polymorphism). The genetic similarities ranged from 52.0% to 93.5% [31].

Our results are applicable to other research work, showing the importance of angiosperms to sustaining humanity by providing a wide range of 'ecosystem services' warrants increased exploration of their genomic diversity. The nearly completed sequences for two species representing the major angiosperm subclasses, specifically the dicot Arabidopsis thaliana and the monocot Oryza sativa, provide a foundation for comparative analysis across the angiosperms. The angiosperms also exemplify some challenges to be faced as genomics makes new inroads into describing biotic diversity, in particular polyploidy (genome-wide chromatin duplication), and much larger genome sizes than have been studied to date [32].

Polymorphism was detected in rice by different analytical methods [13]. The first report on the application of DNA polymorphism analysis [33] to reveal genomic relationship among cultivated Italian rice germplasm was similar to our results. Our results are in correspondence with others who reported that combination and substitution of cereals by pseudocereals lead to nutritional foods and can prevent allergy. Food components may be promoters of positive metabolic mechanisms [34].

A combination of cereals with pseudocereals and soybean is evaluated as an alternative protein-rich ingredients because of its higher nutritive value [35, 36].

Results will be useful for breeding as well for nutritional programs.

Conclusions

1. SDS-PAGE of seed total proteins demonstrates the possibility of distinguishing both dicotyledon plants and members of *Poaceae* family- rice and sorghum.
2. Coefficient of similarity based on seed total protein pattern dicots and monocots (rice + sorghum) is low. It confirms that these groups of plants are phylogenetic distant.
3. The protein pattern of rice differs significantly from other examined species.
4. The genetic distance between sorghum and dicots plants is less than between rice and dicots.
5. Protein patterns of rice and sorghum have confirmed morphological data that subfamilies Oryzeae and Andropogonea are considerable different.
6. SDS-PAGE of total seed proteins might be a useful tool in rice varieties verification.
7. Differences and similarities determined by all used methods can be used in substitution of cereals and pseudocereals.

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