ELECTROPHORETICAL CHARACTERIZATION OF PROTEIN FRACTION AND THEIR QUANTIFICATION IN COMMON AND TARTARY BUCKWHEAT VARIETIES

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Abstract

Electrophoretical study of protein fractions (albumins + globulins, prolamins and sum of glutelins) in SDS-PAGE conditions and their quantification were tested in eight varieties of common buckwheat and in two tartary buckwheat accessions. The electrophoretical visualization showed as a main fraction albumins + globulins with high inter- and intra-varietal polymorphism in tested varieties. Tartary buckwheat accessions showed completely uniform electrophoretical spectra. A low appearance of prolamins was confirmed in all tested varieties (inclusive tartary buckwheat accessions). Spectrum of soluble glutelins in common buckwheat was characterized by lower frequency and intensity of protein bands. In both tartary buckwheat accessions were quite comparable with fraction of albumins + globulins. The overlapping zones were presented in all varieties especially between 20 – 50 kDa. The statistically significant higher values of crude protein were obtained in common buckwheat varieties. All tested common buckwheat varieties showed almost two times higher content of albumins + globulins (5.24-7.51% in d.m. with 43.57-54.37% proportion in crude protein) than accessions of tartary buckwheat (3.36-3.57% in d.m. with 27.84-28.21% proportion in crude protein). Prolamins content was very low in all common and tartary buckwheat. Both tartary buckwheat accessions showed about 20% higher content of sum of glutelins in crude protein (68.38% and 69.58%) than tested common buckwheat varieties (44.15 – 53.66%).

Key words: : Common buckwheat, *Fagopyrum esculentum* Moench, Tartary buckwheat, *Fagopyrum tataricum* Gaertn., Protein fractions, SDS-PAGE,

Abbreviations: M – weight molecular marker, N - ´Špačinská 1´, P - ´Pyra´, KD - ´Kara-Dag´, J - ´Jana´, CZ - Czech Republic, SK - Slovakia, UKR - Ukraine, SDS-PAGE - Sodium dodecyl sulfate polyacrylamide gel electrophoresis Metod, d.m. – dry matter

INTRODUCTION

Buckwheat is an important crop in some regions of the world especially in China, Korea, Russia, Ukraine and Slovenia (Kreft et al., 2003; Ikeda, 2002). Buckwheat belongs to the *Polygonaceae* family (Campbell, 1997) and is taxonomically distant from the *Graminae* family, which cereals such as rice, wheat, and maize belong to (Petr, 1995; Ikeda, 2002; Mazza and Oomah, 2003). Only two species of buckwheat are broadly cultivated around the world: common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* Gaertn.) (Ikeda et al., 1995; Bonafaccia et al., 2003; Javornik et al., 1981).

Seed of buckwheat has high nutritive values with great potential for food and medicine as well (Zeller, 2001). Buckwheat flour contains relatively high content of protein, dietary fiber and is rich in minerals and vitamins (Ikeda, 2002; Pomeranz and Robins, 1972; Joshi and Rana, 1995). Protein content in buckwheat varies from 11-15%, depending on variety and environmental factors during its growth (Mazza, 1993; Edwardson, 1996; Michalová, 1997; Joshi and Rana, 1995). The most of protein is situated in embryo and aleurone layer of dry buckwheat seed (Pomeranz and Robins, 1972).

The high polymorphism of buckwheat storage protein provides good presumption for its utilization as genetic markers. The inter- and intra-varietal polymorphism of buckwheat storage protein was confirmed by several authors (Dontsova a Puasheva, 1979; Dolinšek 1980; Rogl and Javornik, 1996; Zeller, 2001).

Separation of proteins according to their solubility by Osborne (1907) is widely used for the characterization of seed proteins and their subsequent SDS PAGE can be suitable tool for variety identification (Černý and Šašek, 1998).

Buckwheat protein consists of approximately 18.2% of albumin, 43.3% of globulin, 0.8% of prolamin, 22.7% of glutelin, and 5.0% of nitrogen residue (Javornik and Kreft, 1984; Mazza, 1993; Edwardson, 1996). Javornik and Kreft, (1984) confirmed that fraction of albumins had the highest content of lysine in comparison with protein fractions. Especially prolamins were poor in lysine content. Higher contents of leucin and arginin were found in globulin fraction. Study of amino acid sequence of the subunit of 13S globulin of buckwheat confirmed higher presence of lysine and serine (Rout et al., 1997; Bharali and Chrungoo; 2003). The amino acid composition of the protein fractions in tartary buckwheat were described by Guo and Yao (2006) and

albumins were also characterized as a protein fraction with the best amino acids compositions.

The aims of this study were focused on: (a) possibility of electrophoretical characterization of protein fraction of individual seeds in choosen buckwheat varieties with impact on possible variety identification; (b) quantitative evaluation of protein fractions (albumins and globulins, prolamins, sum of glutelins) of bulked samples in tested buckwheat varieties.

MATERIAL AND METHODS

Plant material

The material used in this study was obtained from the Research Institute of Crop Production in Prague, Department of Gene Bank, Czech Republic. There were evaluated four common buckwheat varieties certified in the Czech Republic and four common buckwheat varieties and two accessions of tartary buckwhea.

Common buckwheat:

P - ´Pyra*´ (CZ),
N – ´Špačinská 1*´ (SK),
KD - ´Kara-Dag*´ (UKR),
J – ´Jana*´ (UKR),
E - ´Emka´ (POL),
O - ´Botansoba´ (JAP),
B – ´Bolshevik 4´ (RUS),
LH - ´La Harpe´ (FR) (* certified varieties)

Tartary buckwheat:

T - ´Z51-00012´ (CZ),
Z - ´Z51-00014´ (USA)

Electrophoresis of protein fraction

Protein fractions of several individual seeds were obtained by slightly modified Osborne method.

Extraction of albumins + globulins – grinding seed was extracted in 200 µl of 0.5 M NaCl at 4°C for 15 minutes and then centrifuged at 6500 rp/minutes at 4°C. After centrifugation the portion of 120 µl of albumins + globulins fraction was taken away into new micro centrifuge tube. The rest of seed was rinsed two-times in 500 µl in the same solution for removing residues of this fraction.

Extraction of prolamins – the residue of seed after previous extraction were extracted in 200 µl of 60% ethanol at room temperature for 4 hours. The following steps (with exception of using temperature 20°C) were identical as in the previous extraction.

Extraction of soluble glutelins – the residue of seed after previous extraction were extracted in 200 µl of 0.02 M NaOH at 4°C for 15 min. The following steps were identical as in case of albumins and globulins extraction.

The supernatants of all three fractions were lyofilized 4 hours and homogenized with 200 µl of SDS extraction buffer and analyzed in conditions of discontinuous electrophoresis (SDS-PAGE) according to Laemmli (1970). Electrophoretic phenotypes were digitalized and evaluated by means of specialized software Bioprofil 1D++ (Vilber Lourmat).

Quantification of protein fractions

Quantification of albumins + globulins and prolamins was carried out from bulked samples (at least 300 seeds for each variety) harvested in 2005 according to Dvořáček et al. (2001) and content of protein was measured by Kjeldahl method (ČSN 56 0512-12). Content of third protein fraction -"Sum of glutenins" was calculated as a difference between content of crude protein and sum of albumins + globulins and prolamins.

Analysis of variance (ANOVA) and Tukey HSD test were used for statistical evaluation (software-Statistica 7.0CZ).

RESULTS AND DISCUSSIONS

Electrophoretical evaluation of protein fractions

Comparison of three fractions (albumins + globulins, prolamins and glutelins) in 10 tested buckwheat varieties (accessions) is presented in the Figure 1. The fraction of albumins + globulins showed in both buckwheat species strong and rich protein spectrum of bands. In case of tartary buckwheat the band spectra of glutelins showed comparable intensity as albumins + globulins.

Significantly different protein spectra of albumins + globulins were found between both buckwheat species (tartary and common buckwheat). Molecular weight of albumins + globulins varied from 15.7 kDa to 95.5 kDa and about 15-19 bands were detected in varieties of common buckwheat. These bands showed significant inter- and also intra-varietal polymorphism (Fig. 2). The most polymorphic group of bands was situated in the middle part of the gel with molecular weight from 30 kDa to 55 kDa. The common buckwheat electrophoreograms of albumins + globulins seemed to be very analogous as the band spectrum of total protein of bulked samples conditions and confirmed an important ratio of this fraction in total protein (Figure 1 and 2). Eight albumin and six globulin bands were also detected between 17-61 kDa by Javornik and Kreft (1984).

An important role from the point of healthy complications plays allergenic proteins of common buckwheat with molecular weight 15, 22 and 24 kDa (Morita et al., 2006; Kondo et al., 1998). The molecular weight of some detected protein bands in albumins + globulins corresponded with these published proteins nevertheless their allergicity has to be verified.

Fraction of albumins + globulins in tartary buckwheat was detected in the closer range of molecular weights (21.8 – 55.7 kDa) and tested seeds of each accession were completely uniform, which is caused by self-fertility of this buckwheat species. Clear differences between both tartary accessions were recognized in two band positions 22.3 and 47.2 kDa illustrated in Fig. 3. These differences were not clearly confirmed in electrophoretical evaluation of total protein.

A low appearance of prolamins was confirmed in all tested varieties (inclusive tartary buckwheat accessions)

and protein bands of the fraction were problematically detected on the gel. Only several more intensive bands were found out in varieties ´Jana´ and ´Kara-Dag´ between molecular weight 20.7 kDa – 87.2 kDa. The specific two prolamin bands with molecular weights 53.8 and 57.3 kDa were identified in these two varieties. They did not show any inter- or intra-varietal polymorphism. Polymorphism of prolamin spectra was only presented in both varieties by minor bands with molecular weight from 30 to 50 kDa (Figure 2). Other common buckwheat varieties showed much lower number of bands or no bands in these positions (Figure 1). Javornik and Kreft (1984) confirmed only one protein band in prolamins with molecular weight 17 kDa. Prolamins in tartary buckwheat were also characterized by several weak bands which of gel positions were similar as some band positions of previous protein fraction (albumins + globulins).

The last tested fraction was "soluble glutelins". Spectrum of soluble glutelins in common buckwheat was characterized by lower frequency and intensity of protein bands than in albumins + globulins fraction but it showed higher frequency and intensity of protein bands in comparison with prolamins. Generally 10 more intensive bands with molecular weight from 20.7 to 87.3 kDa were detected in the common buckwheat varieties. Javornik and Kreft (1984) also characterized nine stronger bands in glutelins.

Glutelins spectra of the both tartary buckwheat accessions showed lower band frequency but they were quite comparable in band intensities with fraction of albumins + globulins. Main differences between both protein fractions were detected in upper part of gel characterized by molecular range from 55 to 80 kDa. No band positions were found here in case of glutelins.

Detailed comparison of protein fractions in all varieties indicates presence of protein bands with identical molecular weight creating overlapping zones in electrophoretical analyses of total protein especially between 20 – 50 kDa. The band with molecular weight 20.7 kDa in common buckwheat or the two bands with molecular weights 33.5 and 34.7 kDa in tartary buckwheat are well documented examples (Figures 2 and 3). The fact of nonspecific solubility among these protein fractions was described by Hamer (2003) and 80% purity of extraction it is taken for excellent.

The answer to the question, if there are identical proteins with non-specific solubility for used solvent or quite different protein molecules, is problematic at this moment. Both hypotheses must be verified by other more sensitive analytic methods including a different way of protein separations (2D electrophoresis; HPLC etc.) or eventually amino acids sequencing of isolated protein molecules.

In point of varieties identification, the using of albumins + globulins seems to be especially for tartary buckwheat promising tool. These protein bands showed sufficient polymorphism, that were not strongly influenced by extraction process as in case of prolamins and soluble glutelins extractions and wide overlapping zone created mainly glutelins were eliminated in the middle part of gel (30 – 50 kDa).

Protein fractions quantification

The crude protein content and content of particular protein fractions in bulked seed samples of several buckwheat varieties are described in table 1. The statistically significant higher values of crude protein were obtained in Russian variety ´Bolshevik 4´ (14.79%) and Japanese variety ´Botansoba´ (14.54%). In contrast to these values the crude protein content in tartary buckwheat 'Z51-00012' (11.54%) was the significantly lowest.

Many authors evaluated content of crude protein of common buckwheat in their studies (Ikeda et al., 1991; Eggum et al., 1980; Mazza, 1993; Michalová, 1998). Our values of crude protein content corresponded with the results of Bonafaccia and Kreft (1994), Nevertheless several authors published very low crude protein content in seed (less than 9%) (Hagels, 1999; Zhang et al., 1998) and it is possible to predict significant influence of environment (year, locality, agronomical treatment) on values of this parameter.

Publications reporting about crude protein contents in accession of tartary buckwheat are still limited. In comparison with Chinese authors Zhang et al. (1998), whose values of crude protein contents were about 8%, we obtained significantly higher results.

The content of albumins + globulins oscillated from 3.36% to 7.51% in dry matter of seed. There were observed significantly lower content of this fraction (3.36% and 3.57%) and also its proportion in crude protein (28.21% and 29.11%) in both tartary buckwheat accessions. Their values were comparable with the check variety of common wheat - ´Nela´.

All tested common buckwheat varieties showed almost two times higher values in content of albumins + globulins (inclusive their proportion in crude protein) than accessions of tartary buckwheat mentioned above. The varieties ´Bolshevik 4´ and ´Botansoba´ showed the highest content of this fraction in dry matter (7.66% and 7.36% respectively) and also their observed proportions of albumins + globulins in crude protein were in comparison with other varieties significantly higher. Only tetraploid variety ´Emka´ was exceptional. In spite of its lower content of albumins + globulins in dry matter (6.57%), this variety showed the significantly highest value of percentage proportion of this fraction (54.37%) (Table 1).

The detection of about 50 percentage ratio of albumins + globulins in crude protein corresponded with values obtained by Aufhammer (2000); Zhang et al. (1998); Lee (1995); Glowienke (1997) and confirmed declared high nutritive quality of common buckwheat seed (Javornik and Kreft, 1984). In case of tartary buckwheat our results were significantly different in comparison with Chinese authors Zhang et al. (1998), whose values oscillated about 46% in crude protein.

Significant differences and published contrast cases of albumins + globulins content among common buckwheat varieties (more than 60% or less than 30% in crude protein) by Bonafaccia et al. (1994); Javornik and Kreft (1984) and Wei et al. (2001) could predict certain variability of nutritive value in individual varieties. With respect to lower content of this protein fraction in both tartary buckwheat accessions, their nutritive value will be probably lower than in common buckwheat. Nevertheless this statement should be verified by other analyses.

The obtained electrophoretical results and subsequent prolamin quantification confirmed commonly known fact of low content of prolamins in buckwheat seeds and suitability of buckwheat for persons suffering from celiac disease (Skeritt, 1986).

Prolamins content was deeply below value of this protein fraction (gliadins) in the check common wheat and varied from 0.16 to 0.31% in dry matter. Higher differences were logically observed in recalculated proportions of this fraction in crude protein, which were mainly caused by significantly higher oscillation of crude protein among varieties. The highest proportion of prolamins in crude protein was detected in tartary buckwheat ´Z51-00012´ (2.52%). On the other hand, the lowest percentage proportion of prolamins in crude protein was found in the Czech variety ´Pyra´ (1.21%). Pomeranz (1983); Wei et al. (2001) also documented similar range of prolamins in common buckwheat varieties (0.7-2.0% in crude protein. The high content of prolamins (about 10.5%) in tartary buckwheat mentioned by Guo and Yao (2006) was not confirmed.

High band intensities of soluble glutelins in case of tartary buckwheat predicted significantly higher content of glutelins in this buckwheat species. This presumption was confirmed by obtained calculation of "Sum of glutelins", characterized as a sum of soluble and insoluble glutelins. In comparison with common buckwheat, both tartary buckwheat accessions showed about 20% higher content of sum of glutelins in crude protein (68.38% and 69.58%) than tested common buckwheat varieties (44.15 – 53.66%). Both buckwheat species significantly overtopped the common wheat – 'Nela' in sum of glutelins content. Lower percentage proportion of glutelins (22.73-22.15%) in crude protein in common buckwheat varieties were described by Bonafaccia et al. (1994).

Thus, sum of glutelins, representing in common buckwheat one half and in tartary buckwheat almost two-thirds of total protein and they should not be neglected from point of nutritive value of the seed total protein.

CONCLUSION

Electrophoretical visualization of three protein fractions confirmed high ratio of albumins + globulins in total protein, their high polymorphism and very low ratio of prolamins in both buckwheat species. Electrophoretical spectra of soluble glutelins showed in case of tartary buckwheat less frequency, nevertheless comparable intensity as fraction of albumins + globulins. Wide overlapping zones especially between albumins + globulins and glutelins were detected. High polymorphism and clear band detection of albumins + globulins predestinate this protein fraction as a suitable tool for variety identification.

The found differences in frequency and intensity of protein molecules in tested protein fractions by means of electrophoretical visualization were confirmed by their individual quantification. The detection of low number of prolamin molecules in the gel was confirmed by direct quantification in both buckwheat species as well. Significantly higher content of albumins + globulins in common buckwheat and also significant differences among varieties could declare their different nutritive values. Nevertheless, nutritive value will also be significantly influenced by sum of glutelins comprising 50% (common buckwheat) or even almost 70% (tartary buckwheat) of total seed protein.

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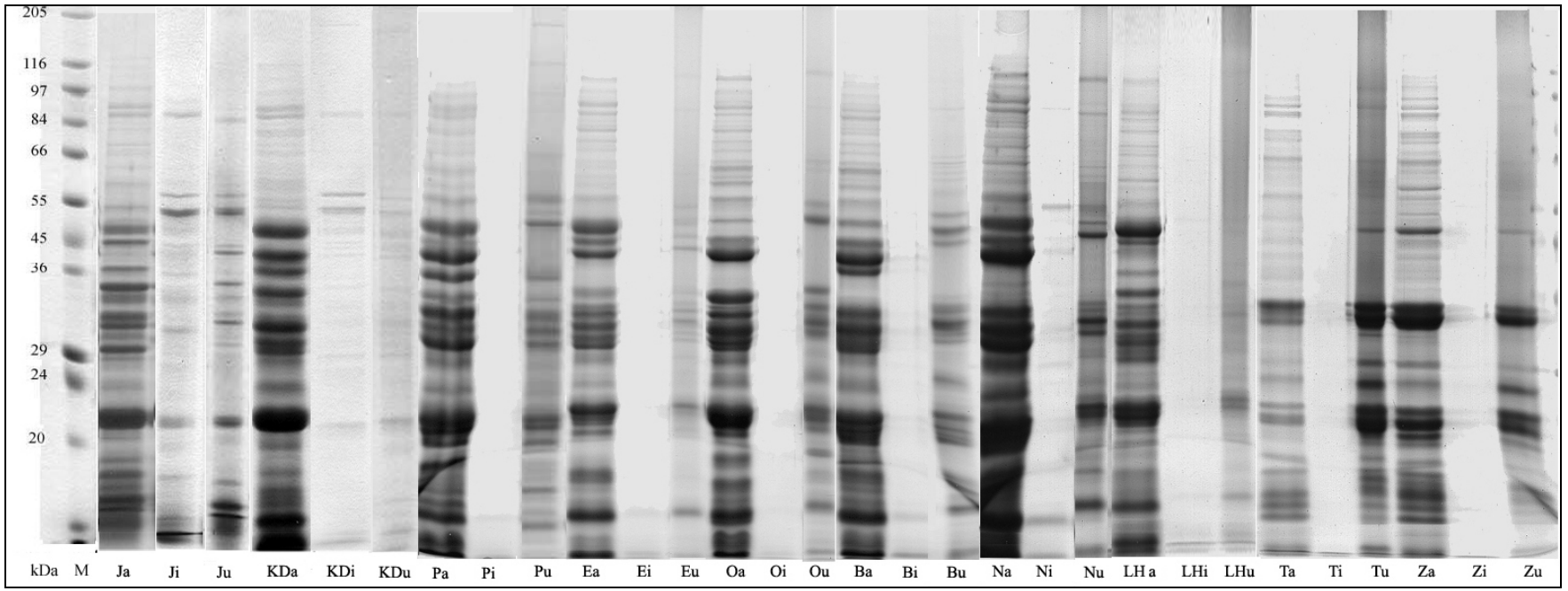
Tab. 1.: Average crude protein content and single protein fractions % in dry matter (d.m.) and their percentage proportion in crude protein of tested buckwheat varieties and tartary buckwheat accessions (ANOVA).

Variety	Crude protein average (% in d.m.)	Alb.+Glob.		Prolamins		Glutelins	
		Average (% in d.m.)	Aver. % proportion in crude protein	Average (% in d.m.)	Aver. % proportion in crude protein	Average (% in d.m.)	Aver. % proportion in crude protein
Pyra	13.23±0.09^{cd}	6.03±0.04^{b}	45.60±0.01^{ab}	**0.16±0.03^{c}**	**1.21±0.21^{c}**	7.04±0.02^{abc}	53.20±0.21^{ab}
Jana	12.03±0.18^{ab}	5.24±0.14^{b}	43.57±0.54^{a}	0.24±0.00^{abc}	2.00±0.03^{abc}	6.55±0.04^{ab}	54.43±0.51^{a}
Špačinská 1	13.77±0.04^{de}	6.20±0.20^{c}	45.04±1.33^{ab}	**0.31±0.07^{b}**	2.26±0.52^{ab}	7.26±0.09^{bc}	52.71±0.81^{ab}
Emka	12.09±0.05^{ab}	6.57±0.01^{c}	**54.37±0.34^{e}**	0.18±0.00^{ac}	1.49±0.00^{ac}	**5.34±0.06^{d}**	**44.15±0.35^{c}**
La Harpe	13.38±0.13^{cd}	6.26±0.03^{a}	46.79±0.65^{ab}	0.25±0.01^{abc}	1.87±0.13^{abc}	6.87±0.17^{ab}	51.34±0.78^{ab}
Bolshevik 4	**14.79±0.11^{e}**	**7.51±0.21^{a}**	50.79±1.82^{de}	0.29±0.04^{ab}	1.96±0.27^{abc}	6.99±0.28^{abc}	47.26±1.55^{cd}
Kara-Dag	11.82±0.32^{ab}	5.26±0.11^{a}	44.52±0.24^{a}	0.22±0.03^{abc}	1.87±0.29^{abc}	6.34±0.24^{a}	53.66±0.59^{a}
Botansoba	14.54±0.66^{e}	7.09±0.18^{ad}	48.80±0.95^{bd}	0.23±0.01^{abc}	1.58±0.03^{abc}	7.22±0.46^{abc}	49.62±0.92^{bd}
Z51-00012	**11.54±0.23^{a}**	**3.36±0.28^{de}**	29.11±1.82^{c}	0.29±0.01^{ab}	**2.52±0.18^{b}**	7.89±0.04^{c}	68.38±1.69^{e}
Z51-00014	12.66±0.33^{bc}	3.57±0.01^{e}	**28.21±0.83^{c}**	0.28±0.03^{abc}	2.22±0.28^{ab}	**8.81±0.37^{e}**	**69.58±1.11^{e}**
Control wheat variety Nela	12.00±0.10^{ab}	3.34±0.03^{b}	27.84±0.23^{c}	4.02±0.08^{d}	33.50±0.71^{d}	4.02±0.08^{f}	38.67±0.94^{f}

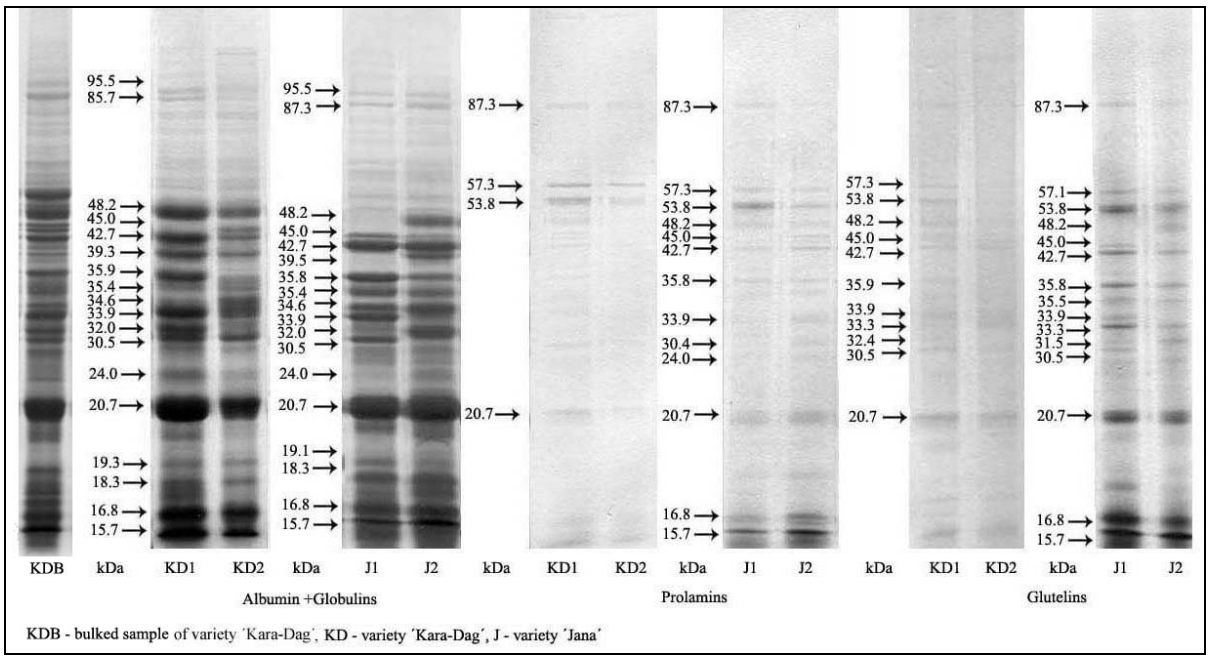
Values of parameters marked by same indexs are not significantly different at $p \leq 0.05$.

Fig. 1.: Comparison of electrophoretical spectra of evaluated protein fractions in individual buckwheat varieties and tartary buckwheat accessions.



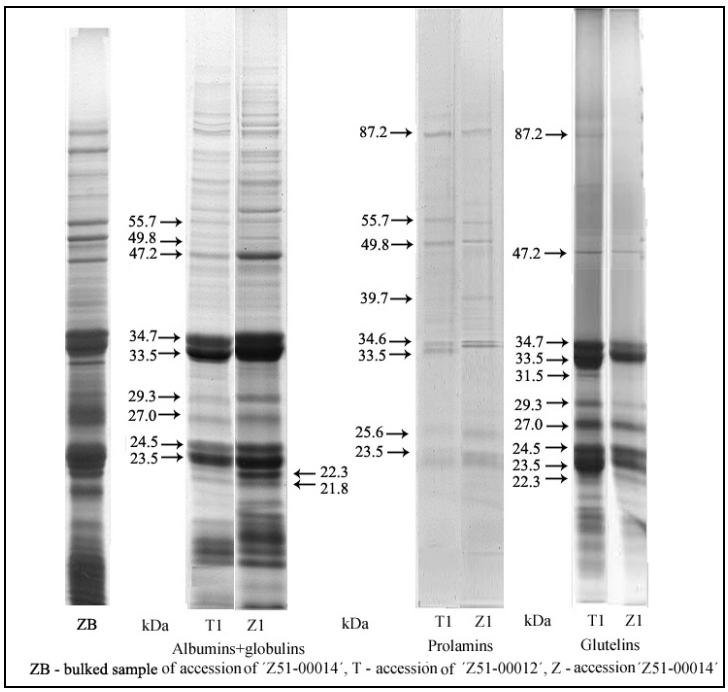
M – marker, a – Albumins+Globulins, i – Prolamins, u - Glutelins

Fig. 2.: Detailed description of protein bands in three protein fractions of buckwheat varieties.



KDB - bulked sample of variety 'Kara-Dag', KD - variety 'Kara-Dag', J - variety 'Jana'

Fig. 3.: Detailed descriptions of protein bands of three protein fractions in tartary buckwheat accessions.



ZB - bulked sample of accession of 'Z51-00014', T - accession of 'Z51-00012', Z - accession 'Z51-00014'