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Endosperm protein polymorphism of common and tartary buckwheat

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ABSTRACT

The endosperm protein polymorphism of seven accessions of common buckwheat (*Fagopyrum esculentum* Moench) and two accessions of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) were studied by SDS-PAGE electrophoresis. The position and the intensity of the main four electrophoretic protein bands (marked as “a”, “b”, “c” and “d”) of cultivar Siva (common buckwheat) were estimated and the polymorphism was studied using seeds from open pollinated heterozygous plants. Two high significant correlations (correlation coefficient 0.559 for “a” and “c” bands, and 0.264 for “a” and “d” bands) were established among the intensities of electrophoretic protein bands of common buckwheat, while other four possible relations were not significant. The results indicate significantly higher protein polymorphism of endosperm within individual accessions than among accessions of common buckwheat. In contrary to common buckwheat, there was in material studied found no variability in endosperm protein bands between or within two accessions of tartary buckwheat.

Key words: *Fagopyrum esculentum*; *Fagopyrum tataricum*; SDS-PAGE electrophoresis; endosperm proteins; polymorphism

IZVLEČEK
POLIMORFIZEM BELJAKOVIN ENDOSPERMA PRI NAVADNI IN TATARSKI AJDI

S pomočjo SDS-PAGE elektroforeze smo analizirali sedem vzorcev navadne (*Fagopyrum esculentum* Moench) in dva vzorca tatarske ajde (*Fagopyrum tataricum* Gaertn.). Pri cv. Siva smo analizirali semena s tujeprašnih rastlin in ocenili pojavljanje in intenzivnost glavnih štirih elektroforeznih črt (označene z “a”, “b”, “c” in “d”). Pri ovrednotenju intenzivnosti pojavljanja elektroforeznih črt, smo ugotovili dve visoko statistično značilni korelaciji (korelacijski koeficient 0,559 pri “a” in “c” elektroforeznih črtah ter 0,264 pri “a” in “d” elektroforeznih črtah), medtem ko ostale štiri možne korelacije intenzivnosti elektroforeznih črt niso bile statistično značilne. Rezultati nakazujejo na visoko statistično značilni polimorfizem beljakovin

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endosperma med analiziranimi semeni cv. Siva, med tem ko med posameznimi vzorci ni razlik v pojavljanju elektroforeznih črt. Med dvema vzorcema in znotraj vzorca tatarske ajde nismo našli razlik v pojavljanju elektroforeznih črt.

Ključne besede: *Fagopyrum esculentum*; *Fagopyrum tataricum*; SDS-PAGE elektroforeza; beljakovine endosperma; polimorfizem

1 INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench) proteins are nutritionally important because of their excellent amino acid composition (Kreft I., 1994; Kreft I., 1995; Bonafaccia et al., 2003a) and thus very high biological value of buckwheat proteins (BV = 86) which is much higher than that of cereal proteins (Skrabanja et al., 2000). The major (13S globulin) and minor classes of storage protein represent about 33 and 6.5% of total seed proteins, respectively (Radović et al., 1996). Buckwheat proteins may prevent gallstone formation more strongly than soy protein isolates, retard mammary carcinogenesis by lowering serum estradiol, and they suppress colon carcinogenesis by reducing cell proliferation (Kayashita et al., 1999; Tomotake et al., 2000; Liu et al., 2001). This is maybe connected with limited digestibility of buckwheat protein (Ikeda et al., 1986; Ikeda et al., 1993; Skrabanja et al., 2000). The high content of protein in some buckwheat grain milling fractions suggests a potential application of these materials for special dietary products. Tannin-protein complexes are in the gastrointestinal tract potent radical cation scavengers and may act as a radical sink (Luthar, 1992; Riedel and Hagerman, 2001). Buckwheat endosperm proteins may contain some selenium (Bonafaccia et al., 2003b), a trace element, essential in human nutrition. As buckwheat does not contain gluten proteins, it is a common supplement for patients with celiac disease (Skerritt, 1986; Wieslander and Norbäck, 2001).

Proteins are along with starch among the main endogenous factors responsible for the textural characteristics of buckwheat products (Ikeda et al., 1997). Correlations between protein content and hardness, cohesiveness, adhesiveness, springiness and chewiness evaluated on buckwheat dough prepared from the endosperm fractions were reported (Ikeda et al., 1997; Ikeda et al., 1999). All textural characteristics, except for adhesiveness, were in significant negative correlation to protein content. Buckwheat is an undemanding low-input plant (Kreft I., 1989; Petr, 1995; Kalinova and Moudry, 2003).

Polymorphism of electrophoretic protein pattern in buckwheat was first reported by Kreft I. et al. (1978). The genetic polymorphism related to buckwheat nutritive value was studied earlier (Michalová, 1998; Michalová, et al. 1998; Baburkova et al., 2000; Kalinova et al., 2002). High polymorphisms of the cotyledon storage proteins, but high endosperm protein homogenity were reported by Rogl and Javornik (1996). However till now there was no research report on polymorphism in endosperm proteins of common buckwheat.

The aim of this investigation was to describe the polymorphism of buckwheat endosperm proteins, as a basis for breeding selection towards the designed endosperm protein constitution.

2 MATERIAL AND METHODS

2.1 Plant material

The material that we used in this study was obtained from the Gene Bank at the Research Institute of Crop Production, Prague-Ruzyně, Czech Republic, and from the gene bank maintained at the Center for Plant Biotechnology, Breeding and Genetics, Biotechnical Faculty, University of Ljubljana. Samples are listed in table 1.

Table 1: List of accessions used for analysis

No. of sample	Accession
1.	Grey buckwheat cv. Siva (SVN)
2.	Z50-0064 La Harpe (FRA)
3.	Z50-0063 Pyra (CZE)
4.	Z50-0062 Bolshevik 4 (RUS)
5.	Z50-0051 Botansoba (JAP)
6.	Z51-0014 Tartary buckwheat (USA)
7.	Z51-0012 Tartary buckwheat (CZE)
8.	Z50-0070 NS-SP-LXH (SVK)
9.	Z50-0111 Emka (POL)

Sample number 1 was common buckwheat cv. Siva, 105 seeds from open pollinated heterozygous plants were analyzed. Samples number 2 – 9 were accessions collected and provided by the Research Institute of Crop Production, Prague-Ruzyně, Czech Republic; approximately 10 seeds from open pollinated heterozygous plants respectively self-pollinated homozygous plants of tartary buckwheat (number 6 and 7) were analyzed.

2.2 Dissection of buckwheat seeds

Prior to dissection the unhusked buckwheat seeds were soaked for 1 hour in distilled water. Under magnifying glass the husk and testa were removed from each analyzed seed and the endosperm was carefully separated from cotyledons (embryo) and placed each endosperm separately into a 1,5 ml micro centrifuge tube.

Each dissected endosperm was smashed in 300 µm of extraction buffer (0.5 M Tris- HCl pH 6.8, 20 % glycerol, 4 % SDS, 3 % 2-merkaptoetanol, Bromphenol blue) with a glass stick. After 2 hours of extraction, the samples were incubated in water bath at 95 ºC for 5 minutes, centrifuged for 15 min at 10 000 rp/min and 15 µm of supernatant was applied for electrophoresis.

2.3 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method

For one run of SDS-PAGE electrophoresis two parts of gels were prepared; separation (10% T) (1.5 M Tris- HCl pH 8.8, 10 % T acrylamide stock, 1% SDS, 1% ammonium persulfate, TEMED) and concentration gel (1.5 M Tris- HCl pH 6.8, 4 % T 1.3 % C acrylamide stock, 1% SDS, 1% ammonium persulfate, TEMED) (Laemmli, 1970).

For one run of electrophoresis, two 1.5 mm thick gels were prepared. The apparatus (for vertical electrophoresis) carried out the electrophoresis at 40 mA for 1 hour, and about 3 hours at 60 mA. The proteins were stained for 24 hours with 0.05% Commasie Brilliant Blue R- 250, 5% ethanol, and 12% TCA, and then destained in deionised water. Intensity of the electrophoretic protein bands was estimated by the scale from 0 (there is no band) to 5 (very thick band). Statistical analysis was performed using Image analysis Excel (Microsoft Co., USA).

3 RESULTS AND DISCUSSION

3.1 Endosperm proteins of buckwheat cv. Siva

The endosperm protein cv. Siva was characterized 3 – 4 strong protein with different intensity and high number of weak bands with molecular weight extent of 20 – 100 kDa. Our population study of cv. Siva endosperm protein variability was focused on clearly visible main 4 bands with molecular weight 95 kDa respectively 50 – 60 kDa. The results of endosperm protein polymorphism of cv. Siva are shown in figure 1.

After the estimation of the intensities of the bands by the scale from 0 (no band) to 5 (very thick band), the correlation between patterns of 105 seeds was established. Correlation coefficients between pairs of the appearances of “a”, “b”, “c”, and “d” bands are presented in table 2.

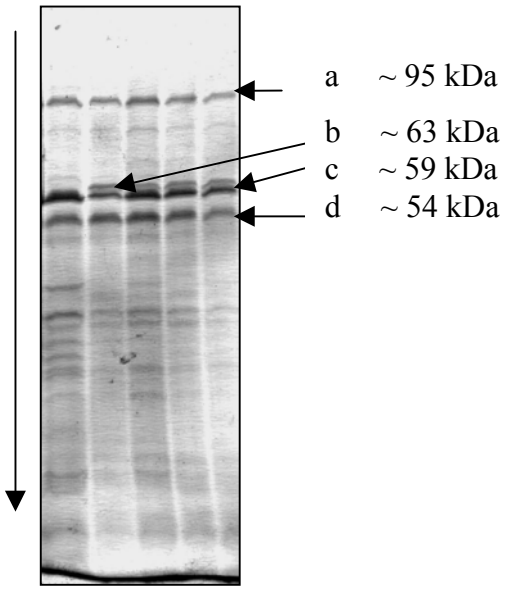


Figure 1: Electropherograms of cv. Siva buckwheat endosperm SDS-PAGE. Among all bands the 4 bands (“a”, “b”, “c”, “d”) are the most intense. Band “b” does not appear in all samples

Electrophoretic band “b” (~ 63 kDa) appears just in some seeds and the intensity is quite variable. Band “a” (~ 95 kDa) appears in endosperm extracts of all studied seeds and here the band intensity is almost in all samples the same. The intensity of “c” (~ 59 kDa) and “d” (~54 kDa) bands was different from seed to seed, but these bands appear always in the combination with the occurrence of band “a”. There was no significant correlation between “a” and “b” or “b” and “c” bands; there was negative, but nont significant relation between “b” and “d” or “c” and “d” bands. Significant correlation appeared between “a” and “c”, and “a” and “d” bands (table 2).

Table 2: Correlation coefficients between pairs of the appearance of a, b, c and d bands in cv. Siva buckwheat endosperm extracts from 105 seeds

band / band	a	b	c	d
a	-	0.1039	**0.5591***	**0.2636***
b		-	0.0347	-0.1770
c			-	-0.0600
d				-

* Correlation significant at $p<0.01$

Buckwheat endosperm proteins are important factors of the final nutritional and functional value and influence value of other constituents with the importance for human health, as rutin (Michalova et al., 1998; Kreft S. et al., 1999; Petr et al., 1999; Park et al., 2000; Fabjan et al., 2003), resistant starch (Skrabanja and Kreft I., 1998; Skrabanja et al., 1998; Skrabanja et al., 2001; Kreft I. and Skrabanja, 2002) and mineral elements (Bonafaccia et al., 2003b). The found correlations among intensity of specific storage protein molecules, respectively presence / absence of "b" band, can have important roll for future finding their relation to quantity or quality grain parameters.

Table 3: Number of different electroforetic phenotypes combinations and their percent ratio in cv. Siva population

	Electrophoretic band				
Combinations of electroforetic phenotypes	**a**	**b**	**c**	**d**	**Percentage ratio of evaluated phenotypes**
1.	++++	/	++++	++++	**13.3%**
2.	+++	/	++++	+++++	**17.1%**
3.	+++	/	+++	+	**2.8%**
4.	++	/	++++	+++++	**6.6%**
5.	++	/	++++	+++	**3.8%**
6.	++	/	++	++++	**3.8%**
7.	++	/	+	+++++	**4.8%**
8.	+	/	++++	++++	**0.9%**
9.	+	/	+	+++++	**5.7%**
10.	+	/	+	++	**2.8%**
11.	++++	++++	++++	+++++	**3.8%**
12.	+++	+	++++	++++	**3.8%**
13.	+++	+	+++	++++	**1.5%**
14.	+++	++	++++	++++	**6.6%**
15.	+++	++	++++	+++	**2.8%**
16.	+++	++	++++	+++++	**0.9%**
17.	++	+++	++++	++	**5.7%**
18.	++	+	++++	++++	**4.8%**
19.	++	+	++++	++	**1.9%**

*Valuation: / (no band, 0); + (intensity 1); ++ (intensity 2); +++ (intensity 3); ++++ (intensity 4); +++++ (intensity 5)

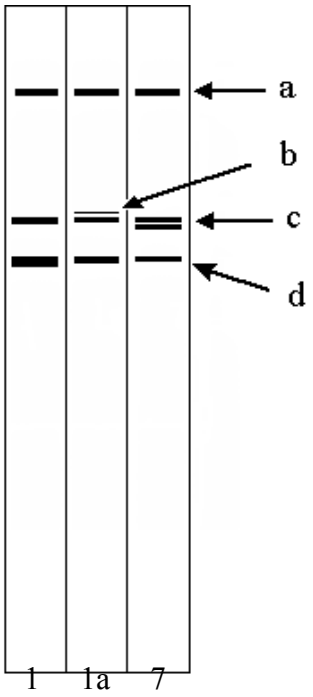
Different genotypes combinations of cv. Siva endosperm and their percent ratio are presented in table 3. On the basis of band positions we only found out 2 genotypes which differed in the presence (ratio 38%) / absence (ratio 62%) of "b" protein band. When we implicated the factor of the band intensity in our estimation, we obtained 19 genotypes with percentage ratio of individual genotypes (electrophoreotypes) from 2% to 17%. That corresponds with biological character of open pollinated heterozygous plants and gives the possibility for selection breeding towards the designed combination of endosperm proteins (Kreft I., 1995).

3.2 Endosperm protein bands of other accessions of common buckwheat

No essential differences of endosperm proteins appeared among other samples 2, 3, 4, 5, 8 and 9 (common buckwheat). In all samples, bands "a", "c", and "d" occurred in the same position and with the similar intensity as in cv. Siva buckwheat, however band "b" does not appear in any of samples 2-5, 8 or 9. This is in agreement with the results of Rogl and Javornik (1996), who find no essential variability in the electrophoretic protein band in common buckwheat. They obtained higher polymorphism of the cotyledon storage proteins. We can also agree with the statemen of Rogl and Javornik (1996), the commonest differentiating feature in the electrophoretic profile of out-breeding crops is quantitative differences in band intensities rather than the complete presence / absence of bands. It can also notice that strictly heterogamy of common buckwheat causes significantly higher intra varietal variability than the variability which we can find among cultivars. It's possible to expect (on the basis of endosperm protein polymorphism) that higher genetic gain (improvement of agronomical or qualitative traits) will be obtained with the selection work inside of individual current cultivars (degrease of genetic basis) than prompt mutual crossing among different cultivars.

3.3 Endosperm proteins of tartary buckwheat

The variation of endosperm proteins in tartary buckwheat was studied and compared to endosperm proteins of common buckwheat cv. Siva (Figure 2). There is no essential difference in the endosperm proteins bands between two accessions of tartary buckwheat. However the important difference was found between common and tartary buckwheat samples. On the electropherogram of tartary buckwheat the band "d" (weight range around 59 kDa) is less intensive than on electropherogram of any common buckwheat and at the tartary buckwheat at the position of "b" there was no band; however at the adjacent position of "c" an intense double band always appears in tartary buckwheat.



Fiure 2: Two different electropherograms of cv. Siva buckwheat endosperm (samples 1 and 1a) compared to electropherogram of tartary buckwheat (sample 7)

4 CONCLUSIONS

The electrophoretical protein evaluation of cv. Siva indicated high polymorphism mainly in band intensity of endosperm proteins. We confirmed significant positive correlation among protein bands “a” - “c”, and “a” - “d” No pronounced difference among endosperm proteins of different accessions of common buckwheat was found. The significantly higher intra varietal protein variability was found in contrast to protein variability among cultivars. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of common buckwheat endosperm proteins seems to be less suitable tool for the identification of the standard buckwheat cultivars. Clear protein difference was only noticed between both different species common and tartary buckwheat.

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