Suitability of oat-seed storage-protein markers for identification of cultivars in grain and mixed flour samples

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ABSTRACT

The objective of this study was an improvement on oat identification procedure for laboratory applications, and the comparison of albumin-globulin and avenin protein patterns in five hulled and naked oat cultivars: Abel (CZ) and Izák (CZ) – naked oats, Auron (CZ), Edmund (D) and Expander (D) – hulled oats. The last object of this study was the authenticity verification of standardly prepared meal samples with various proportions of admixture. It was confirmed that avenins, characterised under SDS-PAGE conditions, are reliable implements for the identification of oat cultivars. It was found that oat grain contains, on the basis of Osborne fractionation, another significant protein fraction – glutelins. The question of the protein fraction analysis that was used for the admixture identification stays still open. In sufficiently different cultivars, the certainty of the admixture detection in meal samples may be high. Nevertheless, in other cases (higher cultivar similarity) it will be necessary to use some other, more sensitive techniques.

Keywords: oat; cultivar identification; protein fraction; electrophoresis; PAGE

Cultivated oats belong to the genus *Avena* L. comprising a polyploid series of wild, weedy, and cultivated species which are found worldwide in almost all agricultural environments. Baum (1977) classified the cultivated hexaploid oats into the taxa *Avena sativa* L. that covers *Avena nuda* L. and *Avena byzantina* L.

Over the last 10–15 years, oat breeders and oat milling industry have shown a considerable interest in the possibility of the development of new oat cultivars containing higher percentages of protein and an improved amino acid balance (Henry and Kettlewell 1996).

The high number of new cultivars and their high mutual similarity do not allow their differentiation on the basis of their phenotype. Genotype distinguishing by electrophoretic separation of seed storage proteins is one of the most efficient methods of the cereal genotype identification. It is largely valid for the identification of the oat genotypes *Avena sativa* L. as well (Gregová et al. 1996).

At present, the breeders are seeking biochemical and molecular markers associated with specific agronomic, qualitative traits. These markers, such as DNA polymorphism or specific protein electrophoretic bands, may be analysed directly, often using tissues from individual plants or the endosperm half of the seed (Howes et al. 1992).

All workers agree that the prolamin (avenin) content of oats is low, with estimates ranging between about 4–14% of the total protein. The water-soluble fraction, albumins, is also a minor component (9–20% of the total protein). The proportion of globulins is not unambiguous. Peter-

son (1976) found 54–56% globulins of the total nitrogen. By contrast, German workers reported that glutelins are the major fraction, and that the concentration of globulins is only 21–27% of total nitrogen (Wieser et al. 1980). Brinegar (1983) reported on characterisation of two main protein fractions, globulins and avenins, and he also described their isolation.

The oat genotypes show valuable and reproducible polymorphism in the prolamin fraction – avenins. Polymorphism in avenin patterns is more heterogeneous than in the globulin fraction (Robert et al. 1983). Most workers have focused their electrophoretic separations on avenins. Compared with wheat, oats produce fewer bands in the protein patterns for the distinction of cultivars. The techniques for oats have been considerably less developed than those for some other species, and the lack of standard methods and the poor reproducibility in individual laboratories are a continuing problem (Peterson 1978). Souza and Sorrells (1990) catalogued 70 North American oat cultivars and genotypes on the basis of avenin patterns by non-denaturing PAGE. At least four loci were found to possess alternative alleles with distinctive electrophoretic mobilities.

Several workers used biochemical markers for the specification of phylogenetic relationships among *Avena* species and variations within species collected from diverse geographical areas. These studies included protein banding patterns and are useful for the breeders attempting to sample germ plasm for useful genes (Souza and Sorrells 1990, Phillips and Murphy 1993).

The objectives of this study were the improvement on the oat identification procedure for laboratory use and the comparison of globulin and avenin protein patterns in 5 evaluated hulled and naked oat cultivars. The last object of this study was the authenticity verification of standardly prepared meal samples with various proportions of admixture.

MATERIAL AND METHODS

Five cultivars of oats were analysed in this study: three traditional Czech cultivars: 1 – Abel (naked oat), 2 – Auron (hulled oat) and 5 – Izák (naked oat), and two German cultivars of hulled oats: 3 – Edmund and 4 – Expander. The individual protein fractions were separated by the standard Osborne method of the year 1907 (Králová et al. 1991). Dual slab electrophoretic system Hoefer SE 600 (gel size – 16 × 18 cm) was used for the analyses. 30 grains of every cultivar were tested.

1. Extraction of seed storage protein in SDS buffer (see point 4), pre-extracted with Na-phosphate buffer. The broken grain was extracted with Na-phosphate buffer (see point 2) and the pellet remaining after lyophilisation was prepared in same way as in point 4. Lyophylisation was carried out in standard way using the equipment ALPHA 1-4 (Martin Christ, Osterode am Harz, SRN) under the following conditions: temperature: –50°C and pressure: 40 mBar, for a period of 24 hours.

2. Extraction of albumins and globulins. Albumins and globulins were extracted with 200 µl of 1M NaCl in 0.1M Na-phosphate buffer, pH = 7, and temperature 4°C. The crude extract was centrifuged (15 minutes, 6000 rpm). Af-

ter centrifugation, 120 µl of supernatant was pipetted into another microcentrifuge tube, and lyophilised for 6 hours.

3. Extraction of avenins. After the above-mentioned procedure, the lyophilised material was extracted with 25% 2-chlorethanol (200 µl per grain) for 4 hours at room temperature. Then the samples were centrifuged (15 minutes, 6000 rpm). After centrifugation, 120 µl of supernatant was pipetted into a fresh microcentrifuge tube and lyophilised for 6 hours.

4. Preparation of samples for SDS-PAGE electrophoresis. The remaining pellet of all protein fractions after lyophilisation was extracted with 190 µl of SDS extraction buffer (0.0625M Tris-HCl, pH = 6.8, 5% BME, 2% SDS) for 4 hours and at temperature 4°C. The sample was centrifuged (10 minutes, 10 000 rps) and the clear supernatant was pipetted into a fresh microcentrifuge tube (100–120 µl) containing 20 µl of loading buffer (5 ml 1.25M Tris-HCl, pH = 6.8, 2.3 g SDS, 10 ml glycerol and 5 mg BPB, 340 µl BME is added per 1 ml of this buffer prior to its use). The sample was boiled for 1 minute (100°C) and pipetted (25–30 µl) on the gel. The SDS discontinuous system was performed according to Laemmli (1970). The electrophoresis was run for 4 hours with maximum voltage 300 V and 30–45 mA per gel.

5. Authenticity verification of standard oat meal samples prepared. Five standard meal samples were prepared with varying proportions (1, 5, 10, 20, 30%) of other cultivar. The main cultivar was chosen the cv. Izák. As the admixture, cv. Edmund was chosen. Data analysis: electrophoretic phenotypes were digitised and evaluated using specialised software Bio-Profil 1D++ (Vilber Lourmart). Similarity matrixes were calculated using Nei & Li coefficient of genetic similarity and dendrograms
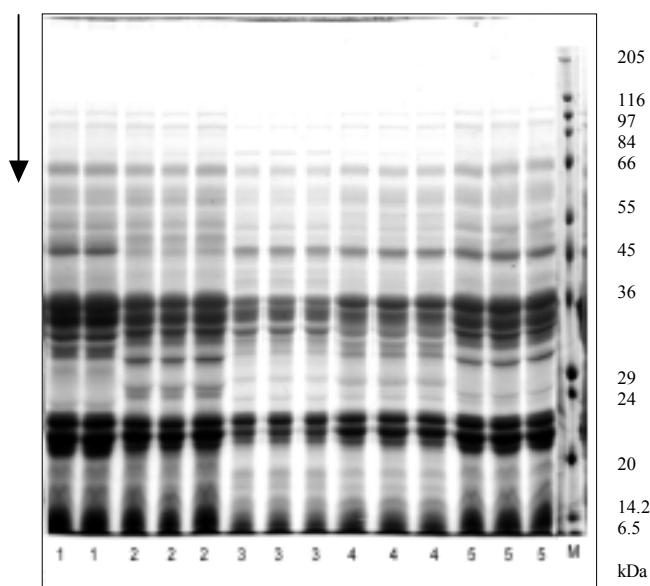

Figure 1. SDS-PAGE of seed storage protein pre–extracted with Na-phosphate buffer
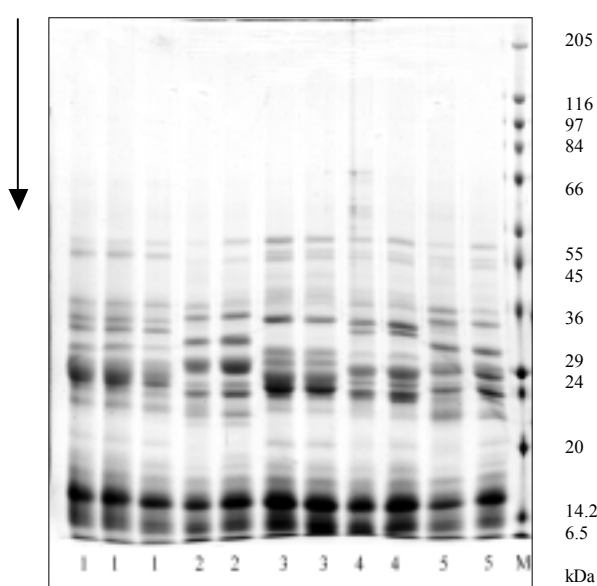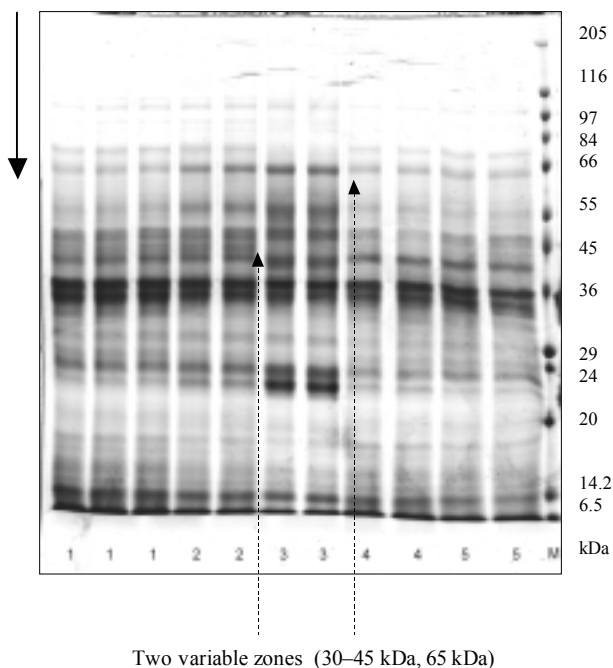
Figure 2. SDS-PAGE on avenins

Two variable zones (30–45 kDa, 65 kDa)

Figure 3. SDS-PAGE of albumins and globulins

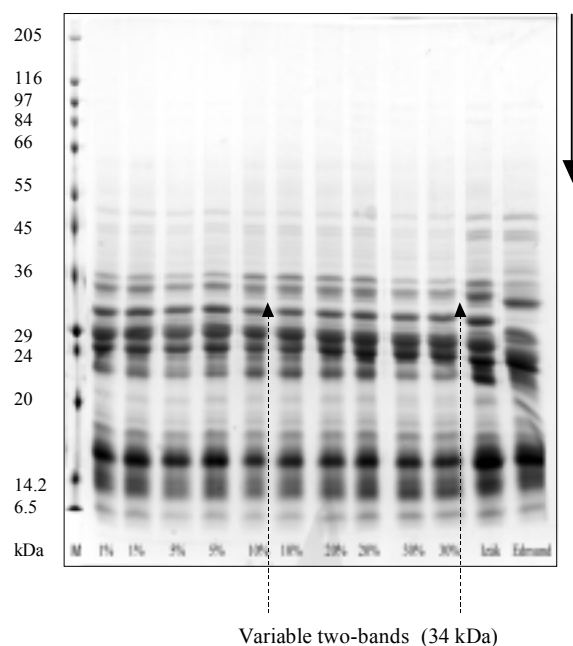

Variable two-bands (34 kDa)

Figure 4. Verification of meal standard – avenins

were calculated using UPGMA method and 95% confidence (Lourmat 1999).

RESULTS

Figure 1 shows the determination of the oat cultivars evaluated on the basis of the pre-extraction method in Na-phosphate buffer. The cultivars differed in the middle and lower gel parts with molecular weights of 36 and 24 kDa. On the resulted dendrogram (see Figure 8), the Czech cultivars Abel and Auron showed the highest mutual similarity (0.89), analogous to both German cultivars (0.88).

The avenin patterns (Figure 2) show an excellent determination of the oat cultivars analysed. The main zones of determination were found in the wide area characterised by molecular weights from 36 to 20 kDa. The cultivars were sorted more logically than by the previous protein pattern. The highest similarity was found between both German cultivars Edmund and Expander 0.90.

The Czech naked oats Abel and Izák were clustered together, on the contrary the third Czech cultivar Auron was sorted separately as the least similar cluster (Figure 9).

The comparison of both Figures 1 and 2 is very interesting. After Na-phosphate pre-extraction, only prolamins, resp. avenins, should stay in the pellet as the main protein fraction. It could be expected, consequently, that both protein patterns should resemble each other. Nevertheless, both protein patterns show clearly different areas.

Figures 3, 6, and 7 show albumin and globulin electrophoretical protein patterns of the oat cultivars tested and a detailed comparison of two oat cultivars, Izák and Edmund. The differences between the cultivars were found in zones 30–45 kDa and 65 kDa. A great difference was found between the cultivars Izák and Edmund (0.78). The differences between other cultivars were minimal and the coefficient of similarity exceeded 0.9 (see dendrogram Figure 10). Only the one variable band with molecular weight 40 kDa was the decisive factor for the determina-
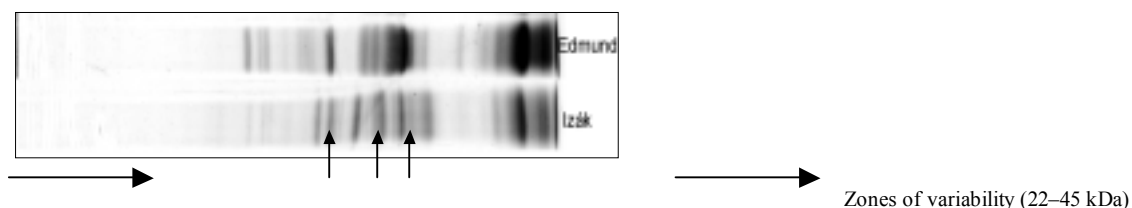

Zones of variability (22–45 kDa)

Figure 5. Eletrophoretic standard patterns – avenins

Variable band (40 kDa)

[1]albumin and globulin protein fractions

Figure 6. Verification of meal standard – globulins (albumin and globulin protein fractions)

Zones of variability (30–40 kDa)

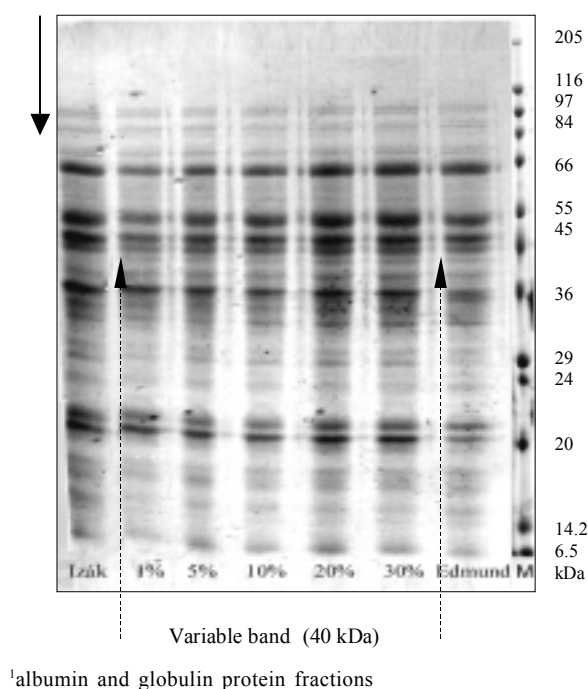[1]albumin and globulin protein fractions

Figure 7. Electrophoretic standard patterns – globulins (albumin and globulin protein fractions)

tion of admixture in the meal sample in this model case. After the addition of 5% meal of the cultivar Edmund to the Izák meal sample, this band was reliably identified.

Figures 4 and 5 show prolamin (avenin) protein patterns of the above mentioned cultivars Edmund and Izák, and also of their meal mixtures. Although in comparison with albumin and globulin protein patterns we found higher variability between the cultivars tested, the resulting mixture of avenin protein patterns did not provide better results in comparison with globulin patterns. We detected, in analogy to globulins, only one variable band with molecular weight 40 kDa in the meal mixture. The admixture of Edmund was reliably identified from its proportion of 10% in the mixture.
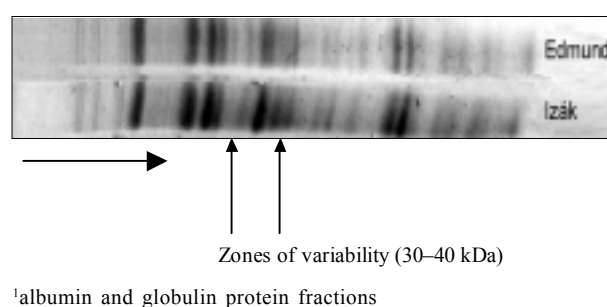
DISCUSSION

For methodical optimisation, the experience was utilised from our own work with spelt wheat (Dvořáček et al. 2001). Based on this, three marker systems were chosen (albumins and globulins; avenins, and SDS seed storage protein with pre-extraction). For the documentation of the high cultivar homogeneity, two or three samples from the same cultivar were tested on the gel. It is also necessary to remind that oat contains extremely high proportions of fat and mucus substances (Webster 1986). That is why it was necessary to use a higher amount of extraction buffer in comparison with other cereals.

The question of the protein composition and its quantification is still problematic. Webster (1986) reported that the protein fractions obtained with Osborne method of protein separation and the homogenous polypeptides detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) are hardly comparable. Also many other authors, e.g. Michael et al. (1961), Völker (1975) and Wieser et al. (1980) used modifications of the original Osborne method which complicates the uniform protein characteristics and other possibilities of their
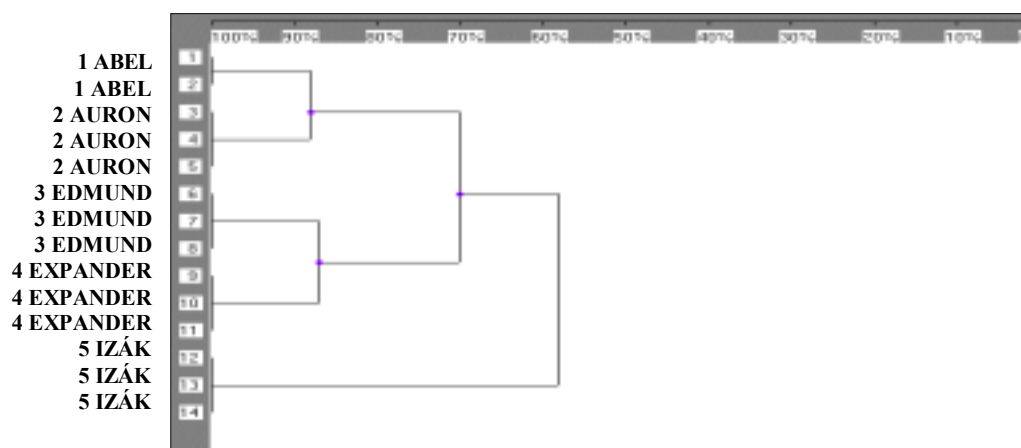

Figure 8. Dendrogram – SDS-storage protein pre-extracted with Na-phosphate buffer, Nei-Li coefficient
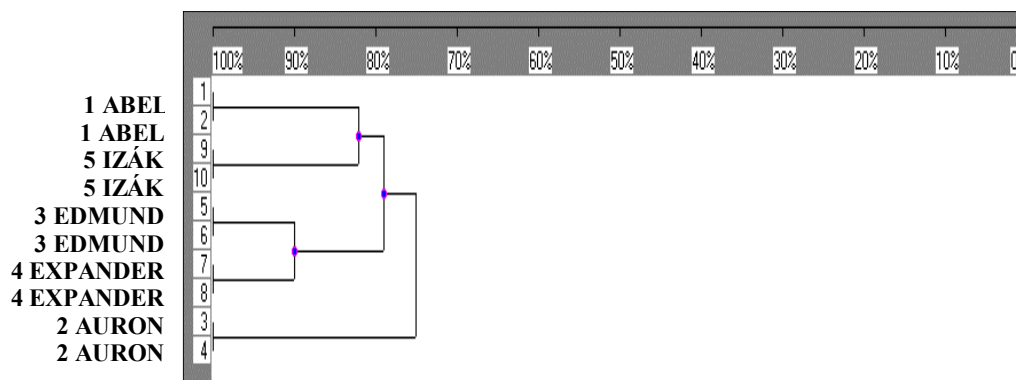
Figure 9. Dendrogram – avenins Nei-Li coefficient

using as markers of economically suitable properties. For comparable results, it will be necessary to observe the extraction conditions very strictly (Pomeranz 1989, Kim and Bushuk 1995).

The explanation for the non-similarity between the avenin protein patterns and the protein fraction remaining after pre-extraction with Na-phosphate buffer (Figures 1 and 2) is very complicated. It seems that, in the case of the first gel (Figure 1), some other protein fraction undissolved in the Na-phosphate buffer stayed in the protein pattern. This third protein fraction, that causes differences in the first protein patterns, could be called, in accordance with German authors Michael et al. (1961), Völker (1975) and Wieser et al. (1980), glutelin fraction.

Albumins and globulins as biochemical markers showed a worse ability of the cultivar identification in comparison with avenins or the protein fraction remaining after pre-extraction (see dendrogram Figure 10). This was confirmed in the report by Králová et al. (1991) concerning the lower degree of albumin and globulin polymorphism.

The problems of a reliable identification of meal mixtures will be in the overlapping of the neighbouring zones. The similarity of cultivars is very high at the present and the differences in the band positions are very small. Nevertheless, we suppose that avenin protein patterns have better expectations for the identification of admixtures in meal in comparison with albumins and globulins, and also with the rich patterns of probable glutelins (see Figure 1). It is necessary to mention that identification of oat admixtures will depend on specific differences between cultivars and biochemical marker used. For a more sensitive analysis it will be necessary to develop new protein extraction technologies focused directly on the differing protein areas. HPLC – methods could be one of these possibilities (Ward et al. 1998).

It was confirmed in analogy to Gregová et al. (1996) that avenins, characterised under the conditions of SDS-PAGE, are a reliable implement for the identifications of oat cultivars. It was also confirmed that oat grain contains another significant protein fraction – glutelins as German authors referred (Michael et al. 1961, Völker 1975, Wieser et al. 1980). The question of the protein fraction used for admixture identification remains still open. In sufficiently different cultivars, the reliability of the admixture detection in a meal sample could be high. Nevertheless, in other cases it will be necessary to use more sensitive techniques.
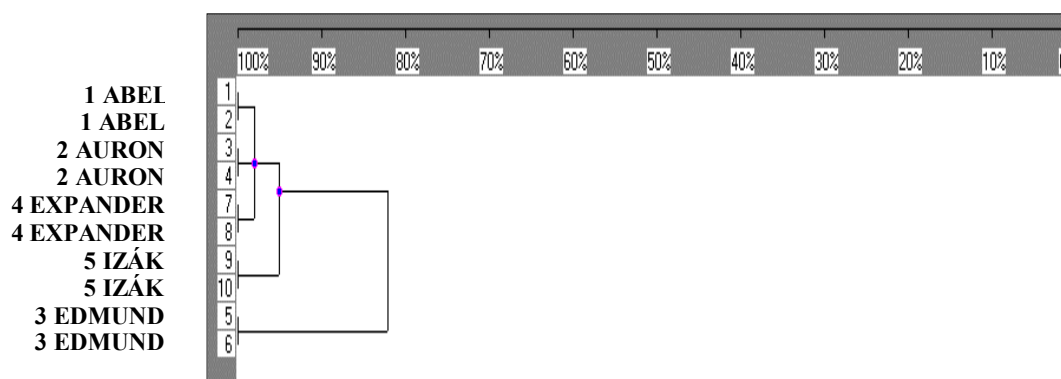

Figure 10. Dendrogram – albumins and globulins Nei-Li coefficient

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ABSTRAKT

Vhodnost zásobních bílkovin zrna jako genetického markeru pro identifikaci odrůd ovsa

Byly vypracovány laboratorní postupy vhodné pro porovnání albumino-globulinových a aveninových proteinových spekter u pěti vybraných odrůd nahého a pluchatého ovsa: Abel (CZ) a Izák (CZ) – nahé ovsy, Auron (CZ), Edmund (D) a Expander (D) – pluchaté ovsy. Součástí studie bylo rovněž ověřit spolehlivost možného odlišení odrůdové příměsi v ovesné mouce u standardně připravených směsných vzorků. Potvrdilo se, že aveniny charakterizované v podmínkách SDS-PAGE jsou vhodným nástrojem pro identifikaci odrůd ovsa. Bylo zjištěno, že zrno ovsa obsahuje na základě Osbornovy frakcionace další významnou bílkovinnou frakci – gluteliny. Otázka identifikace příměsí na základě frakcionace ovesných bílkovin zůstává dosud otevřená. U výrazně odlišných odrůd je jistota detekce příměsi ve směsi mouky vysoká. Nicméně v ostatních případech (vyšší odrůdová příbuznost) bude zřejmě nutné využít jiné citlivější techniky.

Klíčová slova: oves; identifikace odrůd; bílkovinné frakce; elektroforéza; PAGE

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