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SEED PROTEIN ELECTROPHORESIS FOR IDENTIFICATION OF OAT REGISTERED CULTIVARS

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ABSTRACT

The protein diversity of 15 registered oat genotypes (*Avena sativa* L.) was examined. Acid-PAGE (acid polyacrylamide gel electrophoresis) of avenins and SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) of the glutelins were used for seed analyses of oat varieties. The result of this study indicated that the genotypes of oat cultivars could effectively be differentiated on the basis of polymorphism, detected between protein patterns. SDS-PAGE result revealed that glutelins presented a higher differentiation power than avenins and could be used as a rapid method for the identification of oat varieties in breeding programmes. Avenin protein band numbers and molecular weight ranged from 5 to 11, and 8 to 45 kDa, respectively. Genetic similarity analysis based on avenin protein ranged from 0,071 to 1. The tree-cluster analysis illustrates the distribution of varieties in two major groups. The first major group was large with eight varieties and following varieties were determined as identical: Vendelín-Zvolen and Prokop-Václav. The second group consisted of six varieties and Detvan-Hronec and Izak-Važec were identical pairs. The number of glutelin protein markers was 22. Genetic similarity coefficients resulted from comparisons by glutelin pattern and they ranged from 0,125 to 0,929 all oat varieties were different from each other. Two Slovakian varieties Izak and Važec from Breeding Station Viglaš-Ptruša with similarity value 92,9% were grouped with variety Vendelin. Two varieties Kanton and Viliam with similiarity value 92% were grouped with Prokop and Valentin. All analysed cultivars could be distinguished by their glutelin pattern and will be useful in oat breeding process.

Keywords: *Avena sativa* L.; genetic diversity; avenins; glutelins; electrophoresis

INTRODUCTION

The oat belongs to the grass family Gramineae (Poaceae), to the tribe Aveneae, and to the genus *Avena*. It contains 30 different species which form distinct polyploidy series ranging from diploid through tetraploid to hexaploid with base chromosome nuber of seven. Centre of origin of genus Avena L. lies in the western part of the Mediterranenan region where species A. *byzantina* C. Koch originated too. The secundary centre of formation of Avena L. species and origin of cultivated oat (*Avena sativa* L.) is situated within the Asia Minor centre of crop origin **(Loskutov, 2007)**. Proteomic techniques have been applied successfully to the identification of cereal seeds, differentiation of proteins from the wheat kernel **(Šramková et al., 2011)**, identification of hordein from barley (**Bradová et al**., **2001**) and verification of zeins from corn **(Gregová et al., 2013)**. Oats (*Avena sativa* L.) have relatively high protein content (12.4 – 24.5%) and an excellent nutritional score of amino acids can be a good source for protein composition for food application. **(Lásztity, 1996)**. Cultivated oats are hexaploid cereals belonging to the genus *Avena* L., which is found worldwide in almost all acricultural enviroments. Oat grains are mainly used as animal feed and only a few percent are used for human nutrition. Recent results showing the suitability of oats for celiac patient moreover raises the status of oats healthy part of their diet.Oat provides well balanced food for human and has been used for feeding for long time. Oat protein fractions are albumins (10 – 20 %), globulins (12 – 55%), prolamins (12 – 14%) and glutelins (23 – 54%) **(Haard, 1999)**. Globulins and avenins are located in vacuolar protein bodies. Protein belonging to the albumin group are mostly enzymes and functionales proteins. In oat globulin and glutelin fraction were presented as a major components. **Shotwell et al. (1988)** presented the oat 12S globulin is 70 % homologous to rice storage globulins and 30% – 40 % homologous to storage globulins present in legumes. Generally the glutelin fraction is defined as the protein fraction extracted with basic or acidic solution after the removal of water-soluble albumins, salt-soluble globulins and alcohol-soluble prolamins **(Anderson, 2014)**. German workers reported that glutelins are the major fraction and concentration of globulin is only 21 – 27% of total protein **(Weiser et al. 1980)**. Other authors reported that globulins are the major fraction and **Peterson (1976)** found 54 – 56% globulin concentration of the total protein. Storage globulins are the major endosperm proteins in oats and rice, although technically the rice globulins were earlier classified as glutelins due to their solubility properties **(Shewry 1995)**. Polymorphism in the prolamins is more heterogeneous than in the globulin pattern **(Dvoráček et al., 2005; Gregová et al., 1996, Polišenská**

et al., 2011) and has been investigated as a tool for cultivar identification **(Šliková et al., 2015)**. Alcohol soluble prolamins with molecular weights ranging from 22 kD to 33 kD and there are deficient in the essencial amino acid lysine. The avenins are located in the protein bodies **(Pernollet et al 1982)** and have been divided to alpha-avenins (lower molecular weight) and beta-avenins (higher molecular weight) which cannot readily be extracted separately, both types are present in Finnish oat cultivars **(Jussila et al 1992)**. In comparison with wheat gliadins, the avenins have been little studied, and the number of full avenin genes present at the moment in the databases is limited and from few genes. The variability of avenin genes in oats is not well represented. In developing oat endosperms, globulins and avenins are located in vacuolar protein bodies. The globulins aggregate within the protein bodies whereas the avenins aggregate in the rough endoplasmic reticulum **(Lending et al 1989)**. Several workers used reversed-phase high-performance liquid-chromatography **(Lookhart, 1985**, **Lokhart and Bean, 1995)** or two-dimensional polyacrylamide gel electrophoresis for the specification of phylogenetic relationships among *Avena* species and variations **(Nalecz et al**., **2009)**. Western blot analysis with gluten polyclonal antibody is usefull method for qualitative detection of prolamin complex**. (Socha et al., 2011)**. Soluble protein and aggregates in oat was study using asymetric flow field-flow fractionation (AF4) coupled with online UV vis spectroscopy and multiangle light-scattering detection (MALS) and AF4 fraction is usefull for characterization by sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE). AF4 fraction revelaed three peaks, which were determined to be monomeric forms of soluble proteins, globulin aggregates, and B-glucan, respectively **(Runyon et al., 2013)**. In this study, 15 registered oat cultivars were examined based on avenin and glutelin pattern using acid-PAGE and SDS-PAGE techniques for identification and verification.

MATERIAL AND METHODOLOGY

From List of Registered Varieties 12 oat varieties are originated from research and Breeding Station Víglas-Pstruša, two varieties originated from Germany and one originated from Czech Republic. Five varieties (Detvan, Hronec, Izak, Tatran and Važec) were analysed of naked oats *(Avena nuda*) and ten varieties (Flamingsgold, Atego, Kanton, Prokop, Václav, Valentín, Vendelín, Viliam, Vojtech and Zvolen) of husked oats (*Avena sativa*). Acid-PAGE was carried out according to the standard reference ISTA method **(Draper, 1987)**. Proteins were extracted from seeds of 20 mg finely ground powdered seeds with 240 µl extracting solution (0.05 g Methylgreen, 25% 2-chloroethanol), stained overnight at room temperature, and centrifuged for 5 minutes at 5 000xg. From gel solution of 10% acid-PAGE (pH: 3.1) was prepared 0.75 mm think gel, using vertical Hoefer 800 unit. Electrophoresis was carried out at 200 V for 20 minutes, 600 v for 3 hours at 4 °C. Staining of gels was performed in a solution of Coomassie Brilliant Blue R250 dissolved in acetic acid and methanol solution. Gel was scanned with densitometer GS 800 (Bio-Rad) and evaluated with Quantity One-1D Analysis Software.

SDS-PAGE was carried out according to UPOV. Stock extraction buffer was used according to ISTA method for wheat. Extracting solution for oat seed was prepared fresh from 2.8 ml stock extraction buffer, 0.79 ml mercaptoethanol and 1.5 ml DMF (dimethylformamide) with distilled water. Crushed seed (45 mg) was extracted with 1 ml of extracting solution. Extraction was performed at room temperature overnight and heating in boiled water for 5 minutes, centrifugation at 5000 xg for 5 min. 10 µl of extracts were applied to the sample wells. The gel (1.0 mm thick) consists of two parts: stacking gel (3.5% acrylamide, pH = 6.8 acrylamide) and resolution gel (10% acrylamide, pH = 6.8). Vertical unit Bio-Rad protean II xi Cell was used for gel electrophoresis. Protein separation was carried overnight (2 hours at 20 mA, 16 hours at 30 mA at 4 °C). Gels were fixed with fixing solution (water/methanol/acetic acid/700ml:200ml:100ml for 20 minutes and then stained in a solution of Coomassie Brilliant Blue R250, dissolved in acetic acid and methanol solution. Gel was scanned with densitometer GS 800 and evaluated with Quantity One-1D Analysis Software.

RESULTS AND DISCUSSION

Alcohol – soluble seed proteins (avenins) present a typical spectrum of this plant species **(Dumplupinar et al., 2011; Dvoráček et al., 2003; Hansen et al., 1988)**. Avenin proteins have structural homology to the S-rich subgroup α-gliadins and γ-gliadins of wheat, the B-hordeins of barley, and γ-secalins of rye. Prolamin is more variable in structure and sequence than globulin, because the major groups of prolamins in the Triticeae (wheat, barley, rye) maize and millet had a separate evolutionary origins **(Shewry and Halford, 2002)**. In addition rye, wheat and barley contain a high concentration of prolamin and a comparatively small amount of glubulins, whereas the situation is reversed in oat. The relatively low final concentration of prolamins in oat may partly be due to the fact that major avenin synthesis begins approximately one week after that of the glubulins **(Robert et al., 1983)**.

Avenin protein band numbers ranged from 5 to 11. In previous studies 7 to 11 bands were found **(Portyanko et al., 1998)**, 7 to 14 **(Jussila et al., 1992)**, 4 to 16 **(Dumlupinar et al., 2011)**, in *Avena sativa* and 24 to 34 in *Avena fatua* **(Mirza et al., 2007)** with a different method. On the basis of component mobility we evaluated all bands which were used for clustering of oat varieties. Jaccard's similarity coefficient was calculated from the data generated by 2-state scoring of the band patterns obtained in Acid-PAGE system From the similarity matrix a dendrogram was constructed using statistical analysis by means of SPSS software 22 (IBM SPSS, Chicago, Illinois, USA). The tree-cluster analysis illustrates the distribution of varieties in two major groups. The first major group was large with nine varieties from *Avena sativa* L. (Vendelín, Zvolen, Prokop, Václav, Vojtech, Valentín, Viliam, Kanton, and Atego) and following varieties were determined as identical: Vendelín-Zvolen and Prokop-Václav. The second group consisted of five varieties of *Avena nuda* L. (Detvan, Hronec, Izak, Važec and Tatran) and one from *Avena sativa* L. genotype Flamingsgold. The two identical pairs with identical composition of protein pattern were found Detvan-Hronec and Izak-Važec.

Table 1 Genetic similarity between oat varieties estimated by A-PAGE of avenins.

Proximity Matrix

	Jaccard Measure														
Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1:Detvan	1.000														
2:Hronec	1.000	1.000													
3:Flamingsgold	.778	.778	1.000												
4:Izak	.333	.333	.333	1.000											
5:Važec	.333	.333	.333	1.000	1.000										
6:Tatran	.385	.385	.385	.500	.500	1.000									
7:Atego	.071	.071	.071	.154	.154	.308	1.000								
8:Kanton	.273	.273	.273	.167	.167	.333	.300	1.000							
9:Prokop	.182	.182	.182	.083	.083	.364	.333	.375	1.000						
10:Václav	.182	.182	.182	.083	.083	.364	.333	.375	1.000	1.000					
11:Valentín	.273	.273	.273	.077	.077	.333	.300	.714	.571	.571	1.000				
12:Vendelín	.077	.077	.077	.000	.000	.231	.182	.200	.571	.571	.333	1.000			
13:Viliam	.273	.273	.273	.077	.077	.231	.182	.714	.375	.375	.714	.333	1.000		
14:Vojtech	.273	.273	.273	.167	.167	.455	.300	.200	.571	.571	.333	.333	.200	1.000	
15:Zvolen	.077	.077	.077	.000	.000	.231	.182	.200	.571	.571	.333	1.000	.333	.333	1.000

This is a similarity matrix

Table 2 Genetic similarity between oat varieties estimated by SDS-PAGE of glutelins.

Proximity Matrix

	Jaccard Measure														
Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1:Vendelin	1.000														
2:Zvolen	.600	1.000													
3:Detvan	.294	.571	1.000												
4:Hronec	.467	.571	.667	1.000											
5:Izak	.786	.786	.438	.643	1.000										
6:Važec	.733	.733	.412	.600	.929	1.000									
7:Prokop	.533	.438	.400	.400	.412	.389	1.000								
8:Václav	.200	.125	.143	.143	.188	.176	.308	1.000							
9:Tatran	.333	.333	.385	.385	.400	.375	.357	.077	1.000						
10:Atego	.125	.125	.231	.231	.188	.176	.133	.333	.273	1.000					
11:Flamings	.438	.438	.615	.615	.500	.471	.467	.308	.462	.417	1.000				
12:Kanton	.375	.375	.429	.333	.278	.333	.615	.143	.385	.067	.400	1.000			
13:Valentin	.375	.375	.538	.538	.353	.333	.750	.231	.385	.231	.615	.667	1.000		
14:Viliam	.400	.400	.462	.357	.294	.278	.667	.154	.417	.071	.429	.900	.727	1.000	
15:Vojtech	.250	.333	.385	.286	.313	.294	.267	.273	.231	.273	.357	.286	.385	.308	1.000

This is a similarity matrix

Glutelins from oat varieties reveal a higher distinguishing power than acid-PAGE does. The number of protein markers was 22. Jaccard's similarity coefficient was calculated from the data generated by 2-state scoring of the band patterns obtained in SDS-PAGE system. The dendrogram tree (Fig.1) demonstrated the relationship among the fifteen registered oat varieties according to the similarity index detected by glutelin protein patterns, using UPGMA cluster analysis. The dendrogram was divided into two main clusters. The first one contained only two varieties from *Avena sativa* L. (Václav and Atego), while the second cluster contained the rest varieties. The second cluster was divided into 4 subclusters. One group consisted of four varieties from *Avena nuda* L. (Izak, Važec, Vendelín and Zvolen). The second group consisted of one variety from *Avena nuda* L (Detvan) and two varieties of *Avena sativa* L. (Hronec and Flamingsgold). Two Slovakian varieties Izak and Važec from Breeding Station Viglaš-Ptruša with similarity value 92.9% were grouped with variety Vendelin. Two varieties Kanton and Viliam with similiarity value 92% were grouped with Prokop and Valentin. They indicated that most of registered varieties of oat were readily differentiated, some very closely related varieties gave identical glutelin pattern and these could be used as genetic markers.

CONCLUSION

Both techniques may provide useful information on the level of polymorphism and diversity in oat cultivars. 13 registered cultivars originated from Breeding Station Víglaš-Pstruša were very closely related. Glutelins from oat varieties reveal a higher distinguishing power than avenins do. Result from this study show that protein markers are powerful and efficient in characterising and identifying of oat varieties in addition to their usefulness in phylogenetic studies.

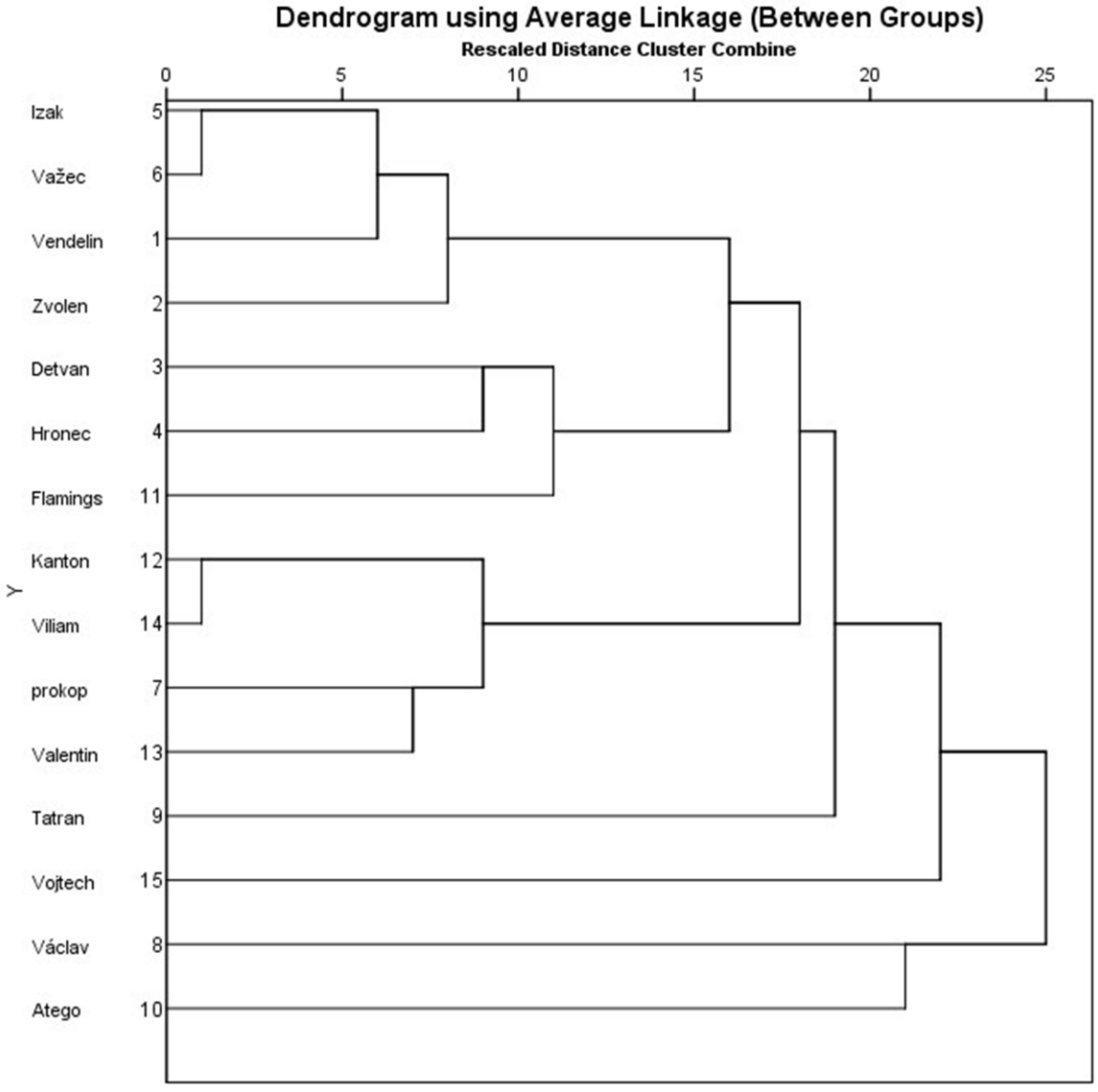

Figure 1 Dendrogram of genetic relationships between 15 oat cultivars, constructed by UPGMA based Jaccard coefficient, based on glutelin patterns.

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