



UTILIZATION OF STORAGE PROTEINS POLYMORPHISM FOR DIFFERENTIATION OF WHEAT, RYE AND TRITICALE GENOTYPES

Milan Chňapek*¹, Marián Tomka¹, Zdenka Gálová^{2,1}, Želmíra Balážová¹

Address: Ing. Milan Chňapek, PhD.,

¹Slovak University of Agriculture, Department of Biochemistry and Biotechnology,
Faculty of Biotechnology and Food Sciences, Tr. Andreja Hlinku 2, 94976 Nitra,
Slovak Republic, phone number: +421 37641 4277.

prof. RNDr. Zdenka Gálová, CSc.,

²Institute of Chemistry, Centre of excellence for white-green biotechnology, Slovak Academy
of Sciences, Tr. Andreja Hlinku 2, 94976 Nitra, Slovak Republic.

*Corresponding author: milan.chnapek@uniag.sk

ABSTRACT

The aim of the paper was to observe and to detect genetic variability of 32 bread wheat genotypes (*Triticum aestivum* L.), 5 rye genotypes (*Secale cereale* L.) and 5 genotypes of triticale (*Triticosecale* Wittmack.) on the basis of polymorphism of storage proteins. Storage proteins consist of two fractions, which are the main part of grain proteins and are used as a marker not only for genetic variability investigation, but also for characterization of genotypes. Polyacrylamide gel electrophoresis with sodium dodecyl sulphate (SDS-PAGE) was used to separate proteins according their molecular weight. Doc-It LS software was used to detect and to calculate variability of genotypes within individual cereal species. The Jacquard coefficient of similarity and the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) algorithm was used for construction of dendrogram of similarity. Our results show, that genotypes of bread wheat are homogenous and single line, but rye and triticale genotypes were heterogenous. There were observed two main clusters and four subclusters in collection of bread wheat and rye genotypes. Analysis of triticale genotypes similarity show two main clusters, but only one of them was further separated into two

subclusters. There were also recorded, that some of the genotypes have no dissimilarity and is necessary to perform additional analysis using 2-D electrophoresis or mass spectrometry. Genotypes PS 28/08 (wheat), Židlochovické ranné (rye) and Benetto (triticale) were the most different.

Keywords: cereal polymorphism, genetic variability, storage proteins of cereals

INTRODUCTION

The cereal grains can be defined as “flowering plants of the Grass Family (*Poaceae* or *Gramineae*), whose seeds are used as food”. Their name is derived from “Ceres”, the Roman goddess of grain. The most important cereal grains are the cool-season crops – wheat, barley and rye as well as the warm-season cereals – rice and maize. Their growing areas take all continents, with the obvious exception of Antarctica and because of that are cereal grains the dominating sources of food for mankind. They can also be used as a major source of feed for animals and for production of nonfood products (Wrigley et al., 2004).

Bread wheat (*Triticum aestivum* L.) is one of the world-wide growing cereal crops in terms of total production used for food. Within the human food is wheat currently the second to rice and ahead of maize as the main human food crop and is the leading source of cereal proteins in human food. Wheat grain is a staple food used to make flour for bread, biscuits, cookies, pasta and noodles (Ortiz et al., 2008).

Rye (*Secale cereal* L.) is cultivated grass plant which is grown worldwide and used as grain and straw. Rye grains are second after wheat in the production of bread. Importance of rye is also in the production of mixed feeds for livestock and for distillation of rye whiskey. Rye has some advantages over wheat in properties related to human nutrition. Flour from rye is milled to a higher extraction rate and has higher content of dietary fiber and lysine. Rye-baked products have stronger flavor and gummier texture and are well liked by many consumers (Bushuk et al., 2004).

Triticale (*Triticosecale* Wittmack.) is the first man-made cereal grain crop species resulting from the hybridization of wheat with rye and combines good qualitative attributes of both parents. Triticale obtained properties for food production from wheat and adaptive properties from rye. Triticale is also a germplasm source for wheat improvement by transfer of desirable rye characteristics. Human consumption of triticale has not yet become widespread.

Flour from triticale has low level of gluten and bread made from it is heavy, because of it must be mixed with rye or wheat flour. Most triticale production is used for animal feed (Furman et al., 2004).

Introduction of productive and improved varieties of cereals caused, that cereals landraces cultivars, which were widely cultivated in the early 20th century are now increasingly being replaced. This has resulted in the loss of genetically diverse, locally well-adapted but unimproved landraces and in an extinction of genetic variability (Royo et al., 2009). Cereals gene pool narrowing leads to an increased risk of vulnerability to diseases and pests. It also leads to decrease in abiotic stress tolerance (Mondini et al., 2010).

Evidence of distribution and extent of genetic variation within and between populations is essential and is necessary to estimate any possible loss of genetic diversity. There is also very necessary, to trace the available genetic variability and its potential in breeding programs. High variability in genetic diversity also depends on selection of possible population for *in situ* and *ex situ* conservation and on evidence of the evolutionary forces influencing natural populations (Semagn et al., 2002).

Cereal storage proteins are characterized by high polymorphism. Their utilization as markers suitable for identification and differentiation of genotypes variability in comparison with other markers of genetic variability has many advantages. They are not so dependent on environmental conditions and ontogenetic stage of the plant. Methods based on storage proteins analysis are not extremely costly in terms of material and instrumentation compared to the detection of polymorphism by DNA markers. Considering these aspects the polymorphism of storage proteins in grain is very suitable for the detection of the genetic variability of cereals (Vyhnánek et al., 2005; Žiarovská et al., 2012).

Great number of genetical, biochemical and technological investigations of cereal storage proteins have been performed using electrophoretic characterization. Electrophoreograms of cereal storage proteins provide information about identity of cultivars, protein polymorphisms and technological quality of grain. Polyacrylamide gel electrophoresis has been developed for identification of cereal cultivars by “fingerprinting” of their storage proteins. The reproducibility of analysis and the high resolution of cereal storage proteins by electrophoresis on polyacrylamide gel are perform electrophoresis as one of the best methods for separation and visualization of proteins (Dukič et al., 2005).

The objective of the present study was to detect the genetic variability by means of spectrums of storage proteins in the grain of wheat, rye and triticale.

MATERIAL AND METHODS

Plant materials

32 genotypes of bread wheat (*Triticum aestivum* L.) originating from Slovakia, Austria, Czech Republic and Netherlands, 5 genotypes of rye (*Secale cereal* L.) originating from Poland, Russia and Czech Republic and 5 genotypes of triticale (*Triticosecale* Wittmack.) originating from Poland, Slovakia, Russia, France and Germany were obtained from the Gene Bank of the Slovak Republic in the Plant Production Research Center Piešťany.

Sample preparation

Proteins were extracted from individual grains according to standard ISTA method (Wrigley *et al.*, 1992).

Seed storage proteins were isolated from whole, dry and mature grains. There were analyzed 20 individual grains from each genotype. Each grain was measured and mechanical homogenized. After homogenization was added 8 µl of extraction solution per 1 mg of grain.

Composition of extraction solution:

- 12,5 ml 1 mol.dm⁻³ Tris-HCl pH 6,8,
- 20,0 ml glycerol,
- 24,1 ml water,
- 4 g SDS,
- 20,0 mg Pyronin Y.

Extraction of storage proteins was performed by 30 min. vortexing in 100 °C following by 10 min. centrifugation (15000 rpm). Supernatant were transferred to a new tube.

Electrophoretical separation of proteins in SDS-PAGE

Seed storage proteins separation was realized in vertical discontinual electrophoretic system Hoefer SE 600 DeLuxe. SDS polyacrylamide gel was used as a separation medium according to standard ISTA method (Wrigley *et al.*, 1992).

5 µl of each sample was loaded into gel. Seed storage proteins separation was running for 20 hours with constant current 10 mA.

Gel staining and image analysis

Protein fraction of separated seed storage proteins were stained in solution of Commasie Brilliant Blue R 250 in ethanol and 10 % TCA. Image analysis of SDS-PAGE gels was carried out using DocIt-LS software (Ultraviolet Products) using an automated process supplemented with occasional manual adjustments. Each lane was first defined where the average intensity across the width of these lanes was depicted as a function of the distance in pixels from the top of the image. The background was subtracted from each profile (parameters adjusted on a case-by-case basis), after which the bands were identified. The DocIt-LS software was also used for statistical interpretation of the eletrophoreograms, i.e. by calculation of Jacquard coefficient of similarity and elaboration of a dendrogram.

RESULTS AND DISCUSSION

Harlan *et al.* (1975) suggested that the genetic variation can be caused by human activities, environmental factors and by dynamic process of hybridization. Physical and biotic environmental factors exert selection pressure on genotypes, leading to the establishment of specific types, which indicates that the genetic variation is different from place to place depending on differences in the environment.

Cereal breeding programs have been focused mainly on quality and quantity of production in last few decades which result to decreasing of genetic variability and narrowing of polymorphism. Therefore, growing genotypes of cereals are high productive with good quality, but their adaptability to environmental conditions and resistance to biotic and abiotic stress factors are on the low level. Polymorphism of individual cereal species is necessary to investigated not only for identification and differentiation of genotypes, but also for searching of new genetic sources which can be utilized in breeding process (**Dimova *et al.*, 2010**).

Most of earlier studies about cereal variability have been based on highly heritable agro-morphological characters, on isozymes and on cytological markers (**Eticha *et al.*, 2005**). Cereal storage proteins are widely used in the research on plant populations due to the genetically determined variability of their characteristic. They are characterized with high level of polymorphism and stability. External factors have no or little effect on their quantity

in mature grain. They are inherited co-dominantly; therefore, the electrophoretic profiles of the seed storage proteins are suitable for identification of cultivars (**Dimova et al., 2010**).

Hailu (2011) recommended utilization of Jacquard coefficient in binary matrix analysis for band presence or absence because it gives more weight to matches than to mismatches. Dendrogram linking together genetically similar clusters of samples and is constructed on the basis of binary matrix analysis, mostly using UPGMA algorithm.

The aim of our research was investigation of seed storage protein polymorphism in 5 genotypes of rye (*Secale cereal* L.), 5 genotypes of triticale (*Triticosecale* Wittmack.) and 32 genotypes of bread wheat (*Triticum aestivum* L.).

It follows from our results (figure 1), that all analyzed genotypes of bread wheat were homogenous and single line. Uniformity of individual analyzed genotypes of bread wheat collection is a result of intensive breeding programs focused on quality and quantity of production. Application of UPGMA algorithm in dendrogram construction has separated collection of bread wheat into two main clusters with dissimilarity 0,180. Each of main clusters was divided into two subclusters with dissimilarity 0,093 and 0,085. Genotype PS 28/08 was the most different, because alone was separated as subcluster with dissimilarity 0,085. Rest of the collection was separated into 6 clusters with dissimilarity from 0,056 to 0,050. Presence of genotypes in clusters varies from 2 to 10. The most different genotypes within clusters were Illias and Pinta, respectively Cornelius and IS Jarissa.

Genotypes with similar HMW-GS composition were different on the basis of storage proteins analyses which correspond with results of authors who referred that gliadins and LMW-GS are the main part of storage proteins (**Branlard et al., 2003; Wang et al., 2011**).

There were observed two large clusters with dissimilarity 0,050 which were consist of 7 respectively 10 genotypes with no dissimilarity. Mass spectrometry analysis or two-dimensional gel electrophoresis seems to be suitable tools for better resolution of individual proteins (**Cunsolo et al., 2011; Mak et al., 2006**) and differentiation of genotypes.

However, our result shows that protein polymorphism of wheat seed storage protein is not variable enough and continuous breeding programs focused on quality and quantity of production may result to production of genotypes which will be productive, but similar and very sensitive to environmental conditions. Therefore, landraces and old genotypes with interesting properties have to be involved into process of hybridization.

Analyse of rye seed storage proteins (figure 2) shows, that genotypes were heterogeneous. There were observed more than one line in each of analyzed genotype. Number of lines in genotypes vary from 3 lines (3 genotypes) to 4 respectively 5 lines (1

genotype). High level of rye seed storage protein polymorphism suggested, that rye is very plastic cereal with very good adaptability to environmental factors and because of that is suitable for extensive system of agriculture. Therefore is rye very popular in Polish and Russian agricultural systems, mainly in areas with colder weather. However, multiline character of rye genotypes may cause problems during processing.

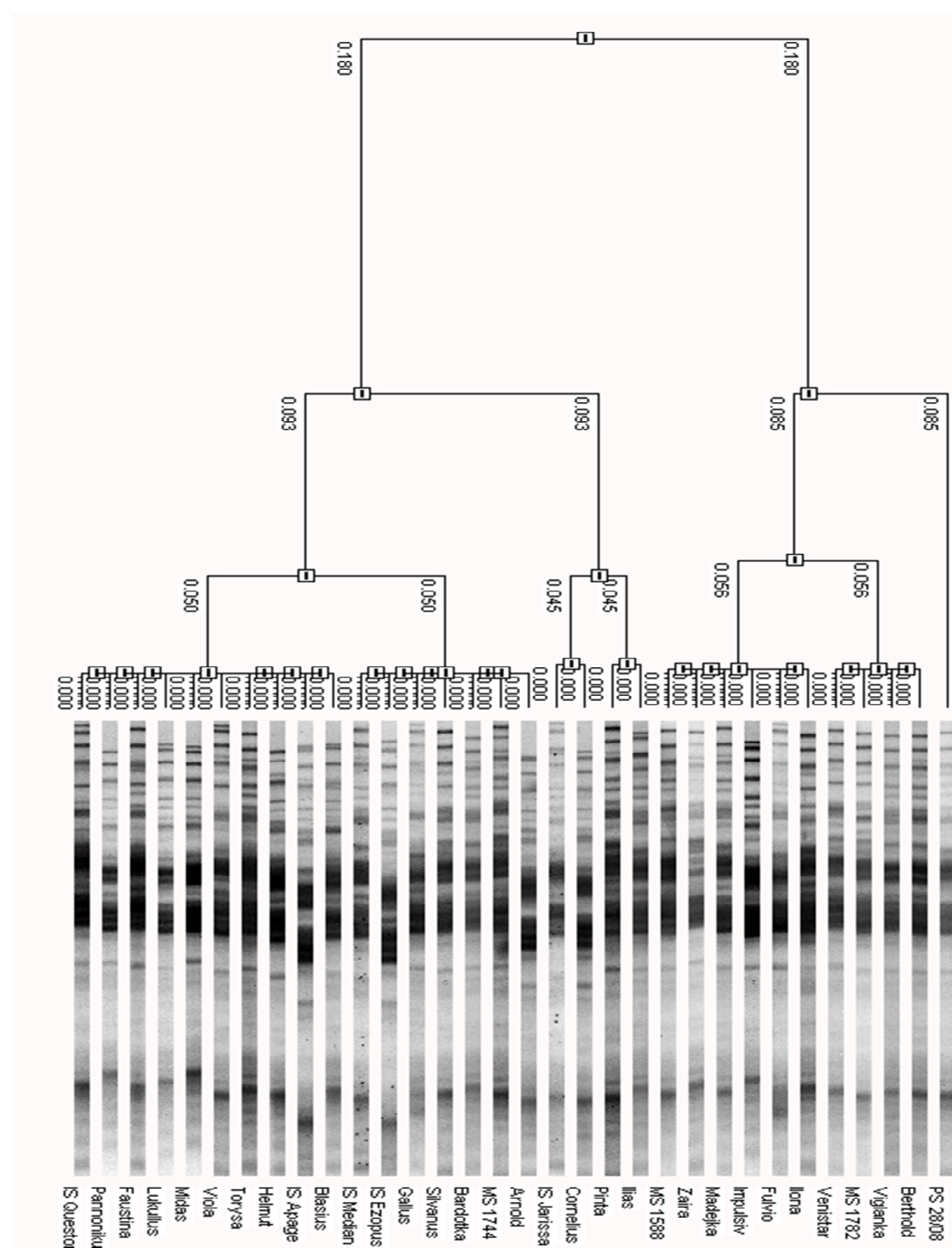


Figure 1 Dendrogram of wheat storage protein polymorphism

Percentage of lines per genotypes has not been calculated, because is necessary to perform more experiments with 100 grains samples, at least.

Application of UPGMA algorithm in dendrogram construction has separated collection of rye genotypes into two main clusters with dissimilarity 0,515. Each of main clusters was divided into two subclusters with dissimilarity 0,198 and 0,358 respectively. Genotype Židlochovické Ranné was the most different with all 4 lines and was observed in subclusters with dissimilarity 0,358, alone. Rest of the collection was separated into 4 clusters with dissimilarity from 0,133 to 0,065. Presence of genotypes/lines in cluster varies from 2 to 5. Determination of the most different genotypes within cluster is very difficult because of multiline character of rye. **Korzun et al (2001)** confirmed our results, that rye is multiline cereal and very good source of valuable characteristics.

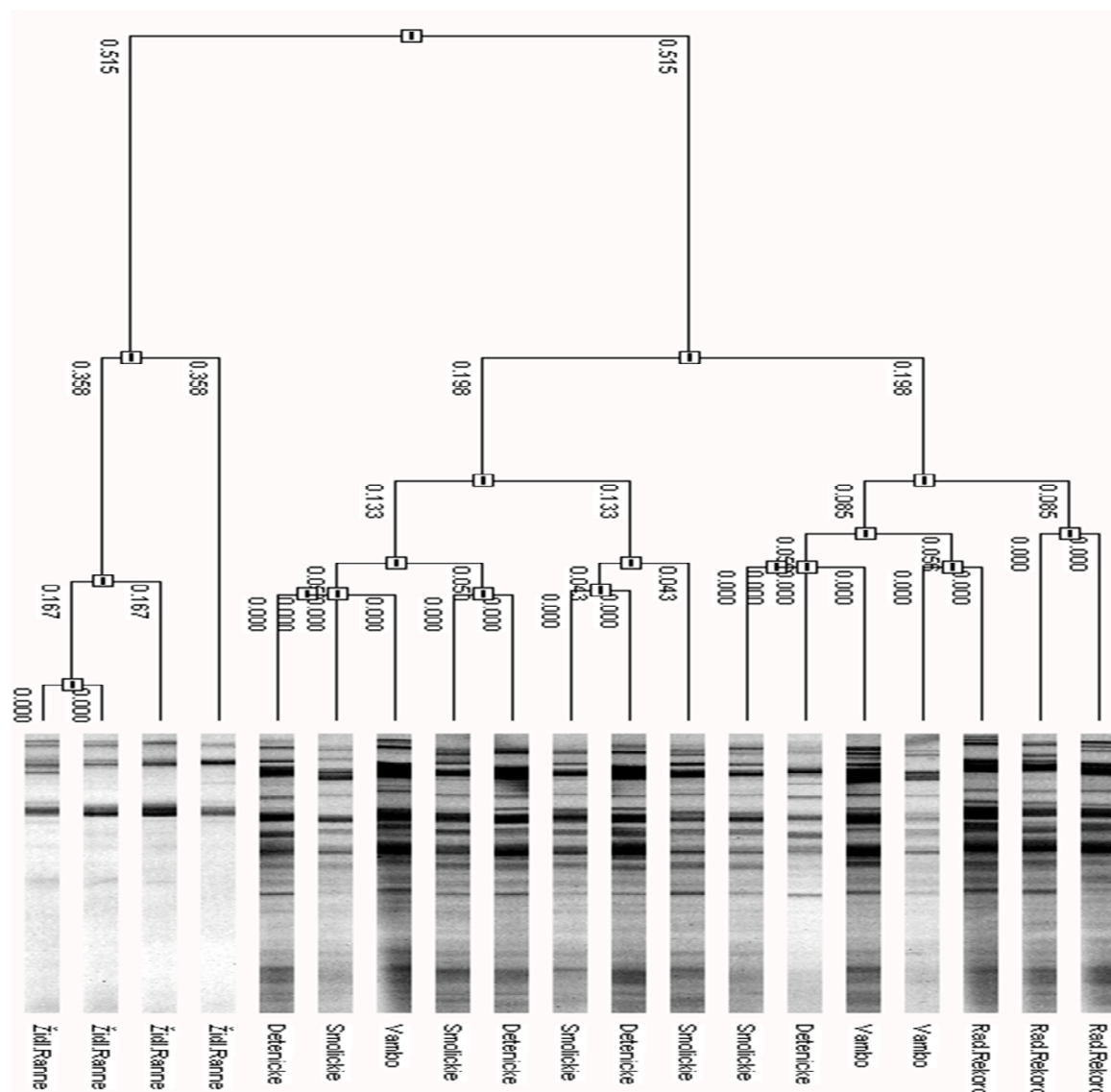


Figure 2 Dendrogram of rye storage protein polymorphism

Triticale is a synthetic type of cereal (Trebichalský et al., 2011) which was created by wheat and rye crossing. Therefore, triticale genotypes combine many of the better features of both of its parents. Therefore, triticale has properties for food production (wheat) and adaptive properties (rye). Our results show (figure3), that 3 of 5 analyzed genotypes were homogenous and single line. Genotype Greneder shows presence of 2 different lines. Genotype Pletomax was the most polymorphic and reported presence of 3 lines. Multiline characteristic of some triticale genotypes is a small disadvantage and together with low level of gluten inhibits their penetration to cereal market. Percentage of lines per genotypes hasn't been calculated because of the same reason as it was with rye.

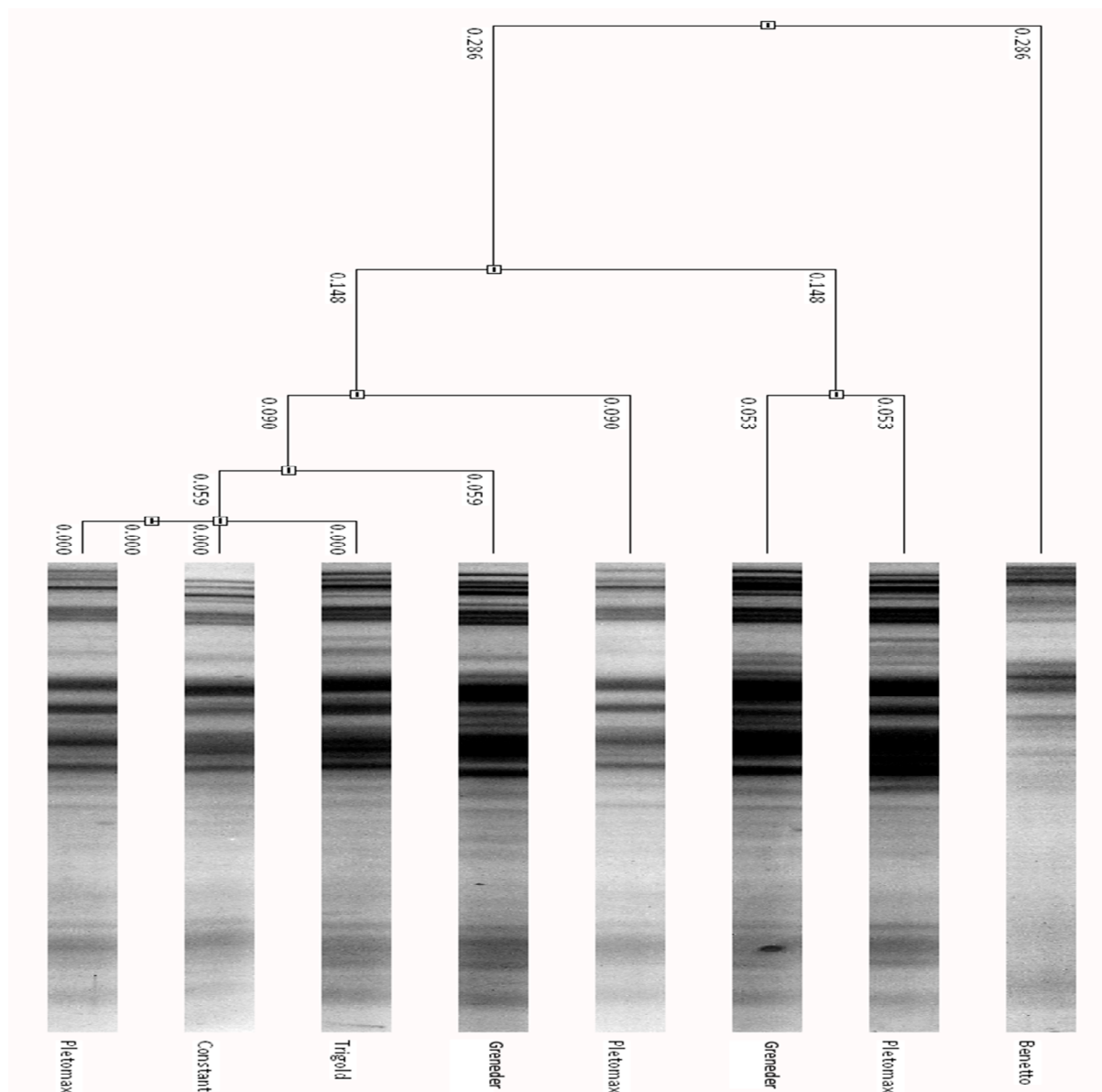


Figure 3 Dendrogram of triticale storage protein polymorphism

Application of UPGMA algorithm in dendrogram construction has separated collection of triticale into two main clusters with dissimilarity 0,286. Genotype Benetto was the most different, because alone was separated as main cluster with dissimilarity 0,286. The second main cluster was divided into two subclusters with dissimilarity 0,148. Each of subclusters was separated into two clusters with dissimilarity 0,090 and 0,053. The most different genotype of this collection was the one line of the genotype Pletomax with dissimilarity 0,090. Presence of genotypes/lines in cluster varies from 1 to 3.

Our results are in suggestion with **Furman (2004)** research, who suggested using triticale as a carrier of important rye genes to wheat.

Wider investigation should be realized to confirm the high level of dissimilarity of cereal genotypes PS 28/08, Židlochovické Ranné and Benetto to eliminate problems connected to extraction, staining or environmental factors.

CONCLUSION

Proteomics and seed storage protein analysis help us to understand polymorphism between different types of cereals as well as gene expression of individual proteins. Observation and evaluation of genetic variability is very important part of hybridization process and helps breeders to cross genotypes with interesting or rare genes which may result in the creation of high adaptive and productive cereal variety.

Acknowledgments: This contribution was supported by the Cultural and Educational Grant Agency of the Slovak Republic KEGA No. 034SPU-4/2012 (50 %) and the project implementation: Centre of excellence for white-green biotechnology, ITMS 26220120054, supported by the Research Development Operational Programme funded by the ERDF (50 %).

REFERENCES

- BRANLARD, G. – DARDAVET, M. – AMIOUR, N. – IGREJAS, G. 2003. Allelic diversity of HMW and LMW glutenin subunits and omega-gliadins in French bread wheat (*Triticum aestivum* L.). 2003. In *Genetic Resources and Crop Evolution*, vol. 50, 2003, p. 669 - 679.
- BUSHUK, W. 2004. Rye. In *Encyclopedia of Grain Science*, 2004, p. 85 - 91, ISBN 0-12-765490-9.

- CUNSOLO, V. – MUCCILLI, V. – SALETTI, R. – FOTI, S. 2011. Mass spectrometry in the proteome analysis of mature cereal kernels. In *Mass Spectrometry Reviews*, vol. 31, 2012, p. 448 - 465.
- DUKIČ, N. – MATIČ, G. – KONJEVIČ, R. 2005. Biochemical analysis of gliadins of wheat *Triticum durum*. In *Kragujevac Journal of Sciences*, vol. 27, 2005, p. 131 - 138.
- ETICHA, F. – BEKELE, E. – BELAY, G. – BÖRNER, A. 2005. Phenotypic diversity in Tetraploid wheats collected from Bale and Wello regions of Ethiopia. In *Plant Gen Res Charac Util.*, vol. 3, 2005, p. 35 - 43.
- FURMAN, B.J. 2004. Triticale. In *Encyclopedia of Grain Science*. 2004, p. 298 - 303, ISBN 0-12-765490-9.
- HAILU, F. – MERKER, A. 2008. Variation in gluten strength and yellow pigment in Ethiopian tetraploid wheat germplasm. In *Genetic Resources and Crop Evolution*, vol. 55, 2008, p. 277 - 285.
- HAILU, F. 2011. Genetic diversity and grain protein composition of tetraploid wheat (*Triticum durum* Desf.) germplasm from Ethiopia. In *Doctoral Thesis*, Swedish University of Agricultural Sciences, Alnarp, 2011, p. 38, ISBN 978-91-576-7646-7.
- HARLAN, J.R. 1975. Our vanishing genetic resources. In *Science*, vol. 188, 1975, p. 618 – 621.
- KORZUN, V. – MALYSHEV, S. – VOYLOKOV, A. V. – BORNER, A. 2001. A genetic map of rye (*Secale cereal* L.) combining RFLP, isozyme, protein, microsatellite and gene loci. In *Theoretical and Applied Genetics*, vol. 102, 2001, p. 709 - 717.
- MONDINI, L. - FARINA, A. - PORCEDDU, E. - PAGNOTTA, M.A. 2010. Analysis of durum wheat germplasm adapted to different climatic conditions. In *Annals of Applied Biology*, vol. 156, 2010, p. 211 - 219.
- ORTIZ, R. - BRAUN, H.J. - CROSSA, J. - CROUCH, J.H. - DAVENPORT, G. - DIXON, J. - DREISIGACKER, S. - DUVEILLER, E. - HE, Z. - HUERTA, J. - JOSHI, A.K. - KISHII, M. - KOSINA, P. - MANES, Y. - MEZZALAMA, M. - MORGOUNOV, A. - MURAKAMI, J. - NICOL, J. - FERRARA, G.O. - ORTIZ-MONASTERIO, J.I. - PAYNE, T.S. - PEÑA, R.J. - REYNOLDS, M.P. - SAYRE, K.D. - SHARMA, R.C. - SINGH, R.P. - WANG, J. - WARBURTON, M. - WU, H. - IWANAGA, H.M. 2008. Wheat genetic resources enhancement by the International Maize and Wheat Improvement Center (CIMMYT). In *Genetic Resources and Crop Evolution*, vol. 55, 2008, p. 1095 – 1140.
- ROYO, C. - ELIAS, E.M. - MANTHEY, F.A. 2009. Durum wheat breeding. In *Cereals*, 2009 p.199 - 226, DOI:10.1007/978/-0-387-72297-9.

SEMAGN, K. 2002. Genetic relationships among ten endod types as revealed by a combination of morphological, RAPD and AFLP markers. In *Hereditas*, vol. 137, 2002, p. 149 - 156.

TREBICHALSKÝ, A. – SCHULMAN, A. – KALENDAR, R. – STRATULA, O. – GÁLOVÁ, Z. – BALÁŽOVÁ, Ž. 2011. Evaluation of IRAP retrotransposon-based markers and BAMY gene polymorphism to find genetic relationship between spring triticale and rye cultivars. In *Applied Natural Sciences* (Medzinárodná konferencia: Aplikované vedy), Papiernička –Častá, 2011, p. 311, ISBN 978-80-8105-266-8.

VYHNÁNEK, T. – BEDNÁŘ, J. 2005. Polymorphism of prolamin proteins of the grain of triticale varieties certified in the Czech Republic. In *Plant, Soil and Environment*, vol. 51, 2005, p. 151 - 155.

WANG, K. – GAO, L. – WANG, S – ZHANG, Y. – LI, X. – ZHANG, M. – XIE, Z. – YAN, Y. – BELGARD, M. – MA, W. 2011. Phylogenetic relationship of a new class of LMW-GS genes in the M genome of *Aegilops comosa* L. In *Theoretical and Applied Genetics*, vol. 122, 2011, p. 1411 - 1425.

WRIGLEY, C. 2004. Cereals. In *Encyclopedia of Grain Science*, 2004, p. 187 - 273, ISBN 0-12-765490-9.

Mak, Y. – Skylas, D. J. – WILLOWS, R. – CONNOLLY, A. – CORDWELL, S. J. – WRIGLEY, C. W. – SHARP, P. J. – COPELAND, L. 2006. A proteomic approaches to the identification and characterization of protein composition in wheat germ. In *Functional & Integrative Genomics*, vol. 6, 2006, p. 322 - 337.

ŽIAROVSKÁ, J. - RAŽNÁ, K. - SENKOVÁ, S. - ŠTEFÚNOVÁ, V. - BEŽO, M. 2012. Variability of *Linum usitatissimum* L. based on molecular markers. In *Journal of Agricultural and Biological Science*, vol. 7, 2012, p. 50 - 58. ISSN 1990-6145.