Journal of Microbiology, Biotechnology and Food Sciences

Tomka et al. 2012 : 1 (February Special issue) 703-710

REGULAR ARTICLE

ANALYSIS OF POLYMORPHISM OF BARLEY STORAGE PROTEINS

Marián Tomka[1], Milan Chňapek[1], Jana Bradová[2], Zdenka Gálová[1]*

Address:[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Trieda Andreja Hlinku 2, 949 76, Nitra, Slovak Republic,

[2] Crop Research Institute, Drnovska 507, 161 06 Prague 6, CzechRepublic

*Correspondingauthor: marian.tomka@uniag.sk

ABSTRACT

Identification and characterization of different genotypes is essential for improving the quality of cultivated varieties in breeding programs. Electrophoretic separation of barley storage proteins allows us to identify and to differentiate the individual genotypes. We used acid polyacrylamid gel electrophoresis (A-PAGE) and starch gel electrophoresis (SGE) to differentiate14 barley genotypes.Using SGE we were able to detect 29 lines while using APAGE we have detected only 22 lines. A total of 43 different alleles were deteceted and based on their combination we observed 20 hordein profiles using each method. The frequency of alleles varied from 0.51 % to 9.64 % for A-PAGE and from 1.0 % to 11.0 % for SGE. On the basis of detected alleles dendrograms were constructed using UPGMA algorithm. These dendrograms revealed genetic relations between varieties and divided collection of barley genotypes into two main groups based on A-PAGE and SGE. Both methods confirmed heterogeneity of barley varieties what allowed us to study intravarietal polymorphism.

Keywords: barley, hordeins, polymorphism, genetic diversity, protein markers

INTRODUCTION

Cereals are the most grown crops in the world. Large number of methods is used to learn more about genomes of individual organisms because knowledge of the genome is a key element for handling with genetic information of the crop.

Identification and characterization of different plant genotypes is the primary assumption for increasing the quality of grown varieties at breeding programs. Also, information about variety origin can be useful for choosing convenient genotype for specific agroclimatic conditions and for final agricultural plant utilization. Barley (*Hordeum vulgare* L.) is one of the most important cereals cultivated at Slovakia. Winter form of barley has higher stability of yield and lower soil requests comparing to spring form **(Žáková andBenková, 2004).**

Prugar and Hraška (1989) present that the hordein electrophoretic image is genetically determined, characteristic for each variety of barley and therefore it is suitable for the differentiation and identification of varieties or lines and marking economically important characteristics and attributes.

The gel electrophoresis is the most widely used and successful biochemical method applied to barley variety identification from the grain. In particular, electrophoresis of the alcohol-soluble seed storage protein fraction (hordeins) has been extensively researched **(Chňapek*et al.*, 2007)** to assess origin of cultivated plants, assign genome structure, realize genome analyses, identify varieties and detect lines and mutants **(Gregová*et al.*,1995).**

The aim of this work was to analyze polymorphism of barley storage proteins extracted from 14 different barley varieties using starch gel electrophoresis (SGE) and acid polyacrylamid gel electrophoresis (A-PAGE).The minor goal was to compare ability of these methods to differentiate barley genotypes.

MATERIAL AND METHODS

We analyzed 14 barley genotypes from Gene Bank of the Slovak Republic in Piešťany, Central and Testing Institute in agriculture in Bratislava (UKSUP) and AGRO BSS s r. o. in Sládkovičovo. All of the genotypes had Slovak origin except variety Jubilant which is older and has it origin in Czechoslovakia. For the A-PAGE we used 10 grains and for the SGE we used 48 grains of each variety.

Starch gel electrophoresis

Storage proteins were isolated from endosperm of whole, dry and mature grains by optimized CSN 46 1085-1 **(Bradová andSýkorová, 2006).**

Electrophoresis, fixation and dyeing of starch gels was realized also by optimized CSN 46 1085-1 **(Bradová andSýkorová, 2006).** Nigrosin dyeing solution was used to detect hordeins in starch gels and after dyeing, gels were evaluated and photographed. Evaluation of the gels and identification of alleles was carried out by comparing gels with collection of standards from Crop Research Institute in Prague. Finally, dendrogram was created by hierarchic cluster analysis using UPGMA algorithm with DARwin 5.0.158 software.

Acid polyacrylamid gel electrophoresis

Electrophoresis was carried out at constant voltage 500 V three times longer than the course of marker Pyronin G at 3^{o}C. All protein fractions separated in A-PAGE were dyed in a solution prepared by mixing 95 ml 10% trichloroacetic acid and 5 ml 0.5% solution of Coomassie Brilliant Blue R250 in ethanol. Excess dye was removed from the gel by washing the gel in distilled water for 12-24 hours. Alleles were marked from 1 to 21 in the order they occurred. Dengrogram was created with the same procedure as for SGE.

RESULTS AND DISCUSSION

Hordeins represents major group of storage proteins in barley endosperm. They are convenient tool for differentiation and identification of lines and varieties as well as for marking some important farm attributes **(Shewry*et al.*, 1980).**

Both methods revealed significant intravarietal polymorphism. From 14 analyzed varieties 5 were heterogeneous and a total of 22 lines were detected using A-PAGE. On the basis of SGE we detected 8 heterogeneous lines with in analyzed collection of barley genotypes with a total of 29 different lines. The number of lines per genotype varied from 1 to 6 and the most lines (6) were detected in the genotype Adran.

Almeida and Molina (2000) detected higher level of intravarietal polymophism when 12 out of 14 tested barley varieties were heterogeneous. On the other hand **Leistrumaitė and Paplauskienė (2007)** founded lower level of hordein polymorphism

because their results showed that in 17 spring barley genotypes 14 hordein profiles were present which means that some genotypes had similiar hordein profile.

On the basis of two types of electrophoresis we were able to identify 54 different alleles from which 33 were detected using starch gel electrophoresis and 21 with acid polyacrylamid gel electrophoresis. Differences between obtained results using these two methods we can demonstrate on Figure 1 and Figure 2.

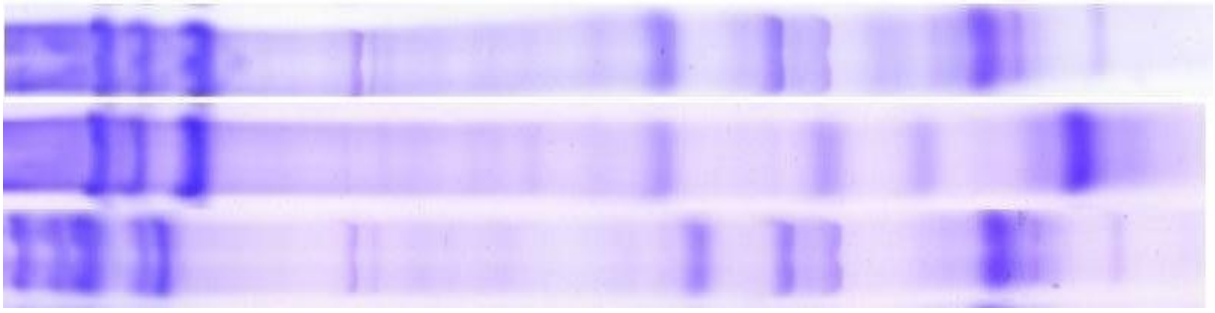

Figure 1 SDS PAGE gel of varieties SK 5451, SK 5835, SK 5840 (up to down respectively)

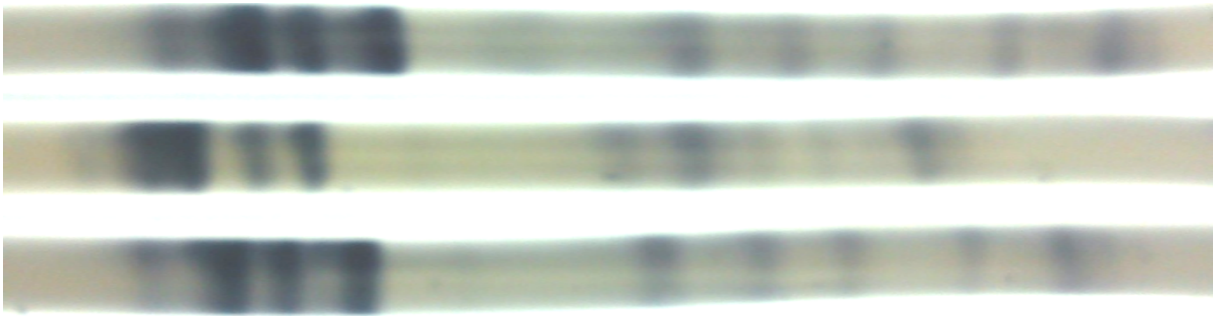

Figure 2 SGE gels of varieties SK 5835, SK 5451, SK 5840 (up to down respectively)

.

A total of 43 different alleles were detected from which 21 were detected using A-PAGE and 22 with SGE. The frequency of alleles varied from 0.51 % to 9.64 % for A-PAGE and from 1.0 % to 11.0 % for SGE (table 1).

Table 1 Frequency and types of detected alleles

Allele (SGE)	A2	A12	A14	A21	A23	A31	AN1	AN2	B3	B5	B19	B21	B29	B34	B47	F1	F2	F3	C	D	E	G
Frequency (SGE)	10	3	2	2	7	2	1	2	5	3	3	2	11	2	3	13	5	11	1	1	10	1
Allele (APAGE)	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	C1	C2	C3	C4	C5	C6	C7	C8	
Frequency (APAGE)	0.51	2.54	4.06	1.02	5.08	8.12	8.12	4.06	2.54	4.57	5.58	6.60	4.06	4.57	3.05	9.64	9.14	5.08	7.61	2.03	2.03	

On the basis of detected alleles a total of 40 hordein profiles were obtained, 20 for each method. Using both methods we have identified the same hordein profile for varieties SK 5397, SK 5407 and 5451 what is also demonstrated with created dendrograms (Fig3, Fig 4). High level of polymorphism in hordein loci, which was detected in our analyses is proved by works of many authors e.g. **Prugar a Hraška (1989)**, **Yin *et al.* (2003)** who detected 72 different alleles within collection of 211 varieties of *Hordeum vulgare* ssp. *agriocrithon* and *H. Vulgare* ssp. *Spontaneum* with frequency from 0.005 % to 0.77 %. **Pomortsev *et al.* (2007)** analyzed 93 Turkish varieties of spring barley and they identified 101 alleles with frequencies between 0.11 % and 41.29 %. **Pomortsev *et al.* (2008)** using starch gel electrophoresis detected 149 alleles in analyzed collection of 140 barley genotypes. These authors detected more alleles than we because of higher number of analyzed barley varieties.

Dendrograms (Fig 3., Fig 4.) were created on the basis of acquired hordein spectra with hierarchic cluster analysis using UPGMA algorithm and they show genetic relations among studied genotypes. Created dendrograms are different in some cases because of numbers of detected lines using A-PAGE or SGE. Differences between number of detected lines are surely caused by number of analyzed seeds and may also be caused by environmental conditions. Only genotype Expres created own subcluster in both dendrograms and also genotypes SK 5451, SK 5407 and SK 5397 are closely linked what is probably caused by similar genetic basis. Except these three varieties we were able to differentiate all others lines using APAGE while using SGE another 11 lines cannot be differentiated because of identical hordein profile. These varieties can be differentiated using polymorphism of esterases.

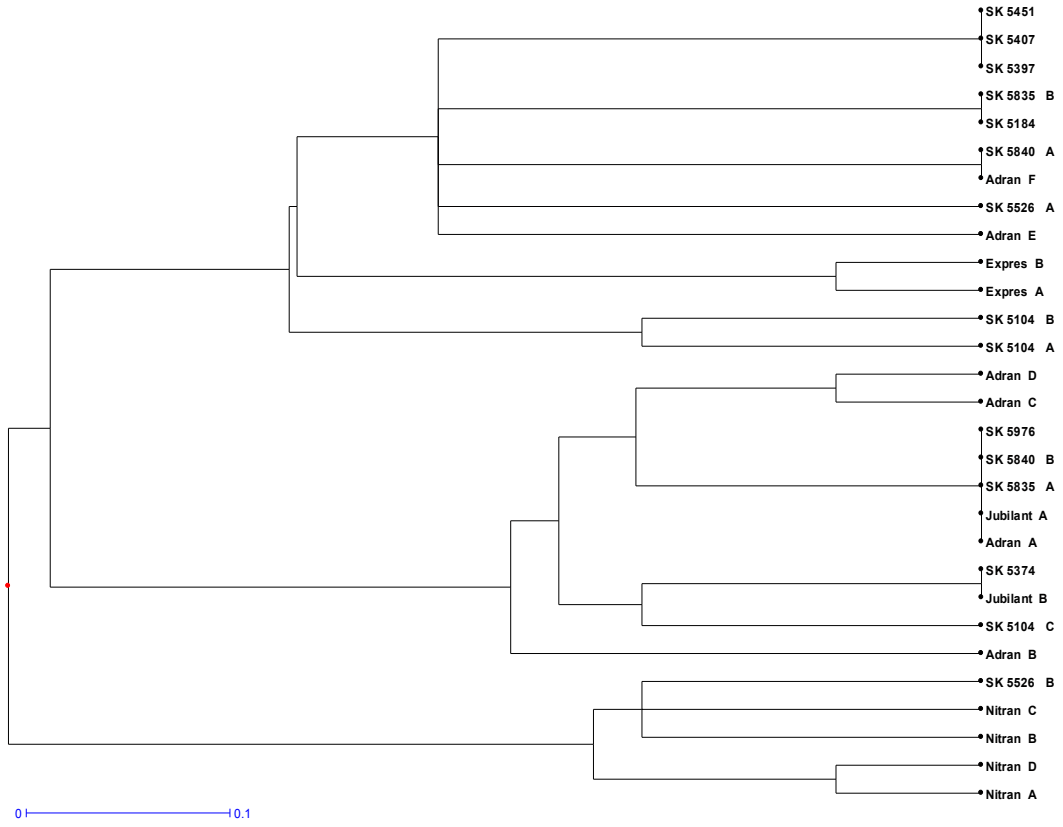

Fig3 Dendrogramofbarleygenotypes on thebasisof SGE

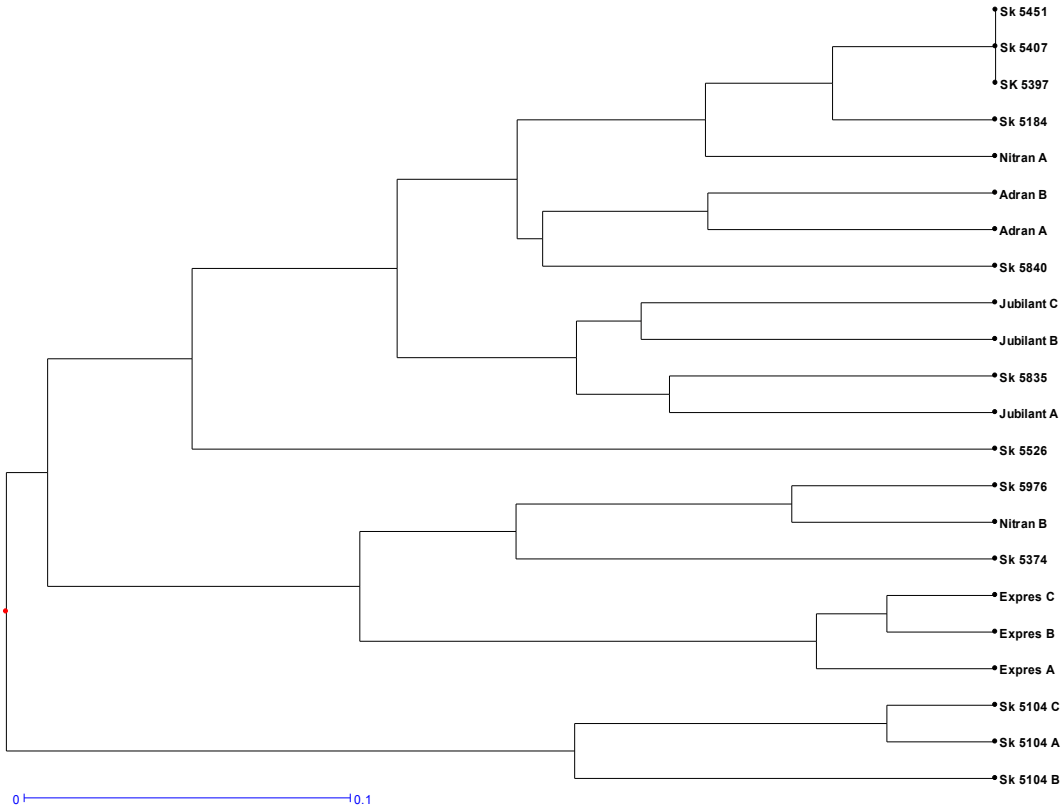

Figure 4 Dendrogram of barley genotypes on the basis of A-PAGE

CONCLUSION

Obtained results show that barley storage proteins can be applied as protein markers for identification and differentiation of spring barley genotypes. High level of detected polymorphism in hordein loci suggests these markers for genetic diversity studies. We can use created dendrograms to study genetic relations among different barley genotypes. We are able to monitor heterogeneity among varieties and to study intravarietal polymorphism using whether A-PAGE or SGE.

Acknowledgments: This contribution is the result of the project implementation: Centre of excellence for white-green biotechnology, ITMS 26220120054, supported by the research Development Operational Programme funded by the ERDF (50%) and VEGA project 1/0471/09 (50%).

We are very thankful to Crop Research Institute in Prague, Czech Republic for useful advice and perfect technical support.

REFERENCES

ALMEIDA, C.E. – MOLINA, S.C. 2000. Hordein variation in Brazilian barley varieties (*Hordeumvulgare* L.) and wildbarley (*H. euclastonSteud*. And *H. stenostachys*Godr.) In *Genetics and Molecular Biology*, vol. 23, no.2, 2000, p. 425-433

BRADOVÁ, J. – SÝKOROVÁ, S. 2006. Optimalizace metod elektroforézy proteinů pro identifikaci odrůd ječmene (*Hordeumvulgare* L.). Praha, VÚRV, 2006, 36 p., ISBN: 80-86555-97-6

CHŇAPEK, M. – VIVODÍK, M. – GÁLOVÁ, Z. – GREGÁŇOVÁ, Ž. 2007. Identification of barley varieties by gel electrophoresis of grainproteins. In *Acta Biochimica Polonica.* Abstracts of the 1st International Conference EUROBIOTECH Biotechnology in Agriculture, Kraków, Poland, vol. 54 Supplement 1, 2007, pp. 44

GREGOVÁ, E. – KRAIC, J. – ŽÁK, I. 1995. Charakterizácia odrôd pšenice pomocou glutenínov. Biochemické, molekulárne a morfologické techniky v identifikácii odrôd rastlín, ÚKSUP, 1995, p.11-14

LEISTRUMAITĖ, A. - PAPLAUSKIENĖ, V. 2007. Genetic resources of spring barley: analysis of hordein polymorphism. In *Biologija*, vol. 53, no. 3, 2007, p. 30-33

POMORSTEV, A.A. – MARTYNOV, S.P. – LIALINA, E.V. 2007. Hordein Locus Polymorphism of Cultivated Barley (*Hordeumvulgare* L.) in Turkey. In *Russian Journal of Genetics,* vol. 43, no. 11, 2007, p. 1294–1300

POMORSTEV, A.A. – MARTYNOV, S.P. – LIALINA, E.V. 2008. Hordein locus polymorphism in near eastern local populations of cultivated barley (*Hordeumvulgare* L.) In *Russian Journal of Genetics*, vol.44, no.6, 2008, p. 815-828

PRUGAR, J. – HRAŠKA, Š. 1989. Kvalita jačmeňa. Príroda Bratislava, 1989, 226p.

SHEWRY, P.R. – FAULKS, A.J. – PARMAR, S. – MIFLIN, B.J. 1980. Hordein polypeptide pattern in relation to malting quality and the varietal identification of maltedgrain. In *Journal of the Institute of Brewing*, vol. 86, 1980, p. 138-141

YIN, Q.Y. – MA, D.Q. – DING, Y. 2003. Analysis of genetic diversity of hordein in wild close relatives of barley from Tibet. In *Theoretical and Applied Genetics*, vol. 107, 2003, p. 837-842

ŽÁKOVÁ, M. – BENKOVÁ, M. 2004. Genetic Diversity of Genetic Resources of Winter Barley Maintained in the Genebank in Slovakia. In *Czech Journal of Genetic and Plant Breeding,* vol. 40, no.4, 2004, p. 118–126