ANALYSIS OF POLYMORPHISM OF BARLEY STORAGE PROTEINS

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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 differentiate 14 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 detected 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 (Žákova and Benková, 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 and Sýkorová, 2006).

Electrophoresis, fixation and dyeing of starch gels was realized also by optimized CSN 46 1085-1 (Bradová and Sýkorová, 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°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 similar 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

<table>
<thead>
<tr>
<th>Allele (SGE)</th>
<th>A2</th>
<th>A12</th>
<th>A14</th>
<th>A21</th>
<th>A23</th>
<th>A31</th>
<th>AN1</th>
<th>AN2</th>
<th>B3</th>
<th>B5</th>
<th>B19</th>
<th>B21</th>
<th>B29</th>
<th>B34</th>
<th>B47</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (SGE)</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>11</td>
<td>2</td>
<td>13</td>
<td>5</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Allele (APAGE)</td>
<td>B1</td>
<td>B2</td>
<td>B3</td>
<td>B4</td>
<td>B5</td>
<td>B6</td>
<td>B7</td>
<td>B8</td>
<td>B9</td>
<td>B10</td>
<td>B11</td>
<td>B12</td>
<td>B13</td>
<td>C1</td>
<td>C2</td>
<td>C3</td>
<td>C4</td>
<td>C5</td>
<td>C6</td>
<td>C7</td>
<td>C8</td>
<td></td>
</tr>
<tr>
<td>Frequency (APAGE)</td>
<td>0.51</td>
<td>254</td>
<td>406</td>
<td>1.02</td>
<td>5.08</td>
<td>8.12</td>
<td>8.12</td>
<td>4.06</td>
<td>2.54</td>
<td>4.57</td>
<td>5.58</td>
<td>6.60</td>
<td>4.06</td>
<td>4.57</td>
<td>3.05</td>
<td>9.64</td>
<td>9.14</td>
<td>5.08</td>
<td>7.61</td>
<td>2.03</td>
<td>2.03</td>
<td></td>
</tr>
</tbody>
</table>

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.
Fig 3: Dendrogram of barley genotypes on the basis of SGE
**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.

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