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Hordein polymorphism between spring barley cultivars by SDS-PAGE electrophoresis

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(Manuscript received 27 April 2017; accepted for publication 28 September 2017)

Abstract. In this study reserve endosperm proteins, hordeins of seven spring barley cultivars with different origin: Zernogradskii (Russia), Bodega, Fink, Scarlett and Barke (Germany), Josefin and Astoria (France) were fractionated and characterized by SDS-PAGE electrophoresis. On the basis of the obtained spectra 19 bands (D + C + B) with different relative electrophoretic mobility and intensity were identified. The electrophoresis profiles of the groups D-, C- and B- hordein are designated as separate types (models) using the index corresponding to hordein blocks. We have established one profile type for D-hordein (D1), two- for C-hordein (C1, C2), and five - for B-hordein (B1, B2, B3, B4, B5). Based on these results hordein formulas (configurations) of accessions are constructed, which enable the expression of specific varietal characteristics and prove the existence of the inter allelic variation (hordein polymorphism) due to the presence or absence of protein components and their different electrophoretic mobility in the profiles of D-, C- and B-hordein.

Keywords: spring barley, storage proteins, hordein types, intervarietal polymorphism, SDS-PAGE

Introduction

The use of biochemical markers of genetic control of useful traits is increasingly used in the breeding of barley (Jones, 1982; Hauser et al., 1982; Stoyanova and Popova, 2002). As a result of long research it was found, that electrophoretic spectra of reserve proteins in barley - hordeins - have been divided into three groups on the basis of their electrophoretical mobility and amino acid composition (Shewry and Milfin, 1985). D-hordeins have the highest molecular weight (105 kD); they are characterized by a high amino acid (glutamine, glicine and proline) content (Shewry and Tatham, 1990). Synthesis of these hordeins is encoded by the Hor 3 locus located on the long arm of chromosome 1H(5) (Kreis et al., 1984). C-hordeins (50–80 kD), rich in glutamine, phenylalanine and proline, and the major B-hordeins (36–45 kD), rich in glutamine, are encoded by the Hor 1 and Hor 2 loci, respectively, both located on the short arm of chromosome 1H(5) (Shewry and Milfin, 1985). The advantages of hordeins for studying the genetic diversity in barley have been described by many authors (Pomortsev et. al., 2002; Výhnanek et al., 2003). The barley storage protein hordein is characterized by a high level of polymorphism (Dimova, 2011; Mihova et al., 2012).

By using electrophoretic SDS-PAGE method spare proteins can be separated into individual fractions (Dimova et al., 2010; Dimova, 2011; Mihova et al., 2012), each of which has a specific relationship with the economic valuable traits. Their expression is stable and independent of environmental conditions (Konarev, 2000; Todorov et al., 2002; Todorov, 2006).

The purpose of this study is to establish hordein polymorphism between seven spring barley cultivars with different origin by SDS-PAGE.

Material and methods

The material object of this study were seven cultivars of spring barley showing a set of valuable traits and qualities (yield, number of fertile tillers per plant, length of spikes, number of grains per spike, number of sterile spikes, mass of grain per spike, mass of grain per plant, mass of 1000 grains, complex disease resistance, vegetative period and resistance to lodging), during the period of study 2012-2015. They are of different origin: Zernogradskii from Russia, Bodega, Fink, Scarlett and Barke from Germany, Josefin and Astoria from France (Table 1).

Table 1. Origin and variety of seven spring cultivars Hordeum vulgare L. subsp. distichon (L.) KOERN

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Origin</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scarlett</td>
<td>Germany</td>
<td>nutans</td>
</tr>
<tr>
<td>Bodega</td>
<td>Germany</td>
<td>nutans</td>
</tr>
<tr>
<td>Fink</td>
<td>Germany</td>
<td>erectum</td>
</tr>
<tr>
<td>Barke</td>
<td>Germany</td>
<td>nutans</td>
</tr>
<tr>
<td>Zernogradskii</td>
<td>Russia</td>
<td>nutans</td>
</tr>
<tr>
<td>Josefin</td>
<td>France</td>
<td>nutans</td>
</tr>
<tr>
<td>Astoria</td>
<td>France</td>
<td>nutans</td>
</tr>
</tbody>
</table>

Table 2. Origin of Boletus edulis species

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scarlett</td>
<td>Germany</td>
</tr>
<tr>
<td>Bodega</td>
<td>Germany</td>
</tr>
<tr>
<td>Fink</td>
<td>Germany</td>
</tr>
<tr>
<td>Barke</td>
<td>Germany</td>
</tr>
<tr>
<td>Zernogradskii</td>
<td>Russia</td>
</tr>
<tr>
<td>Josefin</td>
<td>France</td>
</tr>
<tr>
<td>Astoria</td>
<td>France</td>
</tr>
</tbody>
</table>

* e-mail: neykov.nikolay@gmail.com
Separation of reserve proteins was performed in the laboratory of biochemistry of Dobrudzha Agricultural Institute General Toshevo in 201. The analyses were performed on single grains obtained by self-pollination under the isolator. Attached is a vertical SDS-PAGE electrophoresis.

Hordein extraction is carried out using the method of Singh et al. (1991). Each grain was ground to fine flour (with a pestle in a porcelain mortar), the embryo being previously removed by using a scalpel. Ground kernels were transferred to 1.5 ml Eppendorf tubes. Extraction buffer 0.1 ml 50% (v/v) propanol, 0.08 M Tris – HCl, pH 8.0, containing 1% (w/v) freshly added dithiothreitol (DTT) was added to each tube for the extraction of storage protein of barley (hordein). In the absence of DTT relatively less of the medium molecular weight of hordein bands were extracted especially from seed containing high level of nitrogen. Each sample is mixed for a few seconds of vortex in order to facilitate homogenization and extraction. After incubation for 1 hour at 65°C to each micro tube Eppendorf type 0.1 ml 50% (v/v) propanol was added containing 1.4% (v/v) freshly added 4-vinylpyridine (VP). In this way alkylation of SH-groups was performed in the samples. After incubation for 1 hour at 65°C and centrifugation for 10 minutes at 12000 g, 0.2 ml of each clear supernatant was transferred to a new Eppendorf and 0.2 ml of a solution (sample buffer) containing 2% SDS, 0.08 M Tris - HCl (pH 8.0), 40% glycerol and 0.02% bromophenol blue was added to it. The samples were mixed, incubated for 1 hour at 65°C, centrifuged at 12000 g for 10 minutes, and then they can be used for SDS-PAGE analysis.

The extraction procedure used is carried out in several stages to achieve maximum extraction of alternative endosperm proteins (hordein). Even clearer electropherograms were obtained after further alkylation of the protein molecules prior to being treated with SDS. The main advantage of SDS-PAGE electrophoresis is that it allows simultaneous separation of the B-, C- and D-hordein.

Staining of the gels was performed with a 1% solution of Coomassie brilliant blue (CBB) R 250 in acetic acid, methanol and water at the ratio (1:5:4) overnight. Discoloration of the gel plates was carried out with a solution of acetic acid, methanol, distilled water (1:2:7). Bleach was changed repeatedly to clear the background, then gel plates were scanned.

The gel system adapted was the one described by Laemmli (1970) with some modifications. 12% acrylamide separating gel (pH 8.0) and acrylamide stacking gel (pH 6.8) were used. Forty microliter Temed and 100% 10% APS were used as catalysts. A thirty sample well former (0.75 mm perspex comb) was inserted into the stacking gel and left to polymerize. Hordein extracts from individual kernels (40) were loaded into each sample well with a micropipette. SDS-PAGE was performed at a constant current of 20 mA per plate at room temperature for 18-20 hours. Hordein patterns were classified using the Lallemand–Briand system with modifications (Lallemand and Briand, 1990). Hordein formulas were constructed according to Dimova (2011).

Results and discussion

As a result of the electrophoretic analysis 19 bands with different relative electrophoretic mobility were established. One was identified in the area of D-hordein, three in the area of the C-hordein and fifteen in the area of the B-hordein (Figure 1). The electrophoresis profiles of the D-, C- and B-hordein were indicated by using indices corresponding to the identified hordein blocks (Figure 2). There are 8 types of alternative profile endosperm proteins in the tested cultivars (Table 2, Figure 2). Clearly visible is one profile type for D-hordein (D1), two - for C-hordein (C1, C2), and five - for B-hordein (B1, B2, B3, B4, B5).

There are no identified allelic differences between D-hordein profiles (Hor 3) of the analyzed varieties - Zernogradskii, Bodega, Fink, Scarlett, Barke, Josefin and Astoria, (Table 2, Figure 1, Figure 2). In a study of Nordic electropherograms of barley cultivars the same pattern of the D-hordein was identified for all (Peltonen et al., 1994). The lack of differences between D-hordein profiles of barley cultivars was reported at a later stage in the study by Leistrumati and Paplauskien (2007).

In the area of C-hordein (Hor 1) three band are expressed with different electrophoretic mobility and intensity, two of which are common for hordein models (C1, C2). The electrophoretic profile, type C1, containing two minor and one well expressed subunits, is
The assessment of hordein composition proved the existence of 8 hordein profile types in the tested samples - D1, C1, C2, B1, B2, B3, B4, B5. The analysed spring barley cultivars - Zernogradskii, Bodega, Fink, Scarlett, Barke, Josefin and Astoria have identical profile model of the D-hordein – D1. In comparison with the cultivars Zernogradskii, Bodega and Fink, which have profile type C1, four accessions - Scarlett, Barke, Josefin and Astoria have profile type C2. All cultivars are characterised by a high degree of polymorphism of B-hordein, wherein five different profile types were identified - B1, B2, B3, B4 and B5. The results of electrophoretic analysis and design hordein formulas for each cultivar show that, on the one hand, they are homogeneous, i.e. each variety is characterized only by a formula. On the other hand, intervarietal polymorphism exists, i.e. cultivars Scarlett and Astoria with the same hordein formula are different from the cultivars Zernogradskii, Bodega, Fink, Barke and Josefin, which have specific hordein formulas.

Table 2. Profile types (hordein models) and frequency (%) occurring in barley cultivars.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Profile type</th>
<th>Cultivar</th>
<th>Frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hor 1</td>
<td>C1</td>
<td>Zernogradskii, Bodega, Fink</td>
<td>43.00</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>Scarlett, Barke, Josefin, Astoria</td>
<td>57.00</td>
</tr>
<tr>
<td>Hor 2</td>
<td>B1</td>
<td>Zernogradskii</td>
<td>14.30</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>Bodega, Barke</td>
<td>28.60</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>Fink</td>
<td>14.30</td>
</tr>
<tr>
<td></td>
<td>B4</td>
<td>Scarlett, Astoria</td>
<td>28.60</td>
</tr>
<tr>
<td></td>
<td>B5</td>
<td>Josefin</td>
<td>14.30</td>
</tr>
<tr>
<td>Hor 3</td>
<td>D1</td>
<td>Zernogradskii, Bodega, Fink, Scarlett, Barke, Josefin, Astoria</td>
<td>57.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43.00</td>
</tr>
</tbody>
</table>

Table 3. Hordein formulas (configuration) of the tested varieties of barley

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Hordein formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scarlett, Astoria</td>
<td>C2 B4 D1</td>
</tr>
<tr>
<td>Zernogradskii</td>
<td>C1 B1 D1</td>
</tr>
<tr>
<td>Bodega</td>
<td>C1 B2 D1</td>
</tr>
<tr>
<td>Fink</td>
<td>C1 B3 D1</td>
</tr>
<tr>
<td>Barke</td>
<td>C2 B2 D1</td>
</tr>
<tr>
<td>Josefin</td>
<td>C2 B5 D1</td>
</tr>
</tbody>
</table>

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