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Hordein polymorphism of spring barley genotypes from European-Siberian Genetic Center by SDS-PAGE electrophoresis

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Abstract

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In this study were fractionated and characterized reserve endosperm proteins, hordeins of seven spring barley genotypes with different origin: Zernogradskii (Russia), Bodega, Fink, Scarlett, Barke (Germany), Josefin and Astoria (France) by SDS-PAGE electrophoresis. On the basis of the obtained spectra are identified 19 bands (D + C + B) with different relative electrophoretic mobility and intensity. 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 are constructed hordein formulas (configurations) of accessions, 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

Barley is an important cereal crop used as animal and human feed, as well as cereal grain for malt production in the brewing industry. It is highly important to have methods that can accurately and rapidly identify barley genotypes for the different end-use requirements. These methods are also needed for germplasm screening and genotype identification in barley breeding programs. To date the methods for quantitative and visual identification of barley genotypes are limited because many morphological, physiological and technological characters of the different genotypes are not stable in a given environment. These characters could vary significantly under unpredictable environmental conditions (Yan et al., 2003).

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 & Popova, 2002). As a result of long research it was found, that electrophoretic spectra of reserve proteins in barley – hordeins remain unchanged under the influence of environmental conditions (Konarev, 1983, 1983a; Perovic et al., 2009). Studying the genetic nature of the traits in barley varieties on the basis of polymorphism of reserve proteins is a guarantee for successful breeding program (Konarev et al., 1986). The determination of reserve proteins are widely used in studies of plant populations, since variability in their characteristics is genetically determined. They are characterized by a high level of polymorphism and stability.

Barley storage proteins – hordeins – have been divided into three groups on the basis of their electrophoretical mobility and amino-acid composition (Shewry & 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 & 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 & Milfin, 1985). The advantages of hordeins for studying of genetic diversity in barley have been described by many authors (Pomortsev et. al., 2002; Vyhnanek et al., 2003). The barley storage protein hordeins characterized by a high degree of polymorphism (Dimova, 2011; Mihova et al., 2012).

Different gel and buffer systems were used for hordein electrophoresis (Pomortsev et al., 2002). High performance liquid chromatography (HPLC) and DNA analysis are successfully applied for investigating hordein composition and barley polymorphism (Molina-Cano et al., 2004; Kroth et al., 2005).

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 genotypes with different origin by SDS-PAGE.

Material and Methods

The material object of this study was seven spring barley genotypes with different geographic origin (Table 1).

The spring barley genotypes listed above have different origins: Zernogradskii from Russia, Bodega, Fink, Scarlett, Barke from Germany, Josefin and Astoria from France.

Separation of reserve proteins was performed in the laboratory of biochemistry of Dobrudzha Agricultural Institute – General Toshevo in 2020. The analyses were performed on single grains obtained by self-pollination under the isolator. Attached is a vertical SDS-PAGE electrophoresis.

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

Genotype	Origin	Variety
Scarlett	Germany	nutans
Bodega	Germany	nutans
Fink	Germany	erectum
Barke	Germany	nutans
Zernogradskii	Russia	nutans
Josefin	France	nutans
Astoria	France	nutans

Hordein extraction is carried out using the method of Singh et al. (1991). Each grain was ground to a fine flour (with a pestle in a porcelain mortar), as previously removed the embryo 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 hordein bands were extracted especially from seed containing a high level of nitrogen. Mix each sample 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 type eppendorf was added 0.1 ml 50% (v / v) propanol, containing 1.4% (v / v) freshly added 4-vinylpyridine (VP). It 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 to it was added 0.2 ml of a solution (sample buffer), containing 2% SDS, 0.08 M Tris – HCl (pH 8.0), 40% glycerol and 0.02% bromphenol blue. The samples were mixed, incubated for 1 hour at 65°C, centrifuged at 12 000 g for 10 min, and then can be used for SDS-PAGE analysis.

The main advantage of SDS-PAGE electrophoresis is that it allows for the 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 in the ratio (1: 5: 4) overnight. Discoloration of the gel plates are carried out with a solution of acetic acid, methanol, distilled water (1: 2: 7). Bleach is changed repeatedly to clear the background and then gel plates are scanned.

The gel system adapted was that described by Laemmli (1970) with some modifications. 12% acrylamide separating gel (pH 8.0) and acrylamide stacking gel (pH 6.8) were used. Fourty microliter Temed and 100 µl 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 µl) were loaded into each sample well with a micropipette.

SDS-PAGE 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 & Briand, 1990). Hordein formulas are constructed in accordance with Dimova (2011).

Results and Discussion

As a result of the electrophoretic analysis are established 19 bands with different relative electrophoretic mobility. One has been 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 profile of the D-, C- and B-hordein are indicated by using indexes corresponding to the identified hordein blocks (Figure 2). There are 8 types of alternate profile endosperm proteins in the tested genotypes (Figure 2, Table 2). Clearly visible are one profile type for D- hordein (D1), two – for C-hordein (C1, C2), and five – for B-hordein (B1, B2, B3, B4, B5).

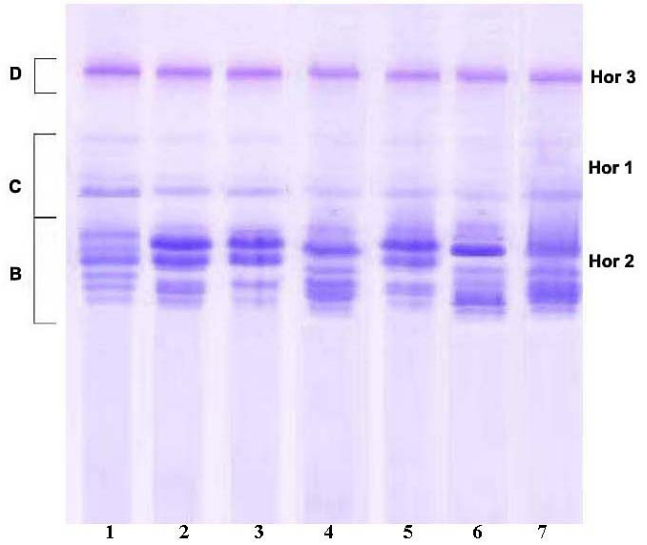

Fig. 1. Electrophoretic spectra of hordein by SDS-PAGE: 1:Zernogradskii; 2:Bodega; 3:Fink; 4:Scarlett; 5:Barкe; 6:Josefin; 7:Astoria

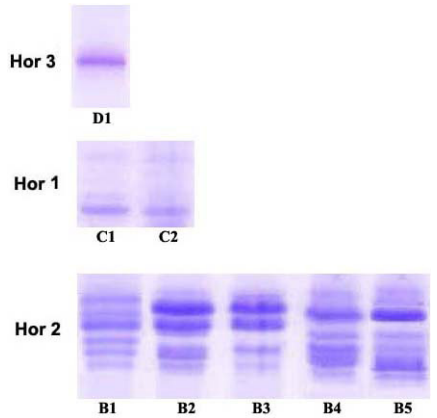

Fig. 2. Allelic states of genes in loci Hor1, Hor2 and Hor3 in spring barley genotypes

Table 2. Hordein models and frequency (%) occurring in barley genotypes

Locus	Profile type	Genotype	Frequency, %
Hor 1	C1 C2	Zernogradskii, Bodega, Fink, Scarlett, Barke, Josefin, Astoria	43.00 57.00
Hor 2	B1 B2 B3 B4 B5	Zernogradskii Bodega, Barke Fink, Scarlett, Astoria Josefin	14.30 28.60 14.30 28.60 14.30
Hor 3	D1	Zernogradskii, Bodega, Fink, Scarlett, Barke, Josefin, Astoria	100.00

No allelic differences were found between D-hordein profiles (Hor 3) of the analyzed varieties – Zernogradskii, Bodega, Fink, Scarlett, Barke, Josefin and Astoria, (Figure 1, Figure 2, Table 2).In a study of Nordic electropherograms of barley genotypes for all is identified the same pattern of the D-hordein (Peltonen et al., 1994). The lack of differences between D-hordein profiles of barley genotypes are reported at a later stage in the study of Algé Leistrumatité and Vanda Paplauskiené (2007).In the area of C-hordein (Hor 1) are expressed three band with different electrophoretic mobility and intensity, two of which are common for hordein models (C1, C2). Electrophoretic profile, type C1, contains two minor and one well expressed subunits, is typical of analyzed genotypes Zernogradskii, Bodega and Fink (Table 2, Figure 2). The other block configuration C2 contains four minor subunits. It has been established for genotypes Scarlett, Barke, Josefin and Astoria (Table 2, Figure 2).

The most significant allelic polymorphism was identified in B-hordein (Hor 2) as compared with D- and C-hordein. Fifteen bands are identified (Figure 2).

Spring genotypes of barley, object of the present study belong to five different profile models in terms of electrophoretic spectra of B-hordein. With greater frequency established type B2 in Bodega and Barke and type B4 in Scarlett and Astoria. The profile type B1 is a typical for Zernogradskii, B3 – for Fink and B5 – for Josefin.

Leistrumatité & Paplauskiené (2007) analyzed seventeen spring barley genotypes and identify the group of C-hordein and in the group of B-hordein – seven different profile types.

In Table 3 are depicted constructed hordein formulas for each of the tested genotypes.

Scarlett and Astoria can be classified into one group, because they have the same hordein configuration. Another

genotypes: Zernogradskii, Bodega, Fink, Barke and Josefin, have specific configurations that differ among themselves and from those of genotypes Scarlett and Astoria.

Table 3. Hordein formulas of the tested varieties of barley

Genotype	Hordein formula
Scarlett, Astoria Zernogradskii	C2 B4 D_1 C1 B1 D1
Bodega Fink Barke Josefin	C1 B2 D1 C1 B3 D1 C2 B2 D1 C2 B5 D1

Presented hordein formulas allow the expression of specific varietal characteristics and prove that hordein polymorphism between the analyzed materials. Intervarietal identified allelic variation is due to the presence or absence of the protein components and their different electrophoretic mobility in the profiles of B- and C- hordein of the studied genotypes.

Conclusions

Electrophoresis of reserve endosperm proteins of seven spring barley genotypes showed the presence of 19 bands with different relative electrophoretic mobility: in the group of D-hordein – one, in the group of the C-horedin – three, in the group of B-hordein -fifteen.

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.

Analyzed spring barley genotypes – Zernogradskii, Bodega, Fink, Scarlett, Barke, Josefin and Astoria have identical profile model of the D-hordein – D1. In comparison with the genotypes Zernogradskii, Bodega, Fink, which has profile type C1, Scarlett, Barke, Josefin and Astoria have profile type – C2. All genotypes are characterized by a high degree of polymorphism of B-hordein, wherein the identified five different profile types – B1, B2, B3, B4 and B5.

The results of electrophoretic analysis and design hordein formulas for each genotype show that on the one hand they are homogeneous, i.e. each variety is characterized only by a formula. On the other hand exist intervarietal polymorphism, i.e. genotypes Scarlett and Astoria with the same hordein formula are different from the genotypes Zernogradskii, Bodega, Fink, Barke and Josefin, which of them have specific hordein formulas.

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