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USING POLYMORPHISM OF GRAIN STORAGE PROTEINS FOR IDENTIFICATION OF FEED BARLEY VARIETIES

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Abstract

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The objective of the present study was to identify feed barley varieties for specific habitats, using polymorphism of grain storage proteins, as well as to construct their hordein formulas. Current biotypes were established in the studied cultivars Veslets, Aheloi 2 and Panagon as well as manifestation frequency. The electrophoretic mobility and molecular weights of the hordein components were found. The results obtained can serve as a basis for future studies on the use of biochemical markers in feed barley breeding and seed production.

Key words: feed barley, hordeins, identification, electrophoresis

Introduction

Storage proteins are widely used in the research on plant populations due to the genetically determined variability of their characteristics. They are characterized with high level of polymorphism and stability. External factors have no or little effect on their quantity in ripe seeds. They are inherited co-dominantly, therefore, the electrophoretic profiles of the proteins, isolated from ripe seeds, are a much better criterion for the characteristics and identification of cultivars alone or in combination with other markers (Alexandrova, 2000; Balashova, 2001). The polymorphism of electrophoretic hordein profiles has been successfully used for cultivar identification. The correct interpretation of electrophoretic profiles requires information on the genetic control of storage proteins. Each protein class is usually coded by a complex locus, gene cluster or family of tandem repeated DNA sequences. Heterogeneity in the electrophoretic profile and/or band intensity might depend on the different number of copies in the cluster, heterogeneity of sequences in the family, different products of the post-translational processing of the proteins in the course of their sedimentation in the protein bodies (Todorovska et al., 2005).

In breeding, biochemical markers serve as a model of genetic control on complex indexes (Todorov et al., 2004; 2006). The use of gel-electrophoresis of seed proteins is a method that has been applied in wheat breeding programs as well as for the identification of genetic variation between barley varieties (Sozinov, 1985; Jones, 1982; Pomortsev et al., 1982; Hauser, 1982; Milkova, 2000; Stoyanova et al., 2002; Todorov et al., 2002; Stoyanova, 2002). The electrophoretic method allows the splitting of storage pro-

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teins into separate fractions, each related to specific economically important indexes. Their expression is stable and independent from ambient conditions. In wheat, there is a direct relationship between the separate protein sub-units and gluten physico-chemical properties (Shewry et al., 2002).

In barley, the polymorphism of storage proteins is used mainly for cultivar identification as well as with reference to some quality indexes, though to a smaller extend (Sozinov, 1985; Grib, 1985; Abdel-Haleem, 2004; Peltonen et al., 1994; Vyhnanek et al., 2003; Wang et al., 2007).

The cultivar characteristics of feed barley in our country is done mainly on the basis of morphological indexes and biological and economical properties (Zapryanov et al., 1996; Mersinkov, 1996; Zapryanov et al., 1997). The use of electrophoresis for variety identification in accordance with international standards would contribute to receiving more ample information about them.

The present study aimed at the electrophoretic study of hordein spectra of feed barley varieties as well as the construction of their hordein formulas.

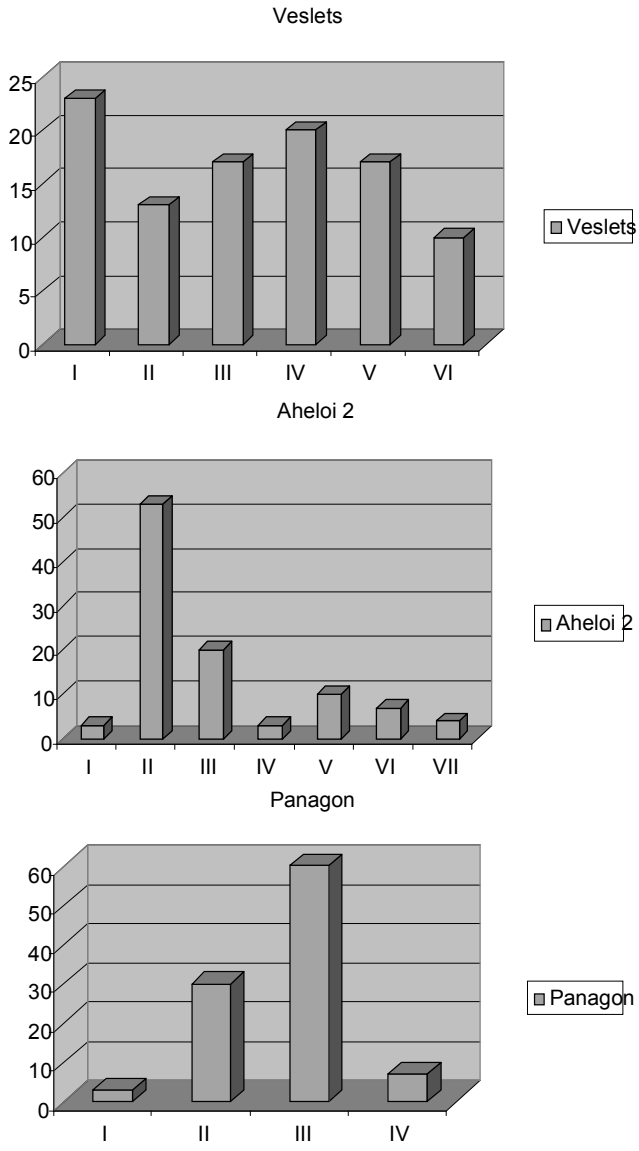


Fig. 1. Frequency of identified biotypes in feed barley varieties

Material and Methods

Subject of the study were 3 major habitat specific winter feed barley varieties Veslets, Aheloi 2 and Panagon, developed at the Institute of Agriculture in the town of Karnobat (Zapryanov et al., 1996; 1997; Mersinkov, 1996). The identification of storage proteins was performed at the Laboratory of Biochemistry of the Dobrudja Agricultural Institute in General Toshevo. The analyses were made with single grains of pre-basic seeds of the varieties, tested and classified as such by the seed control authorities of the Institute of Agriculture of Karnobat.

An SDS electrophoresis as per Laemmli (1971) at 12 % separating gel and 20 mA electric power for 18-20 h is included. Hordein extraction was made by the method of Singh et al., 1991, omitting the first extraction with 15% propanol. To visualize the protein components, 0.03% solution of Coomassie blue R 250 in methanol and acetic acid was used.

The homogeneity and heterogeneity of cultivars as well as the frequency of distribution of hordein genotypes were identified based on the electrophoretic spectra obtained. Molecular marker was used for the identification of electrophoretic mobility (Rf) and molecular weights of the bands.

Indexes, corresponding to the established hordein blocks, were used in the construction of hordein formulas.

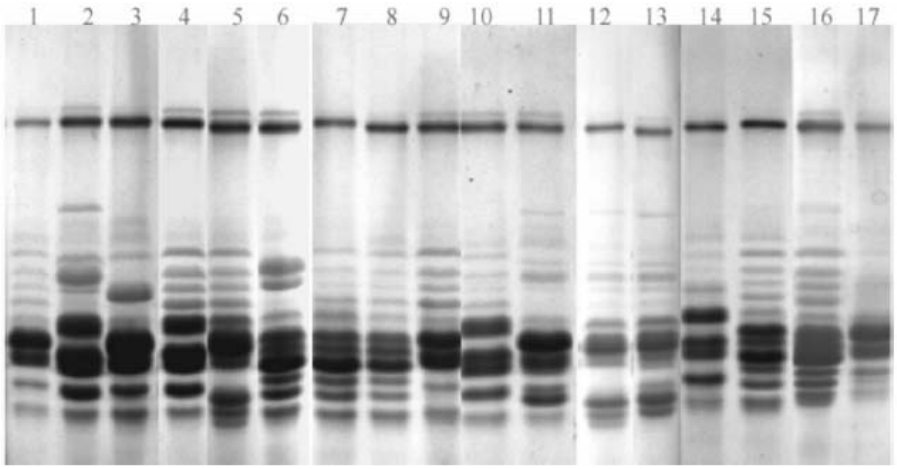
Results and Discussion

Different biotypes were found in the studied feed barley varieties with cv. Aheloi 2 having the highest heterogeneity. The presence of a large number of genotypes could be explained with the fact that poly-row barley varieties have a higher percentage of open flowering, which makes cross-pollination possible as well. The probability of mechanical admixtures was smaller due to the use of pre-basic seed, tested and classified as such by the seed control authorities.

Cv. Aheloi 2 had the largest number of registered biotypes 7, followed by Veslets with 6 and Panagon 4. The frequency of manifestation of separate biotypes was different for each variety (Figure 1). In Veslets, the first biotype showed the highest frequency (23%), the remaining ones were found in almost equal portions. Of the seven biotypes of cv. Aheloi 2, the second (53%) and the third (20%) showed the biggest frequency of manifestation. Panagon was the cultivar with higher homogeneity, compared to the other two. Of 4 biotypes established, the third and the second represented the cultivar and their share was 90%.

The hordein electrophoretic models of the studied varieties are presented on Figure 2. The visualization of storage proteins demonstrated a clearly expressed intervarietal polymorphism that was the result of the presence or absence of protein components and their different electrophoretic mobility. There were differences between the bands in terms of color intensity as well, due to the different protein content. There were significant differences in terms of allele composition in all three varieties.

Figure 3 shows the identified blocks of **D, C** and **B** hordeins in locus Hor 3, Hor 1 and Hor 2. There were 3 alleles identified in Group **D**. All varieties were heterogeneous with reference to this fraction of the prolamins, all three configurations being found in cv. Aheloi 2. **D** hordeins had the smallest spectrum and their electrophoretic mobility was 0.24 and 0.25, while their molecular weights were 102.9 and 99.8 kD, respectively (Table 1). **C** hordeins were represented in



1	Veslets 1	7	Aheloi 1	14	Panagon 1
2	Veslets 2	8	Aheloi 2	15	Panagon 2
3	Veslets 3	9	Aheloi 3	16	Panagon 3
4	Veslets 4	10	Aheloi 4	17	Panagon 4
5	Veslets 5	11	Aheloi 5		
6	Veslets 6	12	Aheloi 6		
		13	Aheloi 7		

Fig. 2. Electrophoretic spectrum of hordeins of habitat specific varieties of winter feed

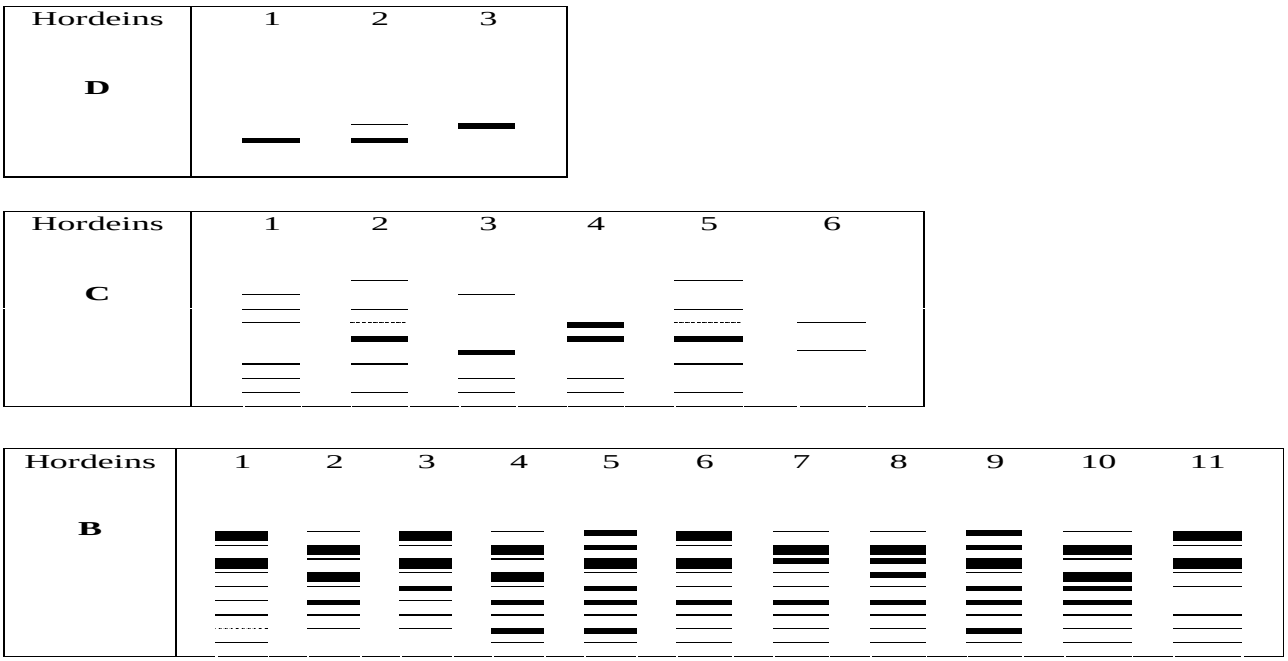


Fig. 3. Blocks found in D, C and B hordeins in feed barley varieties

locus Hor 1 by 6 configurations, containing 9 sub-units (Figure 3).

The electrophoretic mobility of **C** hordeins was within 0.37 and 0.53 at molecular weights of 69.7-46.1 kD. While locus Hor 3 had the smallest number of alleles, **B** hordeins in locus Hor 2 had high rate of polymorphism, which confirmed other studies on polymorphism of storage proteins in barley varieties (Vulcheva et al., 2009). There were 11 sub-units situated in 11 allele conditions. Some of the components were homologous and others were specific for the different biotypes in the studied varieties. A single component was found in the fifth biotype of cv. Veslets in **B** hordeins with Rf of 0.70 (Table 1).

The most frequent configurations were 1 and 2, followed by 3 and 4. At the same time, configurations from 5 to 11 were the allele states of the separate biotypes of the studied varieties. Such specific block configurations of **B** hordeins were recorded in biotype V of cv. Veslets, biotypes V, V^2 and V^{22} of cv. Aheloi 2 and biotypes ^{22}, ^{222} and ^2V of cv. Panagon. Given that biotype V of cv. Veslets had a manifestation frequency of 17% and biotypes ^{22}, ^{222} and ^2V comprised 97% of the hordein model of cv. Panagon, we could say that the established specific allele states of **B** hordeins probably played a significant role in the phenotypic characteristics of the respective variety as well.

Based on the electrophoretic profile of cultivars Veslets, Aheloi 2 and Panagon, the electrophoretic mobility of the bands and indexes of the identified blocks, an attempt was made to construct the hordein formulas of the separate biotypes (Table 2). The identified hordein spectra of the studied varieties and their biotypes, presented by their hordein formulas, enabled the expression of specific cultivar characteristics. The hordein formulas of the six biotypes of cv. Veslets showed that 4 of the registered biotypes were in the second allele state of **D** hordeins. The correlation between the first and second recorded allele states in this variety was 1:2, the percentage of manifestation of the second configuration being 60%. The biotypes of cv. Veslets possessed **C** hordeins of the first, third, fourth and fifth allele state. Four biotypes of the variety with a total frequency of manifestation of 77% had **C** hordeins of the first and third configuration in a ratio of 3:1. The remaining two biotypes were allocated to the established specific individual allele con-

Table 1
Electrophoretic mobility (Rf) and molecular weights of established hordein bands

Hordeins	Electrophoretic mobility /Rf/	Molecular weights /kD/
D	0.24	102.9
	0.25	99.8
	0.37	69.7
	0.43	58.4
	0.44	57.5
	0.46	54
C	0.47	53.2
	0.48	51.6
	0.49	50.5
	0.52	47
	0.53	46.1
	0.56	43.7
	0.57	42.9
	0.58	41.7
	0.59	41.3
B	0.62	38.7
	0.64	37.6
	0.66	36.1
	0.68	35.8
	0.69	35.2
	0.7	34.9

figurations of **C** hordeins. Of the total 11 allele states of **B** hordeins, recorded in cv. Veslets, 5 were identified, the ratio of biotypes from the first to fifth configuration being 1:2:1:1:1. The alleles in the fifth biotype of this variety were of 5 configurations, where the component with the highest electrophoretic mobility and smallest molecular weight was found. Having in mind the specifics of **B** hordeins of the fifth biotype of cv. Veslets as well as the fact that this biotype had a considerable frequency of manifestation (17%), we could assume that this biotype was a carrier of specific varietal characteristics.

The **D** hordeins of cv. Aheloi 2 were in the three allele states in a ratio of 1:4:2. There were 4 biotypes, belonging to the first configuration of **C** hordeins and the remaining ones were in the second allele state. **B** hordeins of the biotypes I to IV of this cultivar were of the first, third and fourth allele state in a ratio of 2:1:1.

Specific individual allele blocks were found in three of the biotypes of this variety with a total frequency of manifestation as low as 21%. Due to their characteristics, they were possibly connected to certain specific peculiarities of the variety as well. Because of the low frequency of manifestation of the biotypes, the probability of a phenotypic relationship to some peculiarities was small.

The **D** hordeins of the biotypes of cv. Panagon in locus Hor 3 were of the first and second configuration in a ratio of 3:1. Alleles of the first configuration of **C** hordeins were registered in biotypes II and III, while I and IV belonged to the third and sixth allele

Table 2
Hordein formulas of feed barley varieties

Varieties	Hordein formulas
Veslets	
² biotype	$D_1\ C_1\ B_3$
²² biotype	$D_2\ C_4\ B_2$
²²² biotype	$D_1\ C_3\ B_4$
²V biotype	$D_2\ C_1\ B_2$
V biotype	$D_2\ C_1\ B_5$
V² biotype	$D_2\ C_5\ B_1$
Aheloi 2	
² biotype	$D_3\ C_1\ B_1$
²² biotype	$D_1\ C_1\ B_1$
²²² biotype	$D_2\ C_1\ B_3$
²V biotype	$D_2\ C_1\ B_2$
V biotype	$D_2\ C_2\ B_6$
V² biotype	$D_3\ C_2\ B_7$
V²² biotype	$D_2\ C_2\ B_8$
Panagon	
² biotype	$D_1\ C_3\ B_2$
²² biotype	$D_1\ C_1\ B_9$
²²² biotype	$D_2\ C_1\ B_{10}$
²V biotype	$D_1\ C_6\ B_{11}$

state. Only the first biotype in Hor 2 belonged to the homologous second configuration and the remaining biotypes belonged to specific individual blocks of **B** hordeins.

Conclusions

The hordein electrophoretic models of cultivars Veslets, Aheloi 2 and Panagon were characterized with clearly expressed intervarietal polymorphism. The intervarietal allele variation was the result of the presence or absence of protein components and their different electrophoretic mobility. The constructed hordein formulas gave the opportunity to express the specific varietal peculiarities of the three cultivars.

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