Hordein Diversity in Spring Barley Genotypes Related to Crude Protein Content
Hordeīna daudzveidības izvērtējums vasaras miežu genotipiem saistībā ar kopproteīna saturu graudos

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Abstract. The aim of the study was to assess the diversity of hordein banding patterns in different spring barley genotypes (covered, hulless, two-row, and six-row) and to identify the relation of hordein patterns and hordein polypeptide bands with total protein content. The study was carried out at the State Stende Cereal Breeding Institute from 2004 to 2006. On the basis of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), 5 D hordein, 16 C hordein, and 28 B hordein banding patterns were detected. In total, 26 hordein polypeptide bands were recognized. Cluster analysis based on hordein patterns classified spring barley genotypes into three groups, and two of them significantly differed in crude protein content. Association was found between definite hordein patterns and crude protein content. There were found five covered varieties with identical hordein banding patterns but with varied crude protein content (111.9 to 154.3 g kg^{-1}). The patterns for these genotypes were distinctly different in the intensity and density of the color of hordein polypeptide bands, especially in C hordein. Among all the genotypes screened, 10 hordein polypeptide bands revealed significance in identifying of genotypes with definite crude protein.
Key words: *Hordeum vulgare*, electrophoresis, hordein diversity, crude protein.

Introduction

The research on new resources of variability and a better knowledge of genetic diversity existing within the available material is of particular interest in different types of barley.

Usually about 40% of the total nitrogen of mature grain is present in the storage protein fraction, termed hordein (Shewry, 1995). In barley, this alcohols-soluble protein, or prolamin, is of poor nutritional quality, notably deficient in the essential amino acid lysine, and is responsible for the poor quality of the whole grain when used as a diet for monogastric animals (Molina-Cano et al., 2000).

Hordein can be classified into three groups of polypeptides called B, C, and D hordeins based on their electrophoretic mobility (Shewry, Tatham, 1990). The B and C fractions account for 70-80% and 10-12%, respectively, of the total hordein, while the D fractions are a minor component (about 5%). Each group of hordein is synthesized from a family of structural genes. These different hordeins differ in molecular weight and amino acid composition (Shewry, 1995). The major B hordeins and C hordeins are encoded by the multigenic loci *Hor2* and *Hor1*, respectively, both located on the short arm of chromosome 5. The D hordein is characterized by high glycine, proline and glutamine content. Their synthesis is encoded by the *Hor3* locus located on the long arm of chromosome 5 (Shewry, Tatham, 1990).

The regulation of grain storage protein synthesis is available for study because these proteins are generally specific to endosperm tissues, the expression of their genes is developmentally regulated, and the proteins have been extensively characterized. Hordeins are largely tolerant to mutations and are selected neutrally. Hordeins show high intergenotypic variation and have been used as a genetic marker (Shewry, 1995).

Any complete study of protein will require methods for protein separation. Diversity in the hordein family has made the analysis of these fractions very useful in evolutionary studies (Yin et al., 2003), for variety identification, and for analyzing the genetic diversity in collections (Shewry et al., 1978). Among biochemical techniques, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a system which is most widely used for separating proteins and for studying the biochemistry and genetics of hordein to describe genetic structure of crop germplazm. This diversity can be explained

through differences within the B and C hordeins that occur between varieties, as well as grain protein levels and environments (Molina-Cano et al., 2001). SDS-PAGE of hordein has been used to characterize covered barley varieties from Brazil (Echart-Almeida, Cavali-Molina, 2000), Tibet (Yin et al., 2003), United Kingdom (Shewry et al., 1978), Yugoslavia (Radovic, Vapa, 1996), and Lithuania (Leistrumaite, Paplauskiene, 2007). Hordein polymorphism in relation to agromorphological traits was also analyzed for hulless barley collection (Atanassov et al., 2001). In these studies several hordein patterns have been fractionated.

Great effort has been made to clarify the relationship between hordein fractions and malting quality (Shewry et al., 1980; Riggs et al., 1983). Peltonen et al. (1994) studied the effect of B, C, and D hordeins on malting quality of northern European barleys and found that the B fraction had some effect on malting quality through changing adjusting diastatic power. Molina-Cano et al. (2000) suggested that both B hordeins and β-glucans were relevant to water uptake. The B and C hordeins have been shown to be associated also with milling energy where increase in C hordein along with a decrease in β-glucan corresponded to a decrease in milling energy, which characterizes such a trait as grain hardness (Molina-Cano et al., 1995).

In the present study, SDS-PAGE analyses of hordein polypeptide patterns were used to analyze genetic diversity of the material of different origin characterized with a wide range of variability in crude protein content. The aim of the study is to assess the diversity of hordein banding patterns in the different spring barley genotypes and to identify the relation of hordein patterns and hordein polypeptide bands with total protein content.

Materials and Methods

There were chosen 52 barley genotypes that represent a broad range of germplazm (two-row, six-row, covered, and hulless) of different origin. Thirty-eight genotypes of covered spring barley, from which 28 with two-row and 10 with six-row ear types, and 15 hulless genotypes were used in this study. Only two-row hulless genotypes were included in this investigation (Table 1).

The barley genotypes were grown at the State Stende Cereal Breeding Institute from 2004 to 2006. The soil at the site was sod-podzolic sandy loam, humus content – 12-15 mg kg^{-1}, soil pH – 6.0-6.7, pre-crop – potatoes, content of available phosphorus P_2O_5 – 201-215 mg kg^{-1}, and available potassium K_2O – 124-147 mg kg^{-1}. Plot size was 2 m^2, two replicates, seeding rate – 400 germinated seeds per m^2. The plots were fertilized with $N_{60}P_{35}K_{50} + S_{42}$ kg ha^{-1}.

Crude protein content (N × 6.25) was determined by the Kjeldahl method (LVS 277/Latvian State Standard). According to results of the previous investigation, all types of barley genotypes included in this study cover a wide range of variation in crude protein content (Bleidere, Grunte, 2008). Analysis of variance suggested that crude protein in this material was more strongly ($p<0.01$) affected

Table 1

Spring barley genotypes used in the study

Barley type	n	Country of origin: No. of genotype
Two-row, covered	27	Latvia: (1)'Abava', (2)'Ansis', (3)'Balga', (4)'Gate', (5)'Idumeja', (6)'Klinta', (7)'Kristaps', (8)'Linga', (9)'Malva', (10)'Rasa', (11)'Ruja', (12)'Sencis'; Australia: (13)'Grimmet'; Austria: (14)'Austrian early', (15)'Landsorte Aus Tirol'; Chile: (16)'379'; Denmark: (17)'Lysimax'; Germany: (18)'Annabell', (19)'Danuta', (20)'Hanka', (21)'Justina', (22)'Polygena'; Great Britain: (23)'Century', (24)'Cork'; Hungary: (25)'Hatvani 45/25'; Portugal: (26)'Lechtaler'; Sweden: (27)'Primus II'
Six-row, covered	10	Latvia: (28)'Druvis'; Bolivia: (29)'Valluno'; Denmark: (30)'Colsess IV', (31)'July'; FIR Macedonia: (32)'IV/192'; Mexico: (33)'Puebla', (34)'Zoapila'; Nepal: (35)'B90A', (36)'RNB-367'; North Korea: (37)'Chosen'
Two-row, hulless	15	Latvia: (38)'L 302'; Canada: (39)'CD Candle', (40)'Gainer', (41)'McGwire'; the Czech Republic: (42)'KM 2084'; Guatemala: (43)'2474', (44)'Clho 7799'; Italy: (45)'Orzo Nudo di Altamura'; Japan: (46)'Sumire Mochi', (47)'Wanubet'; Lithuania: (48)'X-4'; Russia: (49)'C.P.I. 22817'; Sweden: (50)'SW 1291'; Turkistan: (51)'10250'; USA: (52)'Merlin'

by the genotype (Bleidere, 2008). Three-year mean values of crude protein are used in the results of this investigation.

Hordein analysis for each genotype was done from the grain harvest of 2006 in two replications. Barley flour sample for each replication was obtained by crushing grains from one single ear and sieving them through a 0.5 mm sieve. Barley hordein was extracted 1 h at 60 °C by shaking 0.5 g of barley flour with 1.5 mL of extraction buffer 3 times (55% (v/v) propan-2-ol, 2% (v/v) β-mercaptoethanol, 1% acetic-acid). After centrifugation (10' at 14000 rpm), 90 µL of gel-loading buffer (50 mM Tris × Cl (pH 6.8); 100 mM dithiothreitol; 2% SDS; 0.1% bromophenol blue; 10% glycerol) were added to 10 µL of supernatant. Hordeins were separated by vertical sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The stacking gel layer contained 5% of acrylamide, but separating gels contained 10% of acrylamide. The gels were run for 2 h at a 100 mA constant current, then fixed in 50% methanol and 12% acetic acid solution, stained with Coommassie Brilliant Blue G-250, washed in 17% methanol and 3% glycerol solution, and photographed by digital imaging system DigiGenius (*Syngene*). Apparent molecular weights of single polypeptide bands of B, C, and D hordeins were studied by determining their molecular weight (MW) in kilodaltons (kDa) by *GeneTools* gel analysis software (*Syngene*) according to marker proteins β-galaktosidase (MW 116.0 kDa), albumin (66.2 kDa), ovalbumin (45.0 kDa), and lactate dehidrogenase (35.0 kDa) (*Fermentas*). The varieties 'Sulky', 'Atem', 'Nathalie', and 'Iris' were used as checks that correspond to the patterns of the D hordeins group. The variety 'Igri' was run as a control in all gels.

The results of SDS-PAGE for D, C, and B hordeins are shown in diagrammatic manner (Leistrumaite and Paplauskiene, 2007). The hordein banding patterns were determined by scoring the presence and absence of all examined bands. The bands were numbered according to their electrophoretic mobility. The scores were given as 1 for the presence, and 0 for the absence of a band. The presence and absence of bands were entered in the binary data matrix. The crude protein and SDS-PAGE data were analyzed for the comparison of means for quantitative traits using t-test of two samples assuming unequal variance. By elaborating a pairwise similarity matrix using the presence (1) or absence (0) of B, C, and D hordein banding patterns, the dendrogram was constructed by cluster analysis obtained from Euclideant distance and clustered by Neighbor-joining method using software STATISTICA. Coefficient of variation (V%) was calculated to characterize the variation in crude protein for genotypes belonging to a definite hordein banding pattern.

Results and Discussion

Hordein polypeptides have been separated by SDS-PAGE in three fractions which were D, C, and B. Different analysis have indicated that C hordein can be separated into polypeptides with molecular weight ranging between 67 and 86 kDa, B hordein – between 30 and 60 kDa, and D hordein – with molecular weight of about 105 kDa (Shewry et al., 1978; Heisel et al., 1986). Electrophoretic data showed that in this study the molecular weight of the analyzed polypeptides ranged from 35 to 94 kDa. Molecular weight for D hordein polypeptides ranged from 82 to 94 kDa, for C hordein polypeptides – from 49 to 66 kDa, and for B hordein polypeptides – from 35 to 46 kDa. Hordein fractions separated from a range of varieties using SDS-PAGE showed extensive variation in banding patterns within each hordein groups.

There were 5 different banding patterns consisting of 4 bands for D hordein group (Fig. 1). In other studies it was found that the D hordein of European barley varieties consists of single component of polypeptide, but two or three components have been reported in other varieties (Leistrumaite, Paplauskiene, 2007).

Band No.	MW, kDa	Banding pattern				
		D1	D2	D3	D4	D5
1	94					—
2	93				—	
3	92		—	—		
4	82	—	—			

Fig. 1. Diagrammatic representation of SDS-PAGE patterns of D hordein in spring barley genotypes.

Distribution of different types of barley genotypes in each group of D banding patterns is presented in Table 2. Only three six-row barley genotypes (29) 'Valluno', (30) 'Colsess IV' and (35) 'RNB-367' had banding pattern D1 consisting of a single band with molecular weight of 82 kDa.

The D hordein pattern 2 formed from two bands had one covered and three hulless two-row varieties. Most of the varieties from different types of barley belong to hordein banding pattern D3. There were differences in crude protein content between varieties characterized by different banding patterns of D hordein. The lowest crude protein content was for genotypes belonging to the D hordein banding pattern 4 (131.6 g kg^{-1}; V%=4). The highest average crude protein (160.9 g kg^{-1}) was for genotypes with D5 hordein pattern. To D1, D2, and D3 patterns belonged varieties with a wide range of crude protein content, which was indicated by the coefficient of variation – 13, 23, and 14%, respectively. Covered two-row genotypes had 4 bands of D hordein, six-row genotypes had 3 bands, but hulless genotypes – 4 bands of D hordein.

The C hordein group had more polypeptide bands and patterns than D hordeins had. There were determined 16 banding patterns that totally consisted of 11 bands in the C hordein group for different types of barley (Fig. 2).

These 16 banding patterns were formed from 2 to 5 bands. In the C hordein group, 10 different bands and 7 banding patterns were found for 27 covered two-row varieties, 7 bands and 7 banding patterns were found for 10 six-row barley genotypes, and 8 different bands and 7 banding patterns – for 15 hulless genotypes (Table 3). Majority of the varieties

Table 2

D hordein patterns in different types of barley genotypes in relation to crude protein content

Banding pattern*	Barley genotypes, No.			Crude protein, g kg^{-1}	V, %
	covered, two-row	covered, six-row	hulless, two-row		
D1	–	–	38; 40; 48	154.4	13
D2	17	29; 30; 35	–	151.1	23
D3	1; 2; 4; 6; 7; 9; 10; 11; 12; 14; 15; 16; 18; 19; 20; 21; 22; 23; 24; 25; 26; 27	28; 31; 34	39; 41; 43; 44; 45; 50; 51; 52	135.8	14
D4	3; 5; 8	–	42; 47	131.6	4
D5	13	32; 33; 36; 37	46; 49	160.9	11

* banding pattern according to Fig. 1.

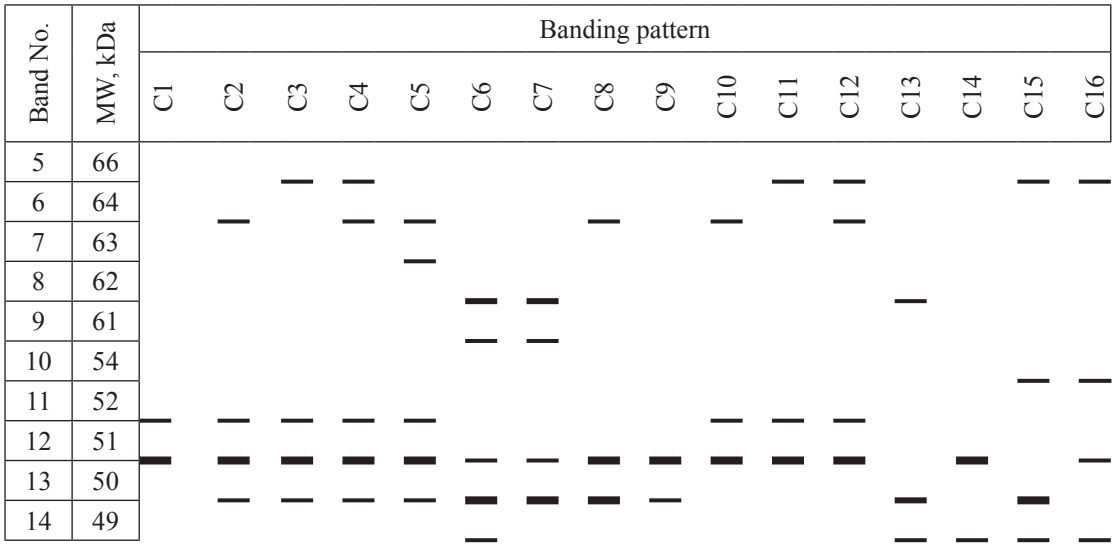

Fig. 2. Diagrammatic representation of SDS-PAGE patterns of C hordein in spring barley genotypes.

from all types of barley were characterized by the C2 and C4 hordein patterns.

Within C hordein, the C2 and C4 hordein patterns were diverse regarding the protein content which was demonstrated by the coefficient of variation (11% and 12%, respectively). Covered two-row genotypes with a comparatively lower average crude protein content (114.0 to 121.7 g kg^{-1}) belong to C11, C12, and C13 hordein banding patterns. Other 7 six-row barley varieties were characterized by different C hordein banding patterns. Six of them had heightened protein content (149.3 to 196.2 g kg^{-1}). C hordein patterns C8 and C16 were specific only for particular hulless genotypes. All hulless genotypes with hordein patterns C5 and C16 had a comparatively high crude protein content – with mean values of 166.2 and 164.4 g kg^{-1} (coefficient of variation – 3% and 5%, respectively).

In total, in B hordein group, 28 hordein banding patterns with 12 polypeptide bands were discriminated. The tested covered two-row barley genotypes were found to possess 12 hordein banding patterns formed from 12 hordein polypeptide bands (Fig. 3, Table 4).

Nine varieties from this type of barley were characterized by B3 hordein banding pattern. All these varieties had low crude protein content (mean value – 122.4 g kg^{-1}; coefficient of variation – 5%). Five covered varieties belonging to B13 hordein banding pattern were diverse regarding crude protein content. The rest of covered varieties had different B hordein banding patterns. For covered six-row and hulless two-row genotypes there was a wide range of diversity regarding B hordein banding patterns that totally differed from each other and also from the covered two-row ones. There were 10 hordein banding patterns which consisted of 9 polypeptide bands found within 15 hulless barley. Only B10 and B24 hordein banding patterns were the same for two two-row and six-row varieties. This result shows that barley varieties included in this study and demonstrating a wide range of variability in crude protein content had high polymorphism of B hordein banding patterns.

The results of polymorphism of different barley genotypes regarding hordein banding patterns are summarized in a dendrogram (Fig. 4).

There were several genotypes with an identical hordein banding pattern (linkage distance 0) eliminating nine similarity groups from 2 to 5 varieties in each group. This was observed not only

Table 3

C hordein patterns in different types of barley genotypes related to crude protein content

Banding pattern*	Barley genotypes, No.			Crude protein, g kg^{-1}	V, %
	covered, two-row	covered, six-row	hulless, two-row		
C1	–	–	46	173.0	–
C2	1; 6; 13; 14; 17; 25	29; 31; 34	47; 49; 50	142.7	11
C3	–	32; 35	–	150.7	12
C4	3; 8; 9; 11; 16; 18; 21; 23; 26; 27	–	40; 42	130.1	12
C5	15	–	38; 45; 48	166.2	3
C6	–	37	–	155.1	–
C7	–	36	–	196.2	–
C8	–	–	39; 41	127.9	5
C9	24	–	52	123.4	8
C10	–	30	–	151.9	–
C11	2; 4; 12; 20	–	–	121.7	4
C12	5; 19	–	–	132.7	–
C13	22	28	–	114.3	3
C14	–	33	–	149.3	–
C15	7; 10	–	–	120.2	4
C16	–	–	43; 44; 51	164.4	5

*banding pattern according to Fig. 2.

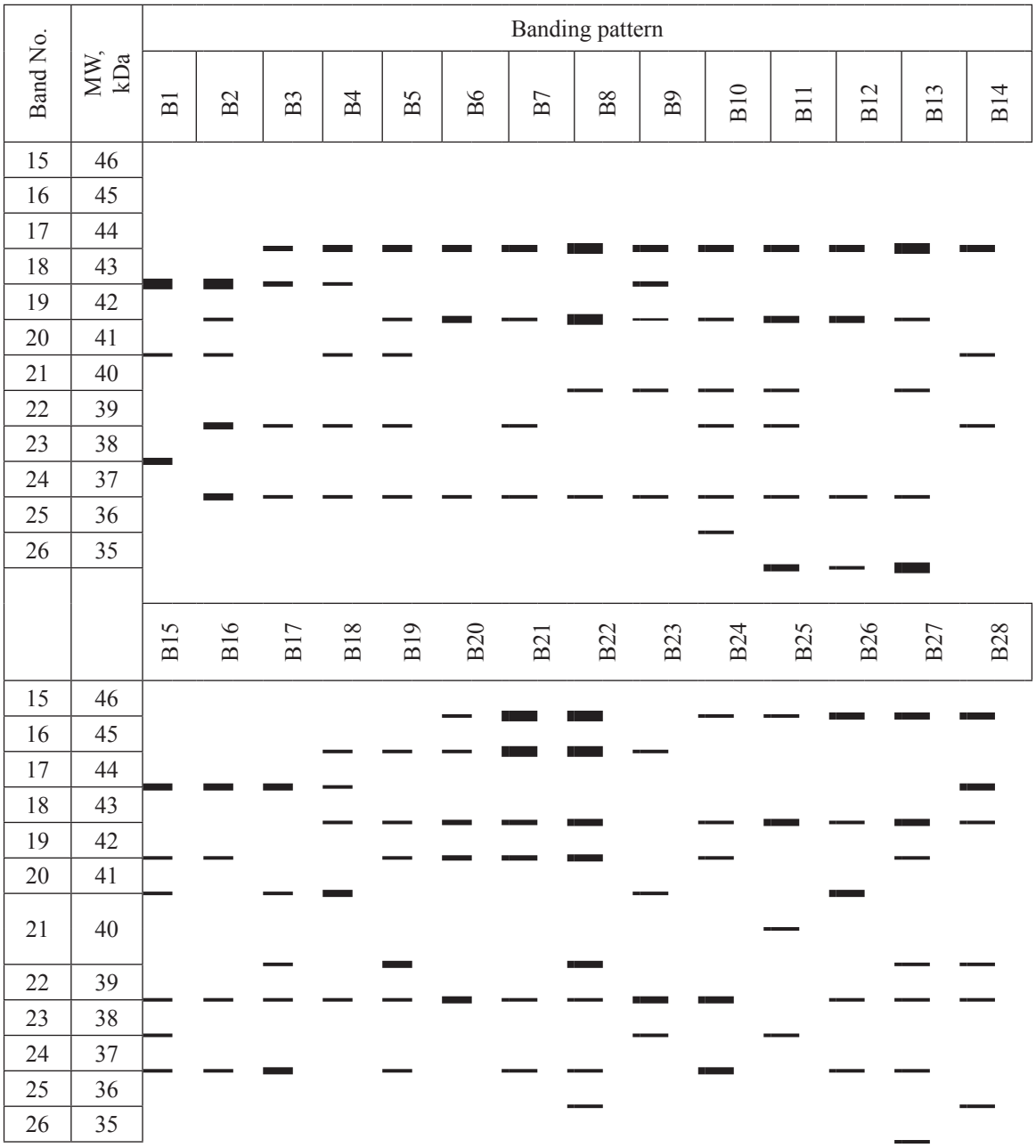

Fig. 3. Diagrammatic representation of SDS-PAGE patterns of B hordein in spring barley genotypes.

between the same types of barley but also between different types of barley (two-row, six-row, and hulless). For example, the covered barley variety (3) 'Balga' had the same hordein banding pattern as the hulless line (42) 'KM 2084', six-row variety (28) 'Druvis' had the same hordein banding pattern as the two-row variety (22) 'Poligena', and covered two-row barley variety (1) 'Abava' had identical hordein pattern with hulless genotype (50) 'SW 1291'. In general, these varieties exhibited also similar crude protein content. Also G. Liu et al. (2000) in his study could not distinctly separate spring and winter barley varieties, as well as 2-row and 6-row barley varieties into two groups by using protein electrophoresis. The hordein polymorphism described in hulless collection was quite similar to that generally observed in covered barley (Atanassov et al., 2001). The created dendrogram allowed observing structuration of diversity. Relatedness between several varieties was found in hordein banding patterns.

Barley genotypes clustered on linkage distance 1.4 are genotypes which have similarity with one or two of the hordein banding pattern groups (D, C, or B). These genotypes are mainly of European origin,

Table 4

B hordein patterns in different types of barley genotypes related to crude protein content

Banding pattern*	Barley genotypes, No.			Crude protein, g kg^{-1}	V, %
	covered, two-row	covered, six-row	hulless, two-row		
B1	14	–	–	170.7	–
B2	–	–	46	173.0	–
B3	4; 7; 9; 10; 11; 12; 20; 23; 24	–	–	122.4	5
B4	–	–	45	164.1	–
B5	–	–	39	120.6	–
B6	1; 13	–	41; 50	137.2	8
B7	6	–	–	136.7	–
B8	–	35	–	159.3	–
B9	17	–	–	111.6	–
B10	22	28	–	114.3	4
B11	–	34	–	157.5	–
B12	5	–	–	132.7	–
B13	16; 18; 21; 26; 27	–	–	137.2	13
B14	–	–	40	133.0	–
B15	–	33	–	149.3	–
B16	–	–	47; 52	134.0	2
B17	–	–	49	155.9	–
B18	–	29	–	144.8	–
B19	–	37	–	155.1	–
B20	2; 3; 19	–	42	123.7	6
B21	25	–	–	165.7	–
B22	–	36	–	196.2	–
B23	8	–	–	129.8	–
B24	15	31	–	161.4	8
B25	–	32	–	142.1	–
B26	–	–	43; 44; 51	164.4	5
B27	–	–	38; 48	165.1	3
B28	–	30	–	151.9	–

*banding pattern according to Fig. 3.

which indicates genetic similarity between them. These genotypes originated two clusters according to hordein banding patterns. Cluster I combined only the covered two-row barley genotypes mainly with a comparatively lower crude protein content (127.3 g kg^{-1}). Cluster II grouped genotypes with a heightened crude protein content (145.1 g kg^{-1}). According to t-test, the difference of means between these two clusters regarding crude protein was significant ($p<0.01$). Cluster III grouped genotypes with unique hordein banding patterns. These barley genotypes are more distant as to the origin of material. To cluster III mostly belong the hulless and six-row covered barley genotypes included in this investigation and they exhibited a heightened grain crude protein content. Also for high lysine barley variety (17) 'Lysimax' the hordein banding pattern differed from other genotypes.

Nevertheless, in cluster I there were found five covered varieties with identical hordein patterns (D3C3B13) but with different crude protein content. When analyzing SDS-PAGE of hordein fractions it was found that the patterns for these genotypes distinctly differed in color intensity and density of hordein polypeptide bands (Fig. 5). For barley genotypes (16) '379', (26) 'Lechtaler' and (27)

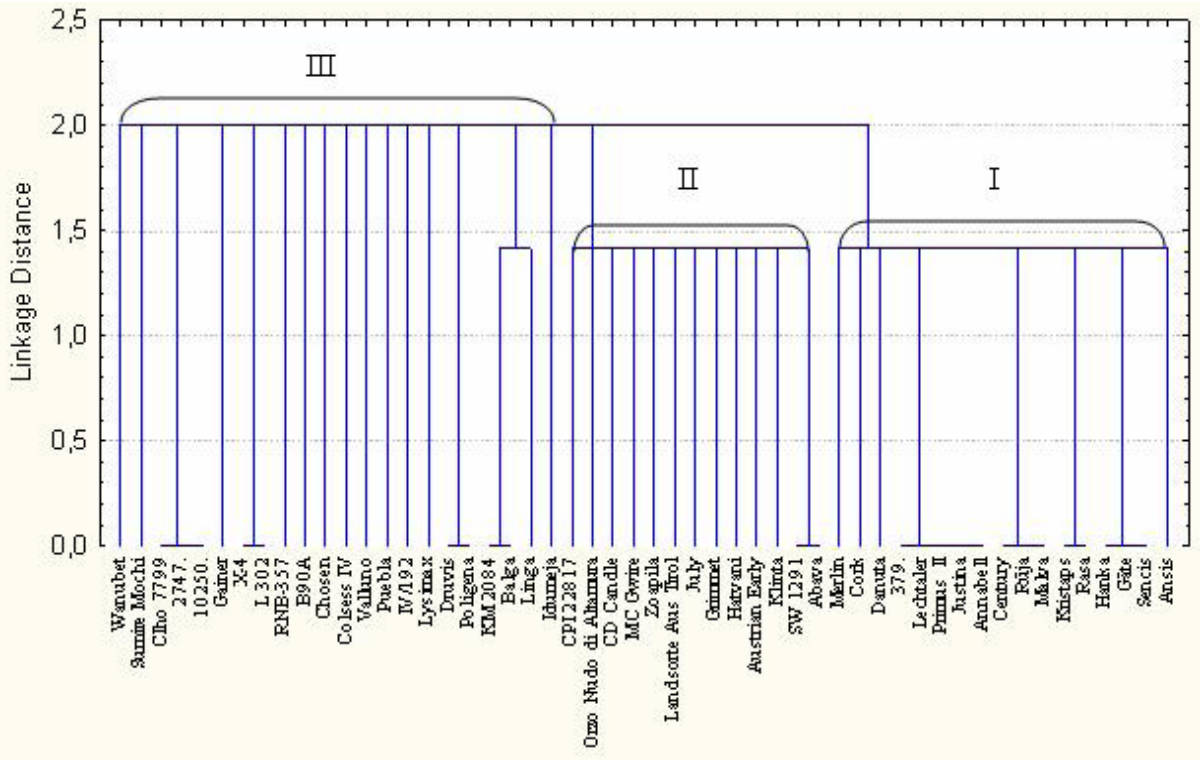

Fig. 4. Dendrogram of 52 different types of barley obtained from Euclideant distance, based on hordein banding patterns and clustered by the Neighbor-joining method.

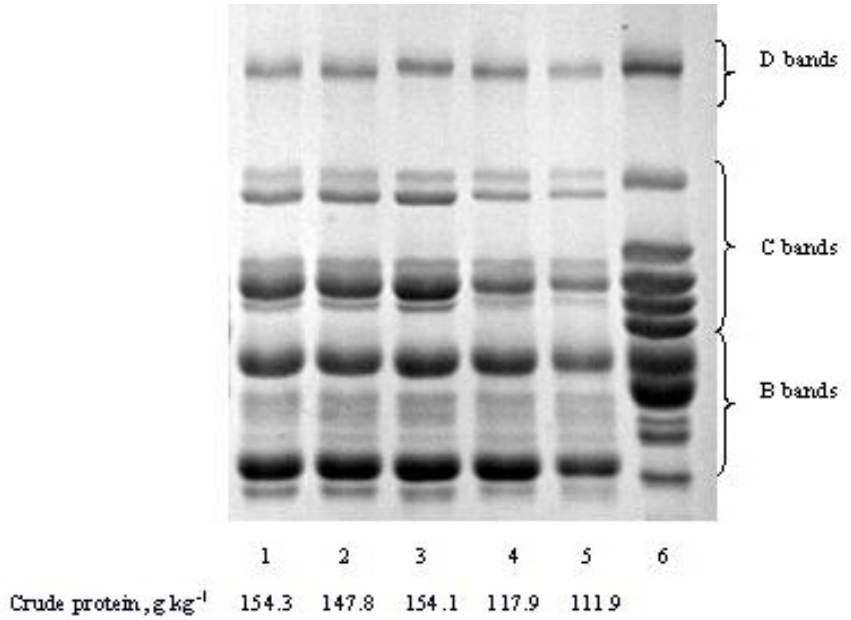

Fig. 5. Spring barley genotypes similar in hordein banding patterns and differing in crude protein content: (1) 'Primus II'; (2) 'Lechtaler'; (3) '379'; (4) 'Annabell'; (5) 'Justina'; (6) 'Igri' (control variety).

'Primus II' which crude protein content according to three-year average was 154.3, 147.8 and 154.1 g kg^{-1}, respectively, the bands were more dense and more intensively colored than for the varieties (18) 'Annabell' and (21) 'Justina' with a significantly lower crude protein content (117.9 and 119.9 g kg^{-1}, respectively). This was found for all hordein fractions but especially for C hordein. According to

Table 5

Associations between crude protein content and single hordein polypeptide bands

Band	Presence of band		Absence of band		Mean difference[1]
	number of genotypes	crude protein, g kg^{-1}	number of genotypes	crude protein, g kg^{-1}	
D hordein					
1	7	160.9	45	137.0	23.9**
2	5	131.7	47	141.1	9.4*
3	37	136.3	15	149.9	13.5*
4	7	147.3	45	139.1	8.2
C hordein					
5	21	128.5	31	148.2	19.7**
6	34	140.5	18	139.5	1.1
7	7	165.7	45	136.9	29.2**
8	4	144.9	48	139.8	5.2
9	2	175.6	50	138.8	36.8
10	5	146.7	47	139.5	7.2
11	40	140.4	12	139.7	0.7
12	48	142.1	4	117.2	24.9**
13	41	139.6	11	142.6	3.0
14	8	140.8	44	140.1	0.7
B hordein					
15	16	151.7	36	135.1	16.6**
16	9	142.9	43	139.7	3.3
17	36	135.2	16	151.5	16.3**
18	29	143.1	23	139.1	4.0
19	30	143.1	22	136.7	6.4
20	16	149.9	36	135.3	14.6**
21	17	145.6	35	137.6	7.9
22	38	149.3	14	137.5	11.8
23	5	139.2	47	140.3	1.2
24	41	141.9	11	133.7	8.2*
25	5	141.5	47	140.1	1.4
26	10	145.5	42	139.0	6.6

[1] *, ** – mean difference significant at the 0.05 and 0.01 level respectively.

the literature, changes in total grain protein content mainly determine the changes in hordein fraction (Shewry, 1995). The accumulation of hordein during grain development is genetically determined and depends on hordein genes expression during grain development stages (Kirkman et al., 1982), which means that for these high-protein genotypes heightened accumulation of grain nitrogen during grain filling is determined genetically.

As indicated by Dailey et al. (1988), the main differences in the increasing of the relative amount of hordein fractions are as visible changes in relative bands' intensity and loss of band sharpness in B hordein region, and replacement of the C polypeptide patterns with a single band. This changes the ratio of hordein B to C, which negatively correlates with the total protein content (Kirkman et al., 1982; Molina-Cano et al., 2001). Therefore analyzing the hordein profile types for different varieties grown under the same growing conditions, also the density of bands that could give information about differences in crude protein content of a definite genotype is important

to be considered for genotypes with similar hordein banding patterns.

Summary of significance of protein subunits for detecting associations between crude protein content and single hordein polypeptide bands by comparison of means using t-test is given in Table 5.

On the basis of combination of various banding patterns, 26 hordein bands were recognized among all the genotypes screened. There was great genotypic variation in the bands of C and B polypeptides, which was determined also in other studies. Shewry et al. (1980) described 21 and 8 different bands of B and C hordein, respectively, in the collection of 183 barley varieties and landraces, whereas Nielsen and Johansen (1986) described 12 C hordein bands and 15 B hordein bands in the collection of 66 varieties commonly grown in Denmark. In the study of Liu et al. (2000), in total, 26 hordein polypeptide bands were observed. The hordein banding patterns of each genotype consisted of 7 to 14 bands. All the 26 bands were polymorphic and, out of these, 10 exhibited significant association with crude protein content in the t-test. The mean value of crude protein increased significantly at the presence of bands 1, 7, 12, 15, 20, and 24, and at the absence of bands 2, 3, 5, and 17. The present study revealed that these protein polypeptide bands could be used in the screening of barley breeding material according to the crude protein content.

As the hordein electrophoresis is possible to be performed also from a single seed, the information about hordein polymorphism and variation of traits of interest considered together would help the breeder obtain useful information not only about genetic diversity of material but also about variability of the crude protein content of genotype already in the early stage of breeding process when the amount of grain is not sufficient for deep evaluation of grain quality. The use of molecular markers to locate the genes controlling quantitative traits, also crude protein, has been considered important in the analysis of such traits. The amount of information provided by marker-based research will depend on the type and number of markers and their linkage relationships. The frequency of these markers based on protein polypeptides for quantitative traits are not commonly observed since these markers based on protein alleles would tend to be simply inherited, whereas grain quality traits such as crude protein are polygenic in nature.

The results of the present study are encouraging for locating the factors that influence expression of crude protein. Hordein polymorphism and variation of traits of interest considered together would indisputably help the breeder to diversify the sources of germplazm and optimize the choice of parents to be used in crossing programs. The breeders can be sure that hordein profile will remain unchanged through different environments. However, the conclusions drawn in this study could be specific to the samples investigated and the environment in which this trait was recorded. It is because of existence of possible variation of crude protein due to genotype and environment interaction. This condition could change associations between single hordein polypeptide band and definite protein level found in this investigation. It would be desirable to continue validation of the obtained results in the next investigations including more barley genotypes characterized with wider variation in grain crude protein.

Conclusions

1. On the basis of SDS-PAGE, 5 D hordein, 16 C hordein, and 28 B hordein banding patterns with 27 bands were discriminated.
2. Cluster analysis based on hordein banding patterns classified three groups of genotypes. Two of them significantly ($p<0.01$) differed in mean crude protein content. Association was found between definite hordein banding patterns and crude protein content.
3. There were found five covered two-row varieties with identical hordein patterns but with varied crude protein content (111.9 to 154.3 g kg^{-1}). The patterns of these genotypes distinctly differed in the intensity of hordein polypeptide, especially in C hordein.
4. On the basis of combination of various banding patterns, 26 hordein polypeptide bands were recognized among all the screened genotypes; out of these, 10 exhibited significant association with grain crude protein content. The present investigation showed that association between biochemical variation and crude protein content could be used for screening of the breeding material and for future exploitation in barley improvement.

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Anotācija

Pētījumu veica Valsts Stendes Graudaugu selekcijas institūtā no 2004. līdz 2006. gadam. Izmantojot nātrija dodecilsulfāta (SDS)-poliakrilamīda gela elektroforēzi, novērtēja rezerves proteīna (D, C un B hordeīna) daudzveidību 52 dažādas izcelsmes vasaras miežu genotipiem (divkanšu, daudzkanšu, plēkšņainajiem, kailgraudu) saistībā ar kopproteīna saturu graudos. Pētījumā iekļautajiem genotipiem konstatēja 26 hordeīna polipeptīdu joslas, kas veidoja 5 D hordeīna, 16 C hordeīna un 28 B hordeīna polipeptīdu joslu profilus. Atrastas būtiskas atšķirības pēc kopproteīna satura graudos starp noteiktiem D, C un B hordeīnu joslu profiliem. Pamatojoties uz datiem par hordeīna joslu profiliem, klāsteru analīzē genotipi sadalījās trīs grupās. Starp divām grupām konstatēta būtiska atšķirība pēc kopproteīna satura graudos. Daļai no pētījumā iekļautajām šķirnēm konstatēts identisks hordeīna joslu profils, bet atšķirīgs kopproteīna saturs graudos. Hordeīna polipeptīdu joslas šīm šķirnēm atšķiras pēc krāsas intensitātes un blīvuma, īpaši C hordeīnā. Pētījumā konstatēts, ka 6 hordeīna polipeptīdu joslu klātbūtne un 4 hordeīna polipeptīdu joslu trūkums būtiski ietekmē kopproteīna saturu graudos. Dati par hordeīna daudzveidību ir izmantojami selekcijas izejmateriāla ģenētiskās daudzveidības novērtēšanā, kā arī selekcijas līniju graudu kopproteīna satura izvērtēšanā selekcijas sākumposmā.