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Genetic resources of spring barley: analysis of Hordein polymorphism

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The objective of this study was to evaluate the hordein prolamin polymorphism of spring barley varieties and breeding lines from the collection of genetic resources. Hordein electrophoresis revealed an important set of polymorphisms. Different banding patterns were obtained with D-, C-, and B-hordein groups. In D-hordein group, there were identified three patterns, and according to their presence three profile types were identified. Spring barley varieties and most of the breeding lines (94.5%) were found to possess D_1 profile types. D_2 and D_3 types were identified in the breeding lines that have hulless barley in their pedigree. In the C-hordeins group there were identified seven patterns, and according to their combination four profile types were distinguished in the varieties and eight profile types in the breeding lines. The C_1 and C_3 types were the most frequent both among the varieties and breeding lines, whereas C_5–C_8 were identified only in individual breeding lines. In the B-hordein group, 14 patterns were determined. The varieties were found to possess seven and the breeding lines 12 profile types. The B_2 and B_6 types were most frequent among the varieties and B_2 and B_{12} among the breeding lines.

While assessing general hordein composition in spring barley varieties, we determined 14 and in breeding lines 28 different profile types. Part of the varieties (47.1%) and breeding lines (34.4%) were polymorphic by hordein profile types, which means that they were composed of 2–4 profile types. The spring barley varieties 'Alsa' and 'Aura' possessed identical hordein profile types, whereas 'Auksiniai-3' and some breeding lines possessed specific hordein profile types.

Key words: spring barley, genetic resources, hordein polymorphism, profile types

INTRODUCTION

Within genetic resources, genetic variability is often present, which is important to know before a resource is utilized in breeding. Agromorphological traits have been used for this purpose, although these are of limited use for assessing the levels of variability because they are frequently influenced by environmental factors and growing conditions. For the assessment of plant genetic variability, different biochemical and molecular methods are used [1]. Biochemical methods, unlike morphological, are more variable, less dependent on the environment and informative at any plant development stage.

Cereal crop prolamins, i. e. alcohol-soluble storage proteins, have been frequently used to study genetic diversity in many species, since they are highly polymorphic and environmentally stable [2]. Barley storage proteins – hordeins – have been divided into groups on the basis of their electrophoretical mobility and amino-acid composition [3]. D-hordeins have the highest molecular weight (105 kD); they are characterized by a high amino-acid (glutamine, glicine and proline) content [4]. Synthesis of these hordeins is encoded by the *Hor*3 locus located on the long arm of chromosome 1H(5) [5]. 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 multigenic loci *Hor*1 and *Hor*2, respectively, both located on the short arm of chromosome 1H(5) [3]. The advantages of hordeins for studying genetic diversity in barley have been described by many authors [6–8].

The barley storage protein hordein is characterized by a high degree of polymorphism. On analysing 211 accessions of wild close relatives of barley, in *Hordeum vulgare* spp. *agriocrithon* and *H. vulgare* spp. *spontaneum* were found altogether 32, 27 and 13 different phenotypes for *Hor*1, *Hor*2 and *Hor*3, respectively [9].

Different gel and buffer systems were used for hordein electrophoresis [7, 10, 11]. High performance liquid chromatography (HPLC) and DNA analysis are successfully applied for investigating hordein composition and barley polymorphism [12–14].

The objective of this study was to evaluate prolamin hordein polymorphism in spring barley varieties and breeding lines from the collection of genetic resources.

MATERIALS AND METHODS

Seventeen spring barley varieties and 32 breeding lines from the collection of genetic resources, mostly of Lithuanian origin, were evaluated in competitive yield trials in 2005. Hordein polymorphism was studied by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Lallemand and Briand (1990). Hordeins were extracted from crushed grain

(400 µl / 50 mg) with 8% mercaptoethanol, 2% SDS, 10% glycerol, 1 M Tris-HCl, pH 6.8, 0.01% bromophenol blue and 15% dimethylformamide. The stacking gel contained 3.5% and the separating gel 13% of acrylamide. The gels were run for 16–18 h on a (12 × 11 × 0.1 cm) gel at a 20 mA constant amperage, using Biometra electrophoresis chambers. Gels were stained in 50% trichloracetic acid (TCA) and 0.01% Coomassie BB R 250 for 30–60 minutes, destained in 7% acetic acid overnight. Ten individual grains were used for the electrophoretic analysis of homogeneous samples and 30 grains for samples that had heterogeneous profiles. Hordein patterns were classified using the Lallemand–Briand system with modifications [15]. As a reference we used varieties with a know hordein composition.

The level of the statistical significance of data was calculated by the method of analysis of variance using ANOVA software adapted by Dr. P. Tarakanovas in LIA [16].

RESULTS AND DISCUSSION

Electrophoresis of the barley storage protein hordein revealed 24 patterns with different electrophoretical mobility. The different patterns obtained with D-, C- and B-hordeins are shown in Figure. There were three different patterns for D-, 7 patterns for C- and 14 for B-hordeins.

No differences were detected between D-hordein patterns of the study varieties. In all varieties only one pattern was identified. Profile type D_1 was common for all varieties (Table 1). In the study of the protein electrophoretical profile of northern barley varieties, no differences were detected among D-hordein patterns, either [17]. In the D-hordein group, we determined three patterns for breeding lines. In most cases, like in variety profiles, we found the D_1 type. The breeding line 160-05 in this hordein group had profile types D_1 and D_2, they constituted 66.7% and 33.3%, respectively. Type D_3 was determined in the profile of the breeding line 161-05. In the hybridization of both breeding lines mentioned above we used hulless barley in whose profiles hordein types D_2 and D_3 were most frequent. In the study of hulless barley hordein polymorphism, for the D-hordein group more patterns were determined [18].

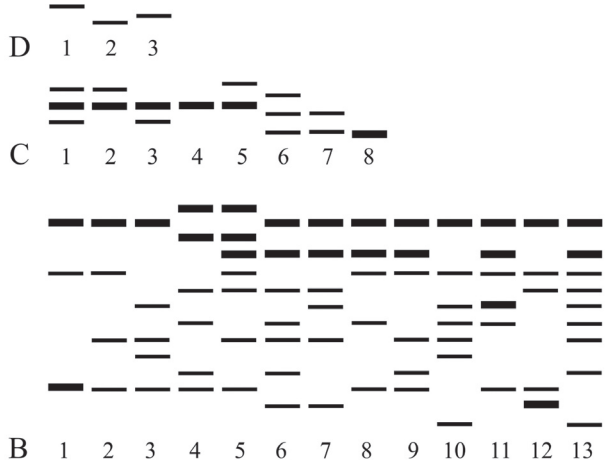

Figure. Diagrammatic representation of SDS-PAGE patterns of D, C and B hordeins in spring barley material

The C-hordein group has more patterns than D-hordeins. In the C-hordein group we determined seven patterns. In the profiles of varieties we determined three patterns, one of them being common for all varieties, and four profile types. For breeding lines in the C-hordein group we determined seven patterns, all of them being polymorphic, and eight profile types (Table 2). The most frequent profile type C_1 possessed all three patterns. In the varieties this type constituted 61.8% and in the breeding lines 55.2%. The varieties 'Auksiniai', 'Vilniečiai', 'Ūla' and some breeding lines had profile type C_2. The profile type C_3 is more frequent in hordein profiles of the varieties (23.5%) than of the breeding lines (15.6%). The varieties 'Luokė' and 'Rudzik' had the profile type C_4 which was represented by only one pattern. This profile type is rare among spring barley breeding lines. The C_5–C_8 types were specific to some lines or their individuals.

The spring barley varieties tested were found to possess 13 patterns and the breeding lines 14 patterns in the B-hordein group. An analogous number of B-hordein patterns was produced in the profiles of hulless barley varieties [18]. In the B-hordein group we discriminated seven profile types in the varieties. A similar number of B-hordein profile types in barley varieties was found by other authors [17]. Breeding lines had a greater diversity of profile types of B-hordein group. There were discriminated 12 profile types. A similar number of B-hordein profile types was determined in the hulless barley variety collection [18]. In both groups of specimens (varieties and lines) B_2 and B_6 profile types were the most frequent. The B_2 profile type was represented by four patterns and was specific to the varieties 'Auksiniai II', 'Gausiai', 'Gintariniai' and many breeding lines. The malting variety 'Otis' and the breeding lines 8242 and 8166-5, characterised by a good malting quality, had six patterns in B-hordeins group and were attributed to B_6 profile type. The breeding lines 8151-7, 8155-7 and 8157-3, in whose pedigree the same parent line was used, had a common B_{12} profile. Profile types B_1, B_4, B_7 and B_9 were specific to only particular varieties or breeding lines.

The total number of patterns from all hordein groups (D + C + B) in individual grains of varieties and breeding lines varied from 5 to 11 and from 6 to 12 patterns in varieties and breeding lines, respectively. Electrophoretic analysis by general hordein composition revealed 14 different hordein profile types in spring barley varieties and 28 of hordein profile types

Table 1. **The number of hordein patterns and their polymorphism**

Sample group	Number of patterns and their polymorphism, %					
	D-hordeins		C-hordeins		B-hordeins	
	1	2	1	2	1	2
Varieties	1	0	3	66.7	13	100
Breeding lines	3	100	7	100	14	100

1 – number of patterns, 2 – polymorphism, %.

Table 2. **Types of hordein profiles and their frequencies (%) in spring barley varieties and breeding lines**

Hordein types	Frequency		Hordein types	Frequency	
	Varieties	Breeding lines		Varieties	Breeding lines
D-types			B- types		
D_1	100	94.8	B_1	2.9	0
D_2	0	1.7	B_2	32.3	21.9
D_3	0	6.9	B_3	14.7	6.2
C- types			B_4	8.8	1.1
C_1	61.8	55.2	B_5	5.9	12.6
C_2	8.8	12.1	B_6	23.5	15.6
C_3	23.5	15.6	B_7	11.8	1.6
C_4	5.9	3.1	B_8	0	7.8
C_5	0	1.0	B_9	0	1.5
C_6	0	5.3	B_{10}	0	3.1
C_7	0	6.2	B_{11}	0	7.8
C_8	0	1.5	B_{12}	0	18.8
			B_{13}	0	3.1

Table 3. **Hordein polymorphism of spring barley varieties and breeding lines**

Sample group	Polymorphic samples, %			
	D-hordeins	C-hordeins	B-hordeins	D+C+B-hordeins
Varieties	0	29.4	41.2	47.1
Breeding lines	3.1	28.1	25.0	34.4

in breeding lines. The spring barley varieties 'Auksiniai II', 'Gintariniai', 'Gausiai' and 'Alsa' with 'Aura' possessed identical hordein profile types, whereas the variety 'Auksiniai3' possessed the hordein profile type specific to only this variety. Specific hordein profile types were identified in breeding lines with a good malting quality (7967-2), resistant to powdery mildew (8239), characterised by yielding stability (8019-4). Breeding lines from the same crossing combination (8056-2, 8056-6) and the breeding lines 8151-7, 8155-7 and 8157-3 in whose pedigree the same parent line was used had identical hordein profile types.

The results of the present study revealed intravarietal hordein polymorphism for Lithuanian varieties and breeding lines. Part of the varieties (47.1%) and breeding lines (34.4%) were polymorphic by hordein profile types, which means that they were composed of 2–4 profile types (Table 3). Particular profile types identified in breeding lines made up 6.3–93.7%. Most polymorphic were the breeding lines 7661-1 and 7695-4. They were represented by 3–4 hordein profile types. The breeding line 7661-1 was monomorphic in the D- and B-hordein groups, but in group C we determined three profile types – C_1, C_2 and C_6 (60.0, 33.3 and 6.7%, respectively). Line 7695-4 had a different profile in the C- and B-hordein groups and according to total hordein had four profile types.

The spring barley varieties had identical D-hordein group profile types, but a higher degree of polymorphism according to C-, B- and total hordein profile types compared with the breeding lines tested.

CONCLUSIONS

Electrophoresis of barley storage protein-hordein revealed 24 patterns with different electrophoretic mobility: in the D-hordein group 3, in C-hordeins 7, in B-hordeins 4 patterns. The spring barley breeding lines examined were distinguished for the variability of hordein profile types. While assessing the general hordein composition in spring barley varieties we determined 14 and in breeding lines 28 different profile types.

Part of the varieties (47.1%) and breeding lines (34.4%) were polymorphic by hordein profile types, which means that they were composed of 2–4 profile types. The spring barley varieties 'Alsa' and 'Aura' possessed identical hordein profile types, whereas 'Auksiniai 3' and some breeding lines possessed specific hordein profile types.

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VASARINIŲ MIEŽIŲ GENETINIAI IŠTEKLIAI: HORDEINŲ POLIMORFIŠKUMAS

Santrauka

Įvertintas vasarinių miežių genetinių išteklių kolekcijos veislių bei selekcinių linijų prolaminų hordeinų polimorfiškumas. Skirtingas elektroforezinių juostų skaičius nustatytas D-, C- ir B-hordeinų grupėse. D-hordeinų grupėje gautos trys skirtingo elektroforezinio paslankumo juostos, pagal jų buvimą nustatyti trys profilių tipai. Vasarinių miežių veislės ir dauguma selekcinių linijų (94,5%) turėjo D_1 profilį. D_2 ir D_3 profiliai gauti selekcinėse linijose, kurias kuriant naudoti plikagrūdžiai miežiai. C-hordeinų grupėje identifikuotos 7 juostos ir nustatyti 4 profilių tipai veislėse ir 8 – selekcinėse linijose. Veislėse ir selekcinėse linijose dažniausiai randami C_1 ir C_3 profiliai, tuo tarpu C_5–C_8 buvo būdingi tik atskiriems selekcinių linijų grūdams. B-hordeinų grupėje gauta 14 juostų, 7 profilių tipai veislėse bei 12 – selekcinėse linijose. Veislėse dažniau randami B_2 ir B_6, o selekcinėse linijose – B_2 ir B_{12} profilių tipai. Pagal bendrą hordeinų elektroforezinį spektrą veislėse nustatyta 14, o selekcinėse linijose – 28 hordeinų profiliai. Dalis (47,1%) veislių bei selekcinių linijų (34,4%) turėjo 2–4 skirtingus hordeinų profilius. Vasarinių miežių veislės ‘Alsa’ ir ‘Aura’ turėjo tarpusavyje tapačius hordeinų profilių tipus, tuo tarpu ‘Auksiniai 3’ ir kai kurios selekcinės linijos turėjo specifinius hordeinų profilių tipus, būdingus tik tai veislei ar selekcinei linijai.