HORDEIN COMPOSITION OF YUGOSLAV BARLEY CULTIVARS

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ABSTRACT

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was applied to study composition of D, C, and B hordein fractions of 33 Yugoslav barley cultivars. In the *Hor3* locus two different D hordein patterns were present, in *Hor1* eight C hordeins and in *Hor2* seven B hordein patterns. On the basis of hordein formulas the cultivars were divided into 18 groups. Twelve cultivars showed unique hordein subunit composition while remainder fell into six groups of 2 to 9 cultivars. Intra-cultivar hordein polymorphism was found in two cultivars.

INDEX WORDS: barley, hordein, electrophoresis, cultivar identification

INTRODUCTION

Hordein, the prolamin fraction of barley, is the major class of storage proteins in the endosperm tissue of mature grains. It accounts for approximately 50% of the total protein and consists of heterogeneous groups, namely D (high molecular weight), C (sulphur-poor), B and γ (both sulphur-rich) hordein polypeptides. D hordein is a minor component, accounting for 1-2% of the total fraction and consists of only one or two polypeptides . C hordein accounts for about 10-20% of the total fraction, and there is extensive genotypic variation in the numbers, molecular weights, and isoelectric points of the component polypeptides (Shewry et al., 1980, 1985). B hordein is the quantitatively major group of storage proteins, and between 8 and 16 major polypeptides together with a number of minor ones can be separated by 2-D electrophoresis (Faulks et al., 1981).

The multigene families encoding the C, B, D, and γ hordein polypeptides have been assigned to chromosome 5 at loci *Hor1*, *Hor2*, *Hor3* and *Hor5*, respectively (Jensen et al., 1980; Kreis and Shewry, 1992). The *Hor2* locus comprises two subloci (Shewry et al., 1990) and maps close to *Hor5*, which also encodes S-rich hordein.

The correct identification of barley cultivars is important to plant breeders for protection of their proprietary rights on cultivars and to crop users for the determination of grain suitability for different end-uses, especially malting. Several approaches have been introduced to enable characterization of genotypes from single or half seeds as a complement to the identification method of visual examination of heritable morphological differences of whole plants or grains. These techniques including electrophoresis (Weiss et al., 1991; Roininen et al., 1992; Peltonen et al., 1994), isoelectric focusing (Shewry et al., 1985; Black and During, 1995) and liquid chromatography (Marchylo and Kruger, 1985; Allison and Bain, 1986), have been used to obtain qualitative information about the variation in hordein and cultivar identification by hordein allelic variation.

In this paper hordein compositions of 33 different barley genotypes were studied using SDS-PAG electrophoresis. The usefulness of this technique for identification of Yugoslav barley cultivars is discussed.

MATERIAL AND METHODS

Twenty seeds of winter and spring barley cultivars selected in the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia, were used for analysis of hordein composition. Barley cultivars Melusine (France), Triumph (Great Britain), Sonate (France), and Menuet (Holland) were used as control.

A method of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) after Laemmli (1970) was applied while sample (rapid type) and gel preparation, staining, and fixation were performed according to Heisel et al. (1986) introducing certain modifications (20 µl samples were applied, 1M Tris-HCl pH 6.8 and 1M Tris-HCl pH 8.8 buffers for gel preparation, and 0.02% Commassie Brilliant Blue R-250 as staining solution). Vertical electrophoresis (Hoefer, USA) was performed, for 4h at 20mA constant current per gel (first hour) and 40 mA till the end.

A modified method after Shewry et al. (1978) for hordein patterns designation of each of three loci, namely *Hor3* (Roman numerals), *Hor1* (alphabet), and *Hor2* (Arabic numerals) was used to determine D, C, and B hordein fraction composition. Also, hordein formula was defined for each cultivar by combining the results obtained for the analyzed loci.

RESULTS AND DISCUSSION

The three polypeptide groups were distinguished on the gel. The first one with two pairs of D hordein bands in each, the second with between four and nine C hordein bands, while B hordeins made the third group with between five and seven bands (Fig. 1). Variability within each locus was estimated on the basis of the presence or absence, as well as position of protein bands on the gel.

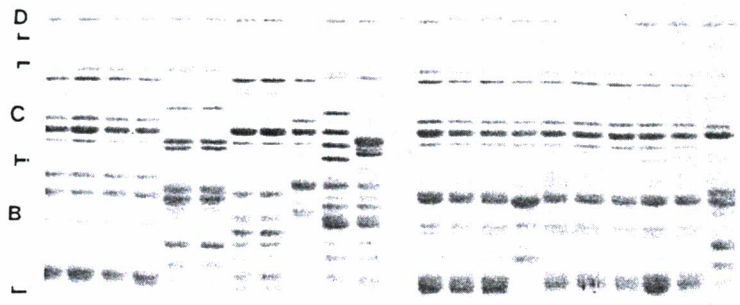

Figure 1. Hordein electrophoregram of barley cultivars

In the *Hor3* locus two D hordein patterns were present (Fig. 2) of which type I in 63.6% while type II in 36.4% of cultivars (Fig. 3a)

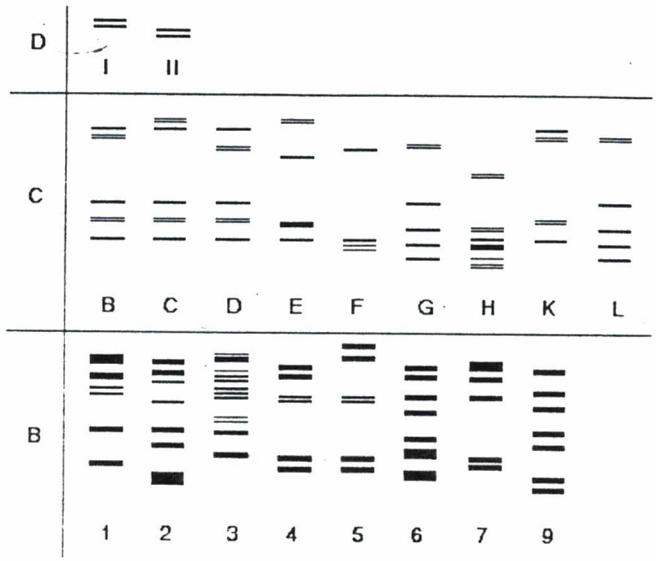

Figure 2. Diagrammatic representation of D, C, and B hordein patterns separated by SDS-PAGE

In *Hor1* eight different C hordein patterns were found (Fig 2). The majority of cultivars belong to B, C, and E types (Fig. 3b).

In *Hor2* seven different B hordein patterns were present (Fig. 2) with the highest frequency of type 1 (Fig. 3c).

Studing hordein polymorphism among European barley cultivars Shewry et al. (1982) revealed 3 different types of D hordein patterns, while Roininen et al. (1992) revealed 6 D hordein patterns among Finnish barley cultivars. As for the variability of C hordeins the number of types varied from 8 (Shewry et al., 1978) to 15 (Heisel et al., 1986) and the number of B hordein types ranged from 13 (Montembault, 1983) to 24 (Shewry et al., 1978). The number of hordein phenotypes revealed in this paper was somehow smaller as a consequence of genetic divergence among parental genotypes or the number of analyzed cultivars. On the other hand, the inconsistencies in electrophoretic methods used by different authors could also cause differences in scoring hordein patterns.

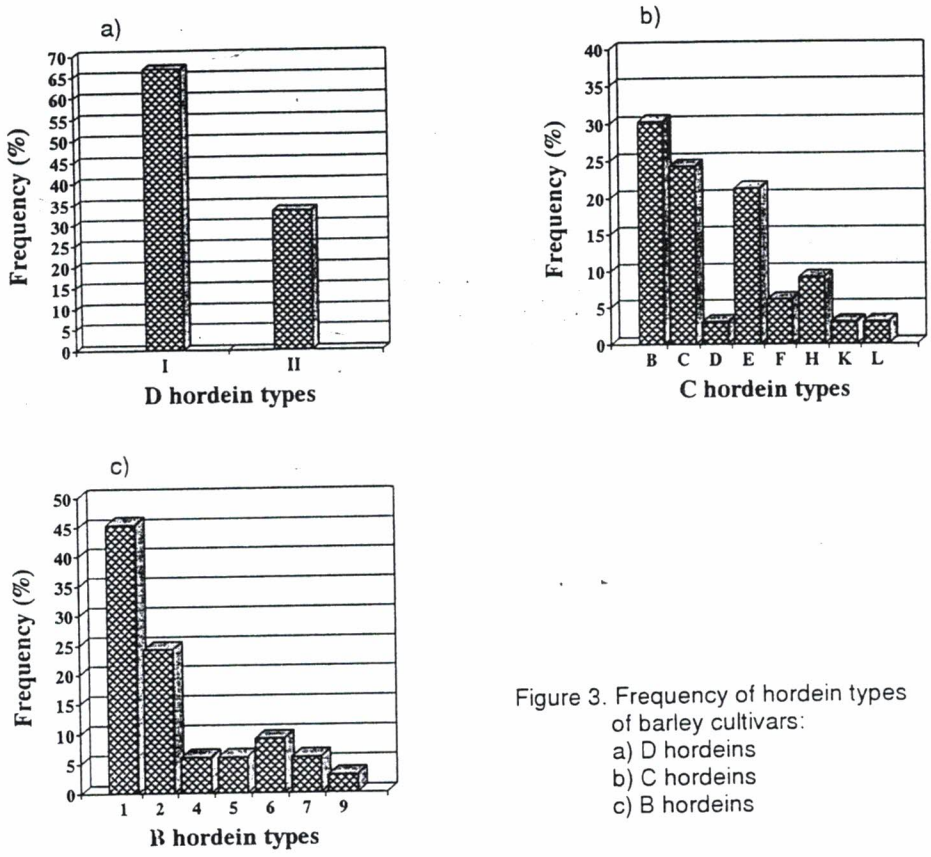

Figure 3. Frequency of hordein types of barley cultivars:
a) D hordeins
b) C hordeins
c) B hordeins

Hordein formulas were designated to all the cultivars. The first symbol in the formula represents D hordein type (I or II) the second C hordein (B-L) while the third symbol represents a B hordein (1-9). On the basis of hordein formulas the cultivars could be divided into 18 groups (Tab. 1). Twelve cultivars had a unique hordein formula, while in the other six groups, from two to nine cultivars were present.

According to C and B hordein formulas for 88 barley cultivars, Shewry et al. (1979) were able to completely identify 15, while the rest of the cultivars were divided into groups with the number of cultivars in each group ranging from 2 to 25. By analyzing hordein variability of 20 barley cultivars Weiss et al. (1991) distinguished 9 different groups of hordein patterns.

Two of the analyzed barley cultivars were heterogeneous with two different electrophoretic patterns each (a variant occurring more frequently is designated as "a" and more rare one as "b"), with the average heterogeneity of 6.1%. In NS 294 the frequency of type "a" was 88.9% while that of type "b" 11.1%. In NS 323 it was 66.7% and 33.3%, respectively.

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Table 1. Hordein fo

Hordein formulas	Culti
I B 1	NS 2 NS 3 NS 7
II B 1	NS 2
I C 1	NS 1
I C 2	NS 7
I C 4	NS 3
I C 5	Vikt
II C 2	Jele
II C 4	Pek
I D 6	NS 3
I E 2	Vihc
II E 1	NS 3
II E 2	NS
I F 7	NS 3
II F 7	NS 3
I H 1	NS 3
I H 6	NS 3
I K 9	NS 3
I L 2	NS
	C
I B 1	Son
I C 2	Triu
I C 4	Mer
I G 3	Mel

Certain degree of intra-cultivar heterogeneity is common among self-pollinated plants (Vapa, 1991; Crosatti et al., 1993). The occurrence of biotypes can be favorable, depending on the cultivar genetic composition, in the process of selection due to increased cultivar adaptability. In analyses of hordein composition of 353 barley cultivars White and Cooke (1992) found out that 9.92% of cultivars were heterogeneous, while Radović (1995) out of the 40 Yugoslav barley cultivars scored 7.5% heterogeneous.

The usefulness of application of electrophoresis of seed storage proteins in cultivar fingerprinting is largely influenced by genetic background. In most cases cultivars with common parents or close genetic background are more probable to have identical electrophoregrams (Roininen et al., 1992), but having in mind the linkage of *Hor* loci to *Ml-k* and *Ml-a* loci for powdery mildew resistance (Jensen et al., 1980) some of the hordein genotypes could be favored during the selection process ("hitch-hiking effect"), regardless of the selection neutrality of *Hor* loci themselves.

Table 1. Hordein formulas of barley cultivars

Hordein formulas	Cultivars
I B 1	NS 27, NS 150, NS 299, NS 313, NS 315, NS 317, NS 329, NS 703 NS 705
II B 1	NS 293
I C 1	NS 135
I C 2	NS 701
I C 4	NS 301
I C 5	Viktor, Lazar
II C 2	Jelen, NS 294
II C 4	Pek
I D 6	NS 310
I E 2	Vihor
II E 1	NS 309, NS 311, NS 331
II E 2	NS 183, NS 307, NS 319
I F 7	NS 316
II F 7	NS 324
I H 1	NS 321
I H 6	NS 292, NS 296
I K 9	NS 306
I L 2	NS 323
Control cultivars	
I B 1	Sonate
I C 2	Triumph
I C 4	Menuet
I G 3	Melusine

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