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Biochemical and Genetic Variation of Hordein Subunits in Korean Barley

Lee Sung Shin*, Jin Baek Kim, Dong Sub Kim*, Jung Hyun Nam***, Jae Chul Kim***, Chung Kon Kim***, Byung Hee Hong*, and Yong Weon Seo*†**

**Dept. of Crop Science, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea*
***Kumho Life and Environmental Science Laboratory, Kwangju 500-712, Korea*
****National Crop Experiment Station, RDA, Suwon 441-100, Korea*

ABSTRACT : One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1D SDS-PAGE) was used to determine whether it would provide improved resolving power of hordein proteins concomitant with improved identification of Korean barley cultivars and germplasms. This system gave rapid and reproducible separations of hordein polypeptides. Total fourteen of clear and easily scorable subunits were identified in Korean barley cultivars and germplasms and their polymorphic constitutions could provide biochemical genetic information in progeny analysis and endosperm quality improvement in barley breeding programs. Each hordein polypeptides residing in B, C, and D hordein pattern designations were scored to prepare a cultivar catalogue of protein patterns. On the basis of this character, 7 hordein polypeptide patterns were constructed from 108 barley cultivars and experimental lines. The molecular weight of hordein subunits in Korean barley cultivars and experimental lines varied in the range of 98 to 48 kDa. In contrast, less polymorphic hordein polypeptides were found in the low protein barley lines including malting barleys than those found in Korean barley cultivars and experimental lines.

Keywords **:** Barley, 1D SDS-PAGE, hordein, cultivar identification

The identification of variations in major cereals is of commercial and agricultural importance. Agricultural research activities to examine seeds, both for the determination of the distinctness of new varieties and for identification of the established varieties are also routinely required (Shewry *et al.*, 1978a).

Several biochemical techniques (Cooker, 1984; Wrigey *et al.*, 1984) have been used to augment morphological examination, and most of them rely on variations among the prolamins (hordeins), the alcohol soluble seed storage proteins. Hordeins have been studied extensively because of their contribution to the low overall nutritive value of barley seed proteins (Munk 1972). They are notably deficient in the essential amino acid lysine, thus responsible for the poor quality of the whole seed as a diet for monogastric animals (Shewry *et al.*, 1979). In addition, their amount and composition influence the suitability and quality of the grain for final end-uses. The quality for malting is negatively correlated with the total amount of hordein storage proteins and is also affected by the hordein composition.

Hordeins consist of the three distinct groups of polypeptides called B, C, and D hordeins on the basis of their molecular weights and differences in their amino acid compositions. The B (mol. wt. 32.4~45 kDa) and C (mol. wt. 49~72 kDa) hordein groups are highly polymorphic mixtures of polypeptides encoded by complex multigenic loci. 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 that are separated by electrophoretic systems (Shewry *et al.*, 1980a, 1985). In spite of this polymorphism, the individual polypeptides have a high degree of structural homology (Shewry *et al.*, 1981) and are all encoded by a multigene family (probably 20~30 copies) at the *Hor 1* locus on the short arm of chromosome 5 (Oram *et al.*, 1975; Shewry *et al.*, 1978b, 1980b; Doll & Brown, 1979; Jensen *et al.*, 1980). B hordein is the quantitatively major group and accounts for about 80% of the storage proteins in the mature grain, and between 8 and 16 major polypeptides together with a number of minor ones can be separated by 2D electrophoresis (Faulks *et al.*, 1981). They are encoded by a multigene family tightly linked to the *Hor 2* locus located on the short arm of chromosome 5. B hordein shows two major double bands of 35 and 46 kDa (called B_1 and B_3) and two minor bands of intermediate molecular weight (B_2). The γ-hordein is a minor component with molecular weight similar to those of B hordein in 1D SDS-PAGE. It is separated to the three bands called γ_1, γ_2, and γ_3 and exhibit allelic variations at *Hor 5*. D hordein is a minor component with higher molecular weights (mol. wt. 100 kDa), accounts for 1~2% of the total fraction, and consists of only one or two polypeptides (Rahman *et al.*, 1982; Shewry *et al.*, 1983). Genetic and molecular analyses show that it is encoded by a single gene or small multigene family located at the *Hor 3*

†Corresponding author: (Phone) +82-2-3290-3005 (E-mail) seoag@korea.ac.kr
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Table 1. Barley cultivars, experimental lines, and germplasms used in 1D SDS-PAGE.

Korean barley cultivars and experimental lines
Sacheon6, Saekangbori, Saessalbori, Saealbori, Saeolbori, Chalbori, Chalssalbori, Tapgolbori, Suwon252, Keunalbori, Chokwangbori, Paldalbori, Suwon18, Suwon185, Suwon187, Suwon189, Suwon200, Suwon201, Suwon202, Suwon203, Suwon204, Suwon205, Suwon206, Suwon207, Suwon208, Suwon209, Suwon212, Suwon214, Suwon216, Suwon229, Suwon231, Suwon232, Suwon234, Suwon235, Suwon238, Suwon239, Suwon241, Suwon242, Suwon243, Suwon244, Suwon249, Suwon254, Suwon256, Suwon258, Suwon259, Songhakbori, Iri20, Iri21, Iri22, Iri23, Iri24, Iri25, Iri26, Iri27, Iri28, Iri29, Iri30, Iri31, Iri32, Iri33, Iri34, Iri35, Iri36, Iri37, Iri38, Iri39, Iri40, Iri41, MilyangCoveredbori, Milyang34, Milyang35, Milyang36, Milyang37, Milyang38, Milyang43, Milyang46, Milyang48, Milyang53, Milyang56, Milyang59, Milyang60, Milyang67, Milyang71, Milyang72, Milyang73, Milyang77, Milyang78, Milyang79, Milyang86, Milyang87, Milyang88, Olbori
Extra-low protein barleys from NSGR
K947, K951, K954, K970, K973, K974
Foreign malting barleys
Foster, Harrington, Karl, Morex, Robust, Stander
Korean malting barleys
Jinyangbori, Namhyangbori, Doosan8, Doosan29

locus on the long arm of chromosome 5 (Blake *et al.*, 1982; Shewry *et al.*, 1983; Bunce *et al.*, 1986). Sometimes some low molecular weight polypeptides, A hordein (mol. wt. <20 kDa), are also extracted with aqueous alcohol from the barley seed, but these are usually not defined as hordeins (Agagoncillo, 1981).

Electrophoretic analysis of hordein polypeptides is known to be a good supplementary technique to visual methods for cultivar identification (Gebre *et al.*, 1986). Shewry *et al.* (1978a) used single kernel analyses of 88 cultivars by SDS-PAGE and urea-PAGE to recognize 32 different groups. Separations have also been achieved on the basis of differences in the isoelectric points of the hordeins by isoelectric focusing and their different hydrophobicities by reversed-phase high-performance liquid chromatography (Marchylo & Kruger, 1984, 1985).

The purpose of this study is to develop electrophoretic system for hordein polypeptides separation and to identify hordein subunits for protein fingerprints which could be used as biochemical genetic markers for progeny analysis and plant identification in barley breeding programs.

MATERIALS AND METHODS

Plant materials

Barley seed samples of 92 cultivars and experimental lines including 4 malting cultivars were provided by the National Crop Experimental Station at Suwon. Six extra-low endosperm protein barley germplasms evaluated at Aberdeen, ID, and 6 malting barley cultivars were provided by USDA-ARS, National Small Grain Research Facility, U.S.A. Seeds were milled by Buhler experimental mill for the laboratory experiments. The names of tested barley cultivars and germplasms are listed in Table 1.

Preparation of hordeins

Hordeins were extracted from 40 mg flour sample with 1 ml 55% (v/v) aqueous isopropanol containing 2% (v/v) β-mercaptoethanol in a 1.5 ml tube. Extraction was conducted by brief vortexing and subsequent sonication at 60°C for 30 min. The mixture was centrifuged by 8400 *g* for 4 min at room temperature and the supernatant was transferred to a new 1.5 ml tube. Hordeins were precipitated from the supernatants by the addition of an equal volume of ice-cold 1.0 M NaCl and incubation on ice bath for 20 min. Precipitated hordein was recovered by centrifugation at 5000 *g* for 10 min at room temperature and the pellet was rinsed with ice-cold deionized distilled water (ddH_2O). Precipitated hordein samples were dissolved in 1 ml of SDS sample buffer [2% (w/v) SDS, 10% (w/v) sucrose, 0.025% (w/v) bromophenol blue, 0.5 mM dithiolthreitol, and 0.025 M Tris-HCl pH 6.8] by heating in boiling water for 5~10 min.

Hordein subunit fractionation

The stacking gel layer contained 4% (w/v) acrylamide, 0.11% (w/v) N, N'-methylene bisacrylamide (BIS), 0.1% (w/v) SDS, 0.5 mM Na_2EDTA, and 0.125 M Tris-HCl pH 6.8. The separating gels contained 14% (w/v) acrylamide, 0.26% (w/v) BIS, 0.1% (w/v) SDS, 0.5 mM Na_2EDTA, and 0.375 M Tris-HCl pH 8.8. The running buffer contained 0.19 M glycine, 0.1% SDS, 1.0 mM Na_2EDTA, and 25 mM Tris-HCl pH 8.3. All gels were polymerized using 0.075% (w/v) ammonium persulfate and 0.038% (v/v) N, N, N', N'-tetram-

ethylethlenediamine. Samples (3 μl each) were loaded in the gel and subjected to electrophoresis at 20 mA. Following electrophoretic separation, gels were silver-stained.

RESULTS

The hordein polypeptides from whole-meal samples of 108 cultivars and experimental lines were separated into the three groups termed B, C, and D by 1D SDS-PAGE (Fig. 1 and Fig. 2). We were able to produce clear hordein subunits by combined use of sensitive silver stain and low protein amounts of gel loading.

The B and C hordein regions showed a great variation in the band patterns. Presence or absence of subunits is easy to score and resulted in producing rich polymorphisms. Therefore, this gel separation system could provide information that enable us to identify barley cultivars and germplasms. However, subunits from D hordein solely did not give enough variation for cultivar groupings. The low molecular weight A hordeins, which are probably not true storage proteins, did not vary qualitatively among the cultivars and were often run off the gel when the gel was run longer to improve resolution of the other groups in this system.

We grouped barley materials based on the place of development and end-use purposes into A) Korean cultivars and experimental lines, B) foreign germplasms with extra-low storage proteins, C) foreign malting barleys, and D) Korean malting barleys (Table 2).

Figure 2 shows hordein subunit profiles of the cultivated malting barleys and germplasms that were known to have low endosperm proteins. The hordein subunits fractionated by 1D SDS-PAGE were scored by the presence of subunits based on their relative molecular weights. Korean barley cultivars and experimental lines showed that the range of hordein subunit presence varied from 98 to 48 kDa (Table 2). Most variable subunits were 68.0, 67.4, 64.8, 63.6 kDa C hordeins: these subunits were present in foreign malting barley but absent in Korean malting barley cultivars. In contrast, less polymorphic hordein polypeptides were found in low protein barley germplasms than those found in Korean barley cultivars and experimental lines. Certain protein bands such as 86.4, 71.5, 70.0, and 57.8 kDa were found rarely in malting barleys. Korean malting barleys showed unique subunits such as 48.5, 58.9, 60.0, and 61.2 kDa bands which were not found in foreign malting barleys.

Analyses of hordeins from 92 barley cultivars, 6 low-protein germplasms, and 10 malting barley cultivars yieled 7 different hordein pattern groups (Fig. 3. A and B, Table 3).

Fourteen components occurring in 7 hordein pattern groups were identified in Korean barley cultivars and germ-

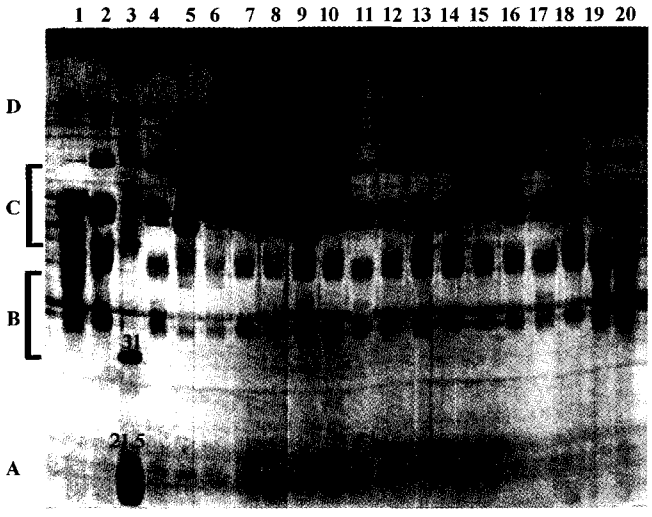

Fig. 1. Genetic variation of hordein subunits used as biochemical genetic markers. Lane 1: Suwon203, lane 2: Suwon204, lane 3: molecular size marker (kDa), lane 4: Suwon205, lane 5: Suwon206, lane 6: Suwon207, lane 8: Suwon209, lane 9: Suwon212, lane 10: Suwon214, lane 11: Suwon216, lane 12: Suwon229, lane 13: Suwon231, lane 14: Suwon232, lane 15: Suwon234, lane 16: Suwon235, lane 17: Suwon238, lane 18: Suwon239, lane 19: Suwon241, and lane 20: Suwon242. The D, C, B, and A designate the range of each hordein.

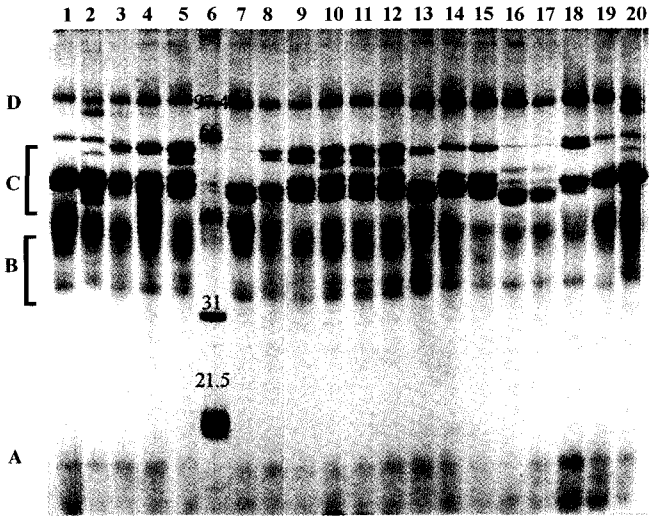

Fig. 2. Hordein subunit profiles of extra-low protein germplasms and malting barley cultivars. Lane 1: K947, lane 2: K951, lane 3: K954, lane 4: Karl, lane 5: Morex, lane 6: molecular size marker (kDa), lane 7: K970, lane 8: K973, lane 9: K974, lane 10: Robust, lane 11: Stander, lane 12: Forster, lane 13: Harrington, lane 14: Jinyangbori, lane 15: Namhyangbori, lane 16: Doosan8, lane 17: Doosan29, lane 18: Olbori, lane 19: K947, and lane 20: K951. Lane 1, 2, 3, 7, 8, and 9: extra-low protein barleys, lane 4, 5, 10, 11, 12, and 13: foreign malting barleys, lane 14, 15, 16, and 17: Korean malting barleys.

Table 2. Occurrence of hordein subunits in the 4 different barley groups.

Subunits (kDa)	A† (%)	B‡ (%)	C§ (%)	D¶ (%)	Subunits (kDa)	A(%)	B(%)	C(%)	D(%)
98.0	90.2	50.0	50.0	100.0	57.9	1.0	-	-	-
97.4	3.2	-	-	-	57.8	23.9	8.3	-	-
86.4	2.1	8.3	-	-	57.5	7.6	16.6	-	-
71.5	10.8	16.6	-	-	56.7	43.4	16.6	41.6	50.0
70.0	11.9	16.6	-	-	55.7	47.8	16.6	50.0	50.0
68.7	56.5	25.0	50.0	50.0	54.7	11.9	-	-	-
68.0	-	16.6	33.3	-	54.5	3.2	16.6	-	-
67.4	6.5	33.3	16.6	-	53.7	39.1	16.6	-	-
66.1	4.3	-	-	-	51.7	44.5	33.3	41.6	50.0
64.8	4.3	8.3	25.0	-	50.7	2.1	-	-	-
63.6	3.2	8.3	25.0	-	49.8	3.2	-	-	-
61.2	6.5	-	-	50.0	48.9	1.0	-	-	-
60.0	-	-	-	50.0	48.5	7.6	8.3	-	25.0
58.9	-	-	-	50.0	48.0	3.2	8.3	-	-

†Korean barley cultivars and experimental lines
‡Extra-low protein barleys from foreign countries
§Foreign malting barleys
¶Korean malting barleys
Shaded boxes are distinct subunits unique to Korean malting barleys

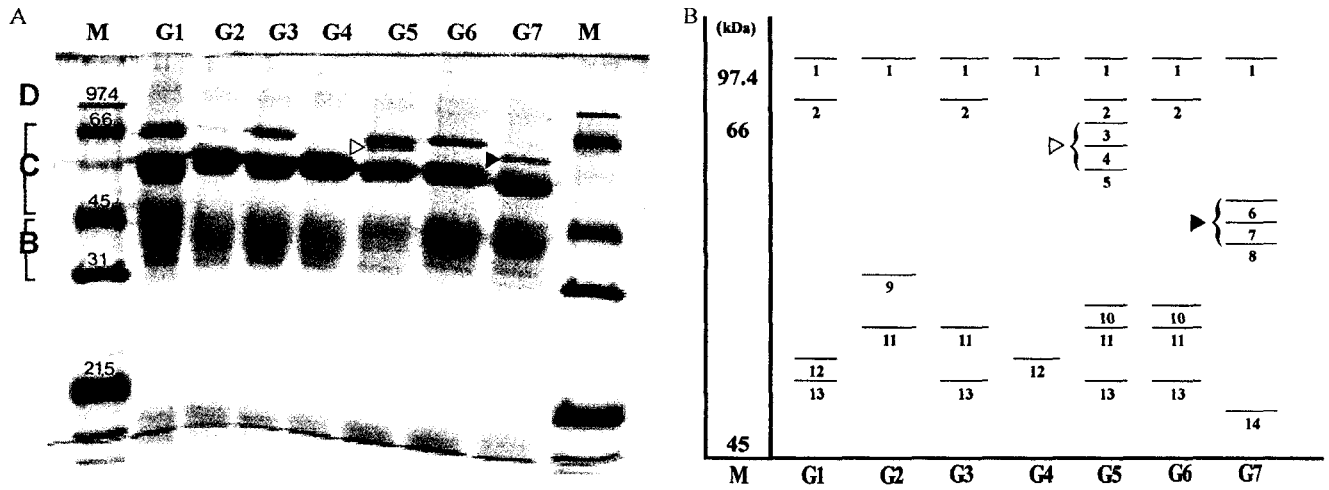

Fig. 3. A. Electrophoretic profiles for 7 hordein polypeptide pattern groups. B. Diagram of gel illustrated in Fig. 3. A (G: group, M: molecular size marker).

plasms and their polymorphic constitutions could provide biochemical markers for progeny analysis and endosperm quality improvement in barley breeding programs (Fig. 3. B). Component 1, a major component, had a molecular weight of about 98 kDa, and occurred in all types. Component 2, 11, and 13 were other major components with molecular weight of about 68.7, 55.7, and 51.7 kDa that occurred in four out of seven types. Component 3 (68.05 kDa), 4 (64.8 kDa), 5 (63.6 kDa), 6 (61.2 kDa), 7 (60.0 kDa), 8 (58.9 kDa), 10 (56.7 kDa), and 14 (48.5 kDa) were observed in malting barley, while component 9 (57.8 kDa) and 12 (53.7 kDa) were present only in barley cultivars.

The results indicate that 1D SDS-PAGE patterns of hordein polypeptides could provide useful biochemical genetic information for the identification or grouping of Korean barley cultivars and experimental lines.

DISCUSSION

An extensive polymorphism in hordein components has been reported earlier (McCausland & Wrigley, 1977). A high level of heterogeneity among the subunits of related

Table 3. Biochemical groupings of 54 out of 108 barley cultivars and germplasms based on the pattern of hordein subunits.

Group	Subunit Presence (kDa)	Cultivars and Germplasms
1	98, 68.7, 53.7, 51.7	Chalbori, Chalssalbori, Chokwangbori, Kuenalbori, Saekangbori, Saeolbori, Tapgolbori, Suwon185, Suwon189, Suwon200, Suwon204, Suwon205, Suwon214, Suwon216, Suwon 229, Suwon231, Suwon232, Suwon235, Suwon241, Suwon242, Suwon252, Milyang53, Milyang59
2	98, 57.8, 55.7	Songhakbori, Iri22, Iri23, Iri24, Iri26, Iri28, Iri29, Iri32, Iri33, Iri34
3	98, 68.7, 55.7, 51.7	Milyang34, Milyang60, Milyang67, Milyang72, Milyang73, Milyang78, Milyang79, Milyang88
4	98, 53.7	Saealbori, Saessalbori, Suwon187, Suwon202, Suwon 203, Suwon726
5	98, 68.7, 68.05, 64.8, 63.6, 56.7, 55.7, 51.7	Foster, Robust, Stander
6	98, 68.7, 56.7, 55.7, 51.7	Jinyangbori, Namhyangbori
7	98, 61.2, 60.0, 58.9, 48.5	Doosan8, Doosan29

storage proteins has also been reported in wheat (Wrigley & Shepherd, 1974) and in maize by Righetti *et al.* (1977). Hordein variation in populations of barley, is the degree to which the occurrence of different alleles at the two complex loci (*Hor 1* and *Hor 2*) is highly associated, or correlated. Such associations have already been reported for alleles in barley (Clegg *et al.*, 1972; Brown *et al.*, 1977) and appear to be a fundamental aspect of the genetic structure of inbreeding populations. The extreme diversity of the two hordein loci and the association between their variation patterns render them extremely usefulness as marker genes.

Using 1D SDS-PAGE it was possible to recognize 7 different hordein polypeptide pattern groups in the 108 barley cultivars investigated here. The use of the hordein polypeptide pattern can be compared with other characters which are at present routinely used for the identification of barley seed and are based on grain morphology (Shewry *et al.*, 1978a). This indicates that the use of both types of characters in conjunction would facilitate the identification of the seed of many more varieties than the use of either type of character alone (Shewry *et al.*, 1978b).

Also, we found a number of indistinguishable groups. The result indicates that reasons might be due to the co-migration of different polypeptides on 1D SDS-PAGE and to closely related ancestry. Some of cultivar groups probably could be subdivided further using two-dimensional chromatographic techniques.

Although limited numbers were included in the test, we found that more diverse subunits were presented in extra-low protein barleys than in malting barley. The result suggests that reduced protein content might not be matter of genetically fixed number of subunits presented but be associated with series of control mechanisms in protein production, transportation, and accumulation.

Malting quality is also related to hordein fraction. Howard *et al.* (1996) studied the relationship between D hordein and malting quality. D hordein displayed the strongest negative correlation with malting extract. Therefore, D hordein offers an alternative measurement to total protein for the prediction of malting quality.

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