(Korean J. Crop Sci.) 44(1) : 78~85(1999)

Analysis of Kernel Hardness of Korean Wheat Cultivars

Byung Hee Hong*† and Chul Soo Park*

ABSTRACT

To investigate kernel hardness, a compression test which is widely used to measure the hardness of individual kernels as a physical testing method was made simultaneously with the measurement of friabilin (15KDa) which is strongly associated with kernel hardness and was recently developed as a biochemical marker for evaluating kernel hardness in 79 Korean wheat varieties and experimental lines. With the scattered diagram based on the principal component analysis from the parameters of the compression test, 79 Korean wheat varieties were classified into three groups based on the principal component analysis. Since conventional methods required large amount of flour samples for analysis of friabilin due to the relatively small amount of friabilin in wheat kernels, those methods had limitations for quality prediction in wheat breeding programs. An extraction of friabilin from the starch of a single kernel through cesium chloride gradient centrifugation was successful in this experiment. Among 79 Korean wheat varieties and experimental lines 50 lines (63.3%) exhibted a friabilin band and 29 lines (36.7%) did not show a friabilin band. In this study, lines that contained high maximum force and the lower ratio of minimum force to maximum force showed the absence of the friabilin band. Identification of friabilin, which is the product of a major gene, could be applied in the screening procedures of kernel hardness. The single kernel analysis system for friabilin was found to be an easy, simple and effective screening method for early generation materials in a wheat breeding program for quality improvement.

Key words : kernel hardness, friabilin, starch granule protein, wheat (*Triticum aestivum* L.)

Kernel hardness, which is defined as the resistance to fracture upon grinding, crushing, slicing, abrasion, or indentation of single kernels or bulk samples (Anjum & Walker, 1991), influences wheat milling performances and flour qualities and has been often used as a key determinant of the end-use quality in wheat. Kernel hardness is related to the tempering of wheat, the distribution of flour particle size, flour density, and milling yield (Hoseney et al., 1988). Therefore kernel hardness is an important index in differentiating wheat classes and the physical characteristics of kernel hardness that affect the milling process and the resultant flour. In general, hard wheats require more conditioning (tempering to a higher moisture content), produce coarser particles, better bran cleanup and higher extraction rates than soft wheats. Hard wheat flours contain a larger mean particle size and a larger amount of mechanically damaged starch than soft wheats. Therefore, hard wheat flours are typically suitable for making the yeast-leavened pan breads, whereas soft wheats are preferred for pastry, cakes, and cookies.

A number of physical methods which evaluate the resistance of a kernel to fracture result from the adhesion forces between starch granules and protein as well as the protein matrix have been used for measuring wheat kernel hardness (Anjum & Walker, 1991). Among these methods, several commercial instruments such as Instron Universal Testing Machine (IUTM, 1985), near-infrared reflectance (NIR) spectroscopy (Norris et al., 1989) and single kernel characterization system (SKCS) (Martin et al., 1993; Smail, 1995) have been used widely in wheat breeding programs and wheat classification tests. The compression test made by IUTM is the most widely known method to measure kernel hardness of individual kernels but it is time consuming. The NIR spectroscopy is suitable for evaluating kernel hardness of bulk samples in early generations. The SKCS determines the average moisture, width, weight, and hardness of samples comprised of 300 individual kernels and is suitable for commercial classification. But these methods are kernel destructive and require a large amount of samples. Since the experimental results of the physical methods vary considerably due to large variability within samples and produce the low reproducibility, the practical application of these methods may not be effective for early selection in wheat breeding programs for quality improvement.

Symes (1965) has reported that kernel hardness is inherited from a single major gene, *Ha*. Law et al. (1978) and Mattern et al. (1973) demonstrated that the major gene is located on the short arm of the 5D chromosome. It was elucidated that the major gene must control the production of water-soluble proteins which are surrounded by starch granules (Barlow et al., 1973; Simmonds et al., 1973). Friabilin, as a water-soluble protein, surrounded by the starch granules and strongly associated with kernel hardness was first identified in soft wheat starch granules by Greenwell & Schofield (1986). Five polypeptide bands of starch granule proteins positioned on the surface of starch granules (5, 8, 15, 19, and 30 KDa) and five starch integral proteins with higher

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Received 4 Mar. 1999.

molecular weight (59, 77, 86, 96, and 149 KDa) were reported by Schofield & Greenwell (1987). They indicated that the gene coding for the friabilin was located on chromosome 5D and was close to *Ha* locus (Jolly et al., 1993).

Friabilin was abundant in all soft wheat starch granules, rare in hard wheat starches and was absent in durum wheat (Jolly et al., 1993). Jolly et al (1993) reported that friabilin correlated perfectly with the three major classifications of the kernel hardness. Morris et al. (1994) and Oda (1994) reported that friabilin is composed of four multiple forms. The major components of friabilin were identified as being similar to puroindolines, Triton X-114-extractable proteins (Blochet et al., 1993), by N-terminal amino acid sequence comparisons (Giroux & Morris, 1997; Oda & Schofield, 1997; Gautier et al., 1994). The significant relationship between the presence of friabilin and kernel softness were found by many researchers (Greenblatt et al., 1995; Morris et al., 1994; Rogers et al., 1993; Bakhella et al., 1990; Morrison et al., 1992). Most previous analytical systems used for evaluating kernel hardness had limitations for quality prediction in breeding programs because the systems required large amount of samples.

The purpose of this research was to develop a reliable method for identification of friabilin with a single kernel sample and compare it to the compression test and the establishment of an evaluation method for friabilin with a single kernel in the Korean wheat varieties and experimental lines, and to provide a basis for an useful biochemical marker system which can be used for assessing kernel hardness in wheat breeding programs.

MATERIALS AND METHODS

Seed samples of 79 Korean wheat varieties and experimental lines were obtained from the National Crop Experiment Station, Suwon, Korea. Flour samples were prepared with the Bühler test mill and water-washed prime starch was isolated by AACC approved methods 38～10 (AACC, 1983).

The compression test was based on the procedures described by Lai et al. (1985). All samples were sieved and equilibrated moisture contents of 10.5%～11.5% at 25℃ for 2 weeks. Fifty kernels were analyzed by a Texture Analyser (TA-XT2i, Version 1.17, Stable Micro Systems, England). For measurement, a load cell pressure of 5 kg, a test speed of 2.0 mm/sec. a test distance of 50% strain were used with a SMS P/5 probe (5 mmø/stainless steel).

The methods of starch isolation from mature single kernel followed procedures described by South & Morrison (1990) with some modifications. About three-fourth of the single kernels, excluding the embryo, were lightly crushed, then transferred into a 1.5 ml microcentrifuge tube and steeped at 4℃ for 6 hours in 1 ml distilled water. After brief centrifugation at 3,000 rpm, the pellets were crushed with a plastic pestle and resuspended in 300 μl distilled water. The suspension was layered on 1 ml of 80% (w/v) CsCl. After centrifugation at 14,000 rpm for 5 minutes, the pellets were resuspended. The resuspended solution with 300 μl distilled water was centrifuged through 80% (w/v) CsCl. The pellets were washed three times with distilled water to removed CsCl, and washed again with acetone to remove water and air-dried.

Starch granule proteins from the water-washed prime starch were extracted by the procedures described by Greenwell & Schofield (1986) with some modifications. Prime starch (200 mg) was transferred to 500 μl of 2% (w/v) sodium dodecyl sulate (SDS) solution and shaken for 1 hour at 55℃. After centrifugation at 14,000 rpm for 5 minutes, the supernatant was mixed with 50 μl dye solution [0.025% (w/v) bromophenol blue and 30% (v/v) glycerol].

Starch granule proteins from the purified starch with single kernel were extracted by the procedures described by Morrison et al. (1992) with some modifications. Purified starch obtained from a single kernel was transferred to 100 μl of 2% (w/v) SDS and shaken for 1 hour at 55℃. After centrifugation at 14,000 rpm for 5 minutes, supernatant was mixed with 10 μl dye solution. Starch granule proteins were separated by gradient (12.5%～20%) SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and detected with silver stain (Graybosch & Morris, 1990). One-fourth of the kernels including the embryo were kept for seeding after the hardness evaluation.

Statical analysis was performed by' Statical Analysis System (SAS) (Kim & Jhun, 1989). For the classification of 79 Korean wheat varieties, the principal component analysis was used with four parameters of compression test measured by Texture Analyser.

RESULTS AND DISCUSSIONS

The list of 79 Korean wheat varieties and experimental breeding lines and the typical curve patterns of compression test measured by Texture Analyser were given in Table 1 and Fig. 1, respectively. The curve patterns of compression test of 50 kernels for Jaraesomaek 1 and Milyang 15 were presented in Fig. 2. The parameters of compression test were maximum force (a measured maximum force required to crush), minimum force (the drop in force occurring as the kernel is crushed), second force (a measure of the maximum force needed to crush the broken pieces of a kernel, effecting a second collapse of the kernel), ratio (a measure of the pattern of the maximum force and minimum force) (Table 1).

The maximum force occurred at 0.23 sec and for Chokwang and 0.51 second for Suwon 276. The distribution of maximum force varied from 5.8 kg for Jaraesomaek 1 to 16.4 kg for Milyang 15. The time of minimum peak occurred at 0.35 second for Chokwang to 0.67 second for Suwon 276. The distribution of minimum force varied from 1.5 kg for Milyang 10 to 6.2 kg for Olmil. The time of second peak varied from 0.45 second for Jinpung to 0.75 second for Suwon 276. The distribtion of second force varied from 3.2 kg for

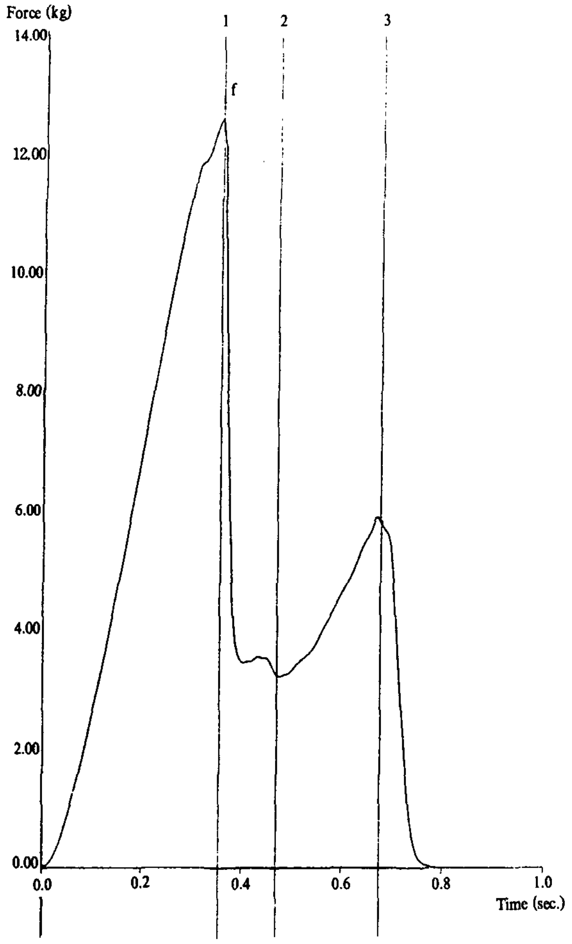
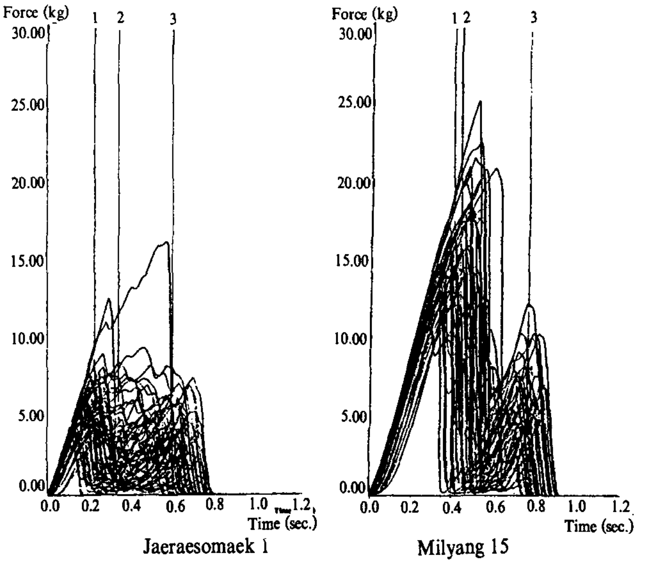


Fig. 1. Typical curve patterns of compression test measured by Texture Analyser (TA-XT2i, Version 1.17, Stable Micro Systems, England). 1 = Maximum peak, 2 = Minimum peak, 3 = Second peak.

Changkwang to 7.5 kg for Suwon 279. The distribution of ratio varied from 11.0% for Milyang 15 to 66.8% for Urimil.

Table 2 shows the results of the principal component analysis on four parameters, maximum force, minimum force, second force, and ratio of minimum force to maximum force, obtained from the compression test performed by Texture Analyser (Table 1). In this study, total variance was fully explained by the first principal component (Z_1, 92.92%) and second principal component (Z_2, 7.08%) and eigenvalues were 5.71 (Z_1) and 0.44 (Z_2), respectively. The coefficients between principal components and four parameters of compression test were also presented in Table 2. The ratio of minimum force to maximum force had the largest coefficient (3.12), followed by minimum force (−0.65), maximum force (0.18) and second force (0.16) in Z_1. In Z_2, the order of coefficient was the same as that of Z_1, and the related coefficients were 3.22, −2.56, 2.46, and 0.18, respectively.



Jaeraesomaek 1

Milyang 15

Fig. 2. Curve patterns of compression test of fifty kernels for Jaraesomaek 1, lowest maximum peak force tested in Korean wheat varieties and Milyang 15, highest maximum peak force by the Texture Analyser (TA-XT2i, Version 1.17, Stable Micro Systems, England). 1 = Maximum peak, 2 = Minimum peak, 3 = Second peak.

Fig. 3 showed the scattered diagram of 79 Korean wheat varieties based on Z_1 and Z_2 score and the classified groups were listed in Table 1. In these results, 79 Korean wheat varieties were classified into three groups based on the principal component analysis. In group Ⅰ, 9 genotypes were included and had the higher ratio of minimum force to maximum force and the lower maximum force than other genotypes. Twenty-seven lines were included in group Ⅱ, this group had the lower ratio of minimum force to maximum force than other genotypes. In group Ⅲ, 43 lines were included and showed the ratio of minimum peak force to maximum force between group Ⅰ and Ⅱ.

The maximum force is frequently used as a measure of hardness of processed foods (Naewbanji et al., 1983). But the maximum force alone may not be used to distinguish Korean wheat into precise kernel hardness groups due to considerable similarities of breeding pattern. Generally, the pattern of the minimum force of the hard wheats is much lower than that of soft wheats (Lai et al., 1985). The minimum force alone cannot be used to identify kernel hardness in Korean wheat varieties because there was considerable overlap. The distribution of minimum force demonstrated there was also considerable overlap in pat-

Table 1. Parameters of compression test measured by Texture Analyser in 79 Korean wheat varieties and experimental breeding lines.

		Compression Test								Compression Test					
No.	Varieties	Max[†] (kg)	Min (kg)	2nd (kg)	Ratio (%)	MC (%)	Group[‡]	No.	Varieties	Max (kg)	Min (kg)	2nd (kg)	Ratio (%)	MC[‡] (%)	Group
1	Urimil	9.3	6.2	7.0	66.8	11.0	Ⅰ	41	Suwon 234	9.4	2.6	5.8	28.5	10.8	Ⅲ
2	Tapdongmil	14.3	1.9	5.3	13.7	11.1	Ⅱ	42	Suwon 236	12.5	3.5	5.1	29.1	10.5	Ⅲ
3	Eunpamil	14.8	3.3	5.2	22.5	10.7	Ⅱ	43	Suwon 239	8.0	2.1	4.8	26.5	11.0	Ⅲ
4	Geurumil	13.6	2.1	4.0	15.7	10.6	Ⅱ	44	Suwon 241	8.3	2.5	4.8	30.8	11.5	Ⅲ
5	Chokwang	9.9	6.0	7.0	60.5	11.5	Ⅰ	45	Suwon 243	10.1	3.0	5.3	29.8	11.1	Ⅲ
6	Namhaemil	8.9	5.6	6.2	65.5	10.7	Ⅰ	46	Suwon 244	8.4	2.7	5.4	32.8	10.8	Ⅲ
7	Cheonggaemil	8.0	4.6	5.6	57.5	10.5	Ⅰ	47	Suwon 245	10.0	3.9	5.8	39.2	11.5	Ⅲ
8	Dahongmil	9.7	6.0	7.2	60.0	11.0	Ⅰ	48	Suwon 246	8.9	2.7	4.9	29.8	10.7	Ⅲ
9	Olmil	9.8	6.2	6.8	62.4	11.0	Ⅰ	49	Suwon 249	14.4	1.8	7.1	12.4	10.5	Ⅱ
10	Alchanmil	14.0	1.8	5.4	12.8	11.0	Ⅱ	50	Suwon 252	8.5	3.2	5.8	38.0	10.5	Ⅲ
11	Olgeurumil	10.6	5.5	6.6	51.7	11.0	Ⅰ	51	Suwon 258	13.9	4.7	6.1	34.3	10.5	Ⅲ
12	Jaeraesomak 1	5.8	2.3	3.3	39.1	10.5	Ⅲ	52	Suwon 259	8.7	2.6	5.7	29.7	10.5	Ⅲ
13	Changkwang	6.2	1.8	3.2	26.5	10.5	Ⅲ	53	Suwon 260	9.3	3.1	4.6	33.5	10.5	Ⅲ
14	Jinpuoong	7.7	3.1	4.9	37.9	10.5	Ⅲ	54	Suwon 261	13.9	3.9	6.6	30.0	10.6	Ⅲ
15	Shinkwang	8.2	3.3	5.3	38.6	10.9	Ⅲ	55	Gobunmil	13.0	2.3	4.9	18.2	10.5	Ⅱ
16	Naemil	9.8	3.3	5.6	35.5	10.6	Ⅲ	56	Suwon 263	8.0	2.7	4.8	33.6	10.5	Ⅲ
17	Yungkwang	8.3	2.8	4.3	32.6	10.5	Ⅲ	57	Suwon 264	8.9	2.2	5.1	24.4	11.0	Ⅱ
18	Kyungkwang	12.6	2.7	5.6	22.8	10.5	Ⅱ	58	Suwon 265	12.1	2.8	6.7	22.9	10.5	Ⅱ
19	Jaeraesomaek	11.8	2.7	5.6	23.1	11.0	Ⅱ	59	Suwon 266	13.3	2.5	6.2	18.0	10.5	Ⅱ
20	Jaeraejong 1	8.5	3.4	5.3	40.5	10.5	Ⅱ	60	Keumkangmil	14.2	2.5	4.8	17.4	10.5	Ⅱ
21	Somaekjaerae	7.5	2.3	4.9	30.6	10.8	Ⅲ	61	Suwon 268	7.4	2.1	4.5	28.3	10.7	Ⅲ
22	Jaeraeneulmil	8.4	2.5	5.1	29.0	10.9	Ⅲ	62	Suwon 269	8.2	2.4	4.6	30.2	10.5	Ⅲ
23	Jaeraemil	7.9	2.3	5.6	29.1	10.8	Ⅲ	63	Suwon 270	8.8	3.7	5.0	41.4	10.5	Ⅲ
24	Chungnamjaerae	10.3	3.3	6.0	33.2	10.6	Ⅲ	64	Suwon 271	8.7	3.3	5.5	33.8	10.5	Ⅲ
25	Tongmil	8.8	3.8	5.3	42.6	10.7	Ⅲ	65	Suwon 272	14.5	3.6	5.7	26.0	10.5	Ⅱ
26	Jaeraejong	7.7	1.9	5.5	24.6	10.9	Ⅱ	66	Milyang 11	8.9	3.7	5.9	42.3	11.5	Ⅲ
27	Jaeraejong 2	11.3	2.2	4.6	19.9	10.5	Ⅱ	67	Milyang 14	8.7	2.4	5.0	26.6	11.2	Ⅲ
28	Suwon 85	9.4	2.8	6.0	29.8	11.3	Ⅲ	68	Milyang 15	16.4	1.8	6.1	11.0	10.8	Ⅱ
29	Suwon 86	8.9	3.3	5.8	36.7	10.6	Ⅲ	69	Milyang 27	7.5	1.9	4.6	25.1	10.5	Ⅱ
30	Suwon 185	8.4	2.6	5.2	29.7	10.6	Ⅲ	70	Milyang 12	7.5	2.1	4.5	28.4	10.5	Ⅲ
31	Suwon 205	13.6	3.3	5.5	24.7	10.5	Ⅱ	71	Milyang 10	8.3	1.5	5.2	18.0	10.5	Ⅱ
32	Suwon 207	7.6	1.8	4.6	22.5	11.0	Ⅱ	72	Suwon 273	13.5	2.8	4.7	20.6	10.5	Ⅱ
33	Suwon 209	8.8	4.6	5.6	53.3	11.5	Ⅰ	73	Suwon 274	11.7	3.5	6.1	30.0	10.5	Ⅲ
34	Suwon 210	15.9	2.1	4.5	13.5	10.5	Ⅱ	74	Suwon 275	9.7	4.0	6.7	41.2	10.6	Ⅲ
35	Suwon 211	8.2	2.6	4.7	32.0	11.0	Ⅲ	75	Suwon 276	14.0	6.2	7.2	44.9	11.5	Ⅲ
36	Suwon 213	8.3	3.0	5.4	35.5	11.5	Ⅲ	76	Suwon 277	12.6	1.9	5.8	15.4	11.1	Ⅱ
37	Suwon 218	14.6	2.8	4.5	20.9	11.3	Ⅱ	77	Suwon 278	16.3	3.1	6.5	19.1	11.0	Ⅱ
38	Suwon 225	7.8	2.4	5.1	31.3	11.5	Ⅲ	78	Suwon 279	11.4	5.5	7.5	48.2	11.5	Ⅰ
39	Suwon 229	12.8	2.7	5.3	22.9	10.5	Ⅱ	79	Suwon 280	13.5	4.0	7.2	30.9	10.5	Ⅲ
40	Suwon 230	12.8	3.2	6.4	24.7	10.8	Ⅱ								

[†] : Max = maximum force, Min = minimum force, 2nd = second force, Ratio = ratio of minimum force to maximum force, MC = moisture content.

[‡] : Varietal groups were classified by principal component analysis(Fig. 3.) with parameters of compression test measured by Texture Analyser.

tern. Lai et al. (1985) proposed that the ratio of the minimum force over the maximum force seems to be potentially the best single characteristic to be able to differentiate between hard and soft wheats. This result is similar to that based on the principal component analysis in this study, although the distribution of ratio showed also a

Table 2. Eigenvalues of two principal components and their contributions to total variance from parameters of compression test measured by Texture Analyser in 79 Korean wheat varieties and experimental breeding lines.

Item	Z_1†	Z_2
Eigenvalues	5.71	0.44
Proportion (%)	92.92	7.08
Cumulative proportion (%)	92.92	100
Parameters	…… Eigenvectors ……	
Max.‡	0.18	2.46
Min.	−0.65	−2.56
2nd	0.16	0.18
Ratio	3.12	3.22

† : Z_1 = 1st principal component, Z_2 = 2nd principal component.
‡ : Max = maximum force, Min = minimum force, 2nd = second force, Ratio = ratio of minimum force to maximum force.

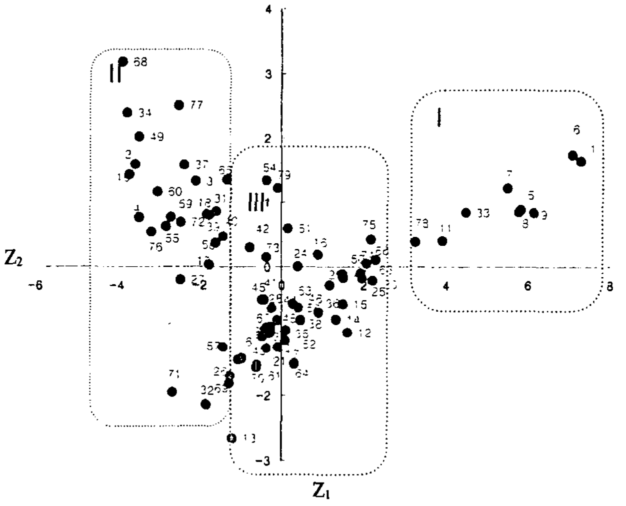


Fig. 3. Scattered diagram of the 1st (Z_1) and 2nd (Z_2) principal components based on parameters of compression test measured by Texture Analyser in 79 Korean wheat varieties and experimental breeding lines. Numbers were identical as in Table 1.

considerable overlap in 79 Korean wheat varieties and experimental breeding lines.

Hong & Baik (1995) proposed that the compression test is affected by characteristics of seed coat, kernel size and depth of crease in some Korean wheats. Miller et al. (1984) showed that correlation coefficients estimated between total protein content and kernel hardness are either very low or insignificant. Yamazaki & Donelson (1983) found high positive correlation coefficients between kernel hardness and the moisture content of wheat samples within a soft wheat cultivar. Compression test of individual kernels is affected by many factors not directly related to inherent differences in hardness and is subjected to large sampling errors (Lai et al., 1985) They also proposed that the compression test requires a great number of individual kernels, is often difficult to interpret, and is related to the performance of bulk samples.

Simmonds et al. (1973) established that the physical difference between hard and soft endosperm lies in the adhesive strength between the starch granules and surrounding protein matrix. Greenwell & Schofield (1986) reported that all the soft wheats possessed a strong 15 KDa polypeptide band (friabilin), a possible role for controlling kernel hardness, whereas this band was weak or absent in hard wheats. It was also proposed that the gene coding for the friabilin is located on chromosome 5D and is a major determinant of wheat kernel hardness (Schofield & Greenwell, 1987).

A newly developed method for determining friabilin with a single kernel of wheat was applied in Korean wheat lines for classification of kernel hardness. Electrophroratic analysis of friabilin extracted from water-washed prime starch (200 mg) of 13 Korean wheat flours with conventional method as described in material and methods is presented in Fig. 4. This electrophoregram of Fig. 4 which shows integral (including 60 KDa granule-bound starch synthase) and surface starch granule pro-

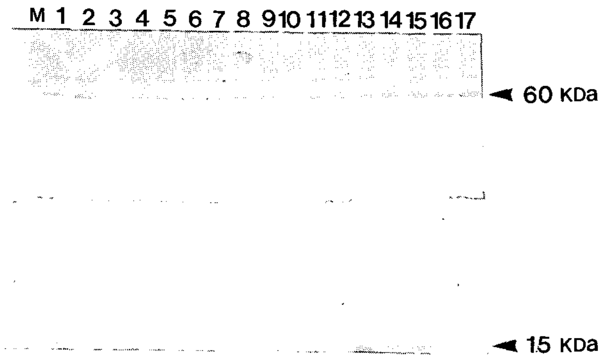


Fig. 4. Electrophoregram of friabilin extracted from water-washed prime starch (200 mg) with 2% (w/v) SDS, separated by gradient (12.5%~20%) SDS-PAGE and silver-stained in Korean wheat lines. Varieties corresponding to each lane were : lane 1 and 11; Urimil, lane 2 and 12; Chokwang, lane 3 and 13; Cheonggaemil, lane 4 and 14; Olmil, lane 5; Geurumil, lane 6; Eunpamil, lane 7 and 9; Olgreumil, lane 8; Gobunmil, lane 9; Olgreumil, lane 10; Alchanmil, lane 15; Suwon 275, lane 16; Keumkangmil, lane 17; Suwon 278. Lane M contains molecular weight markers of 97.4, 66.2, 45, 31, 21.5, and 14.4 KDa and was lightly loaded. Arrows indicate friabilin band (15 KDa) and granule-bound starch synthase (60 KDa).

teins (including *friabilin*) was identical polypeptides to those reported by Greenwell & Schofield (1986). Protein is a minor constituent of wheat starch granules and typically constitutes 2 g/kg of the dry weight. Since these proteins including friabilin represent less than 1% of the total flour protein (Greenwell & Schofiled, 1986; Schofiled & Greenwell, 1987), large amounts of flour samples are required for extraction of friabilin with conventional extraction methods. However, head or plant selected materials in early generations produce only small amounts of samples, but some simple and effective analytical methods to determine kernel hardness with a single kernel or extremely small amounts of samples in breeding procedures are urgently needed.

Because the surface areas of the B-type starch granules were 0.7∼0.9 m^2/g and A-type starch granules were 0.2∼0.3 m^2/g, the small B-type starch granules had more friabilin than the large A-type starch granules roughly in proportion to their surface area (Sulaiman & Morrison, 1990). The single kernel analysis for friabilin with CsCl centrifugation during starch purification gave clean starch fraction without loosing B-type starch granules (Sulaiman & Morrison, 1990). The results of electrophoration of friabilin from a single kernel of Korean wheat varieties and experimental lines are given in Fig. 5. With this method, it was possible to have better resolution of a friabilin band with a single kernel analysis system (Fig. 5) than that of conventional methods (Fig. 4). But, due to a difference in the amount of extraction sample, the result of a single kernel analysis showed a faint band of integral starch granule protein compared to the conventional method (Fig. 4). Therefore, the analysis of friabilin by using a single kernel is suitable for application in breeding programs without ambiguous results. Since the single kernel analysis system required only 3/4 of a single kernel and took less running time than that of the conventional methods, this single kernel analysis system can be utilized effectively for early generation selection in wheat quality breeding programs.

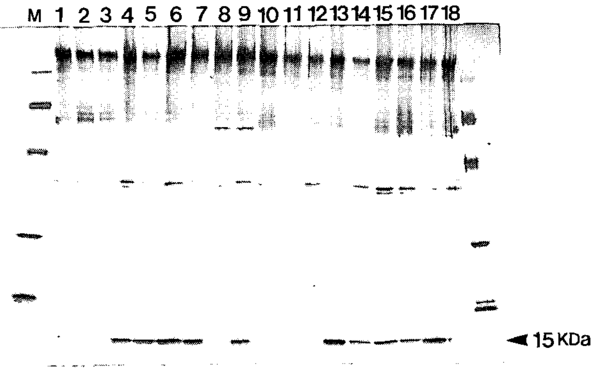


Fig. 5. Electrophoregram of friabilin extracted from single kernel with 2% (w/v) SDS, separated by gradient (12.5%∼20%) SDS-PAGE and silver -stained in Korean wheat varieties and experimental breeding lines. Cultivars corresponding to each lane were : lane 1; Gobunmil, lane 2; Tapdongmil, lane 3; Eunpamil, lane 4; Shinkwang, lane 5; Chokwang, lane 6; Namhaemil, lane 7; Cheonggaemil, lane 8; Suwon 265, lane 9; Olmil, lane 10; Alchanmil, lane 11; Kyungkwang, lane 12; Keumkangmil, lane 13; Urimil, lane 14; Dahongmil, lane 15; Changkwang, lane 16; Yungkwang, lane 17; Tongmil, lane 18; Olguerumil. Lane 18 is poor resolution and lane M contains molecular weight markers of 97.4, 66.2, 45, 31, 21.5 (doblet), and 14.4 KDa. Arrow indicates friabilin band.

As the single kernel analysis system is used for detecting the presence of friabilin in 79 Korean wheat varieties the friabilin band was present in 50 varieties (63.3%) and absent in 29 varieties (36.7%) (Table 3). Twenty-two of 29 genotypes, friabilin-absent varieties belong to group Ⅱ, This group had a lower ratio of minimum force to maximum force than other varieties in the scattered diagram of the principal component analysis (Fig. 3). The other six varieties such as Suwon 236, 258, 261, 274, 276, and 280, were also friabilin-absent varieties

Table 3. Identification of friabilin in 79 Korean wheat varieties and experimental breeding lines using SDS-PAGE

Friabilin	Korean wheat varieties and experimental lines
Presence	Changkwang, Cheonggaemil, Chokwang, Dahongmil, Chungnamjaerae, Jaeraejong 1, Jaeraejong, Jaeraemil, Jaeraeneulmil, Jaraesomaek 1, Jinpoong, Naemil, Olmil, Namhaemil, Olgeurumil, Shinkwang, Urimil, Tongmil, Somaecjaerae, Yungkwang, Milyang 10, Milyang 11, Milyang 12, Milyang 14, Milyang 27, Suwon 85, Suwon 86, Suwon 185, Suwon 207, Suwon 209, Suwon 211, Suwon 213, Suwon 225, Suwon 234, Suwon 239, Suwon 241, Suwon 243, Suwon 244, Suwon 245, Suwon 246, Suwon 252, Suwon 259, Suwon 260, Suwon 263, Suwon 264, Suwon 268, Suwon 269, Suwon 270, Suwon 271, Suwon 275
Absence	Alchanmil, Eunpamil, Geurumil, Keumkangmil, Gobunmil, Jaeraejong 2, Jaeraesomaek, Kyungkwang, Tapdongmil, Milyang 15, Suwon 205, Suwon 210, Suwon 218, Suwon 229, Suwon 230, Suwon 236, Suwon 249, Suwon 258, Suwon 261, Suwon 265, Suwon 266, Suwon 272, Suwon 273, Suwon 274, Suwon 276, Suwon 277, Suwon 278, Suwon 279, Suwon 280

but they were classified into group Ⅲ (Fig. 3). Those varieties showed the higher ratio of minimum force to maximum force than group Ⅱ and also exhibited a higher maximum force than group I (Fig. 3). Suwon 279, an friabilin absent variety demonstrated high maximum force and was classified into group Ⅰ due to the higher ratio of minimum force to maximum force. This result demonstrated that there were close relationships between friabilin-absent and the lower ratio of minimum force to maximum force. Therefore, it seemed possible to postulate that the genotypes showing high maximum force and lower ratio of minimum force to maximum force tend to be a friabilin-absent variety. In this regard, as reviewed in the results of the compression test, testing of friabilin for determinination of kernel hardness is more precise and effective in practical use. Futhermore, as the friabilin test can save part of the seed, it is possible to test the seed and use the same seed in the actual breeding program while the compression test destroys the seeds.

The pattern of friabilin occurrence in Korean wheat variety was similar to that of 133 "Norin" varieties developed in Japan (Oda et al., 1992). Oda et al. (1992) reported that Norin varieties with a faint friabilin band tended to be hard wheat and produced a coarse flour, whereas other wheat varieties expressing a prominent friabilin band tended to be soft wheat and produced a fine flour. Nakamura et al. (1990) also reported that the relationship between the presence of high molecular weight glutenin (HMW-Glu) subunit 2.2 and the particle size of flour. A number of varieties contain a HMW-Glu subunit 2.2 and the presence of the subunit 2.2 may affect the kernel hardness in Japanese wheat varieties (Oda et al., 1992). About fifty percent of Korean wheat varieties with a friabilin band contained the HMW-Glu subunit 2.2 (data not shown) and nothing has been reported on the biochemical-genetic relationship between the presence of HMW-Glu subunit 2.2 and kernel hardness.

Lookhart et al. (1985) proposed that gliadin patterns are related to grain morphology as well as to genetic background and gliadin content seems to affect borderline (intermediate) hardness values. Endo et al. (1992) compared the structure of the kernel cross-section and gliadin components between registered Japanese and Australian wheat varieties. Huebner & Gaines (1992) reported that hardness correlates with at least one gliadin fraction and the differences in hardness among single kernels of a varieties may result from variation in protein synthesis in kernels of different spike positions. Rogers et al. (1993) proposed that gliadin proteins are not useful in differentiating end-use characteristics but friabilin consistently predicted suitability for cookie baking. They also reported that it is not clear whether friabilin is responsible for the difference in baking quality or is just a useful marker. Since kernel hardness may vary greatly among varieties of the same class or even among kernels of a variety, it may be difficult to explain the difference of kernel hardness with only friabilin. Elucidation of the relationship between the occurrence of friabilin and properties of seed storage proteins will also contribute to quality enhancement in future wheat breeding programs.

Since physical-chemical mechanism and genetic control of kernel hardness in wheat are not well understood, research on the interaction of friabilins with starch and other components, such as polar lipids, is required. Oda and Schofield (1997) reported that friabilin is comprised of a mixture of puroindoline polypeptides and α-amylase inhibitor proteins. Though the puroindolines and α-amylase inhibitors from cereal proteins were characterized as low molecular weight cystein-rich proteins, the roles of these proteins in the molecular mechanisms with kernel hardness are not known. Therefore, the roles of these proteins and their relationship with kernel hardness on the molecular basis should be elucidated in future research.

ACKNOWLEDGMENTS

We sincerely thank our colleagues at the Wheat and Barley Division, National Crop Experiment Station for providing material for experiments.

We wish to acknowledge the financial support of the Korea Research Foundation made in the program year of 1997 (997-001-G00085).

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