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Characteristics of Biochemical Markers and Whole-Wheat Flours Using Small-Scaled Sampling Methods in Korean Wheats

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ABSTRACT : To investigate the application of biochemical markers and small-sample methods using whole-wheat flours for screening in early generation in Korean wheat breeding system, 74 Korean wheats, including cultivars, local breeding lines and experimental lines, were analyzed. Seed storage protein and amylose contents of grains were evaluated. Biochemical makers, including granule bound starch synthase (GBSS), high molecular weigh glutenin subunits (HMW-GS) and friabilin were also evaluated by using one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a single kernel. The small-sample methods, including modified SDS-sedimentation test (MST), micro-alkaline water retention capacity (AWRC) and whole-wheat flour swelling volume (WSV) were also tested in this study. Protein content, MST and AWRC was 11.0 - 15.8%, 2.7 - 26.2 ml and 71.9 - 109.7%, respectively. Apparent and total amylose content and WSV was 20.6 - 25.0%, 26.1 - 32.4% and 9.0 - 16.9 ml, respectively. There were highly significant correlations between MST and AWRC (r=0.592, P<0.001), but Korean wheats showed no significant difference in protein content, amylose content and small-sample methods. In the biochemical markers, Korean wheats contained all three GBSS encoded by *Wx* loci, except for Suwon 252. Korean wheats showed the high frequency (58.1%) of 1Dx2.2 + 1Dy12 subunits of HMW-GS. Friabilin band was present in 46 lines (62.2%) and absent in 28 lines (37.8%). Friabilin-absence lines showed the higher MST (14.9 ml) and AWRC (92.1%) value than friabilin-presence lines (8.5 ml and 82.4%, respectively).

Keywords: wheat, modified SDS-sedimentation test (MST), micro-alkaline water retention capacity (AWRC), whole-wheat swelling volume (WSV), granule bound starch synthase (GBSS), high molecular weigh glutenin subunits (HMW-GS), friabilin

Rapid and easy screening methods are very important for the characterization of end-use quality in wheat breeding programs. Traits related to the end-use quality have some limitation in application of early-generation because large amount of samples usually are needed to test and screen. Recently, biochemical markers and small-sample methods using whole-wheat flours, which are easy to measure, highly heritable and meaningfully related to end-use quality, have been contributed for identification, characterization and selection of varieties and breeding lines in wheat breeding programs.

High molecular weight glutenin subunits (HMW-GS), friabilin and granule bound starch synthase (GBSS) are considered as useful tools to characterize wheat varities in breeding programs. These biochemical markers can be directly used in selection of breeding lines with different end-use qualities in Korean wheat breeding programs. The differences in bread-making quality have been strongly correlated with allelic variations in HMW-GS (Payne, 1987). The significant relationship between the presence and absence of specific HMW-GS and bread-making quality of wheat varieties has been now well established. GBSS, responsible for the synthesis of amylose, also plays as an important factor to determine starch characteristics and end-use quality of food products. Reduced quantity of amylose due to decreased number of GBSS isofroms profoundly affects gelatinization and pasting properties of starch (Zeng *et al.*, 1997). Because both waxy and reduced-amylose wheat starch could have potential for commercial utilities, the availability of waxy or reduced-amylose wheat starch could allow the development of food products, especially noodle which is one of the major manufactured food products processed from soft wheat in Korea, Japan and China (Seo *et al.*, 1998). Friabilin, as a water-soluble protein found in surface of the starch granules, was abundant in all soft wheat starch granules, rare in hard wheat starches and was in absent in durum wheat (Jolly *et al.*, 1993). Although up to date the relationship between friabilin and the control of kernel hardness has been unknown, the relationship between the occurrence of friabilin and kernel hardness remained unbroken.

Small sample is generally used for evaluation of flour quality in early-generation of wheat breeding programs. As

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the method to predict protein quality related to bread-baking quality, sodium dodecyl sulfate (SDS)-sedimentation test is more convenient to use and provides better information than the Pelshenke and Zeleny-sedimentation test (Axford *et al.*, 1979). A modified SDS-sedimentation test (MST) is introduced by Dick & Quick (1983) for rapid estimation of gluten strength in early-generation durum wheat breeding lines. Alkaline water retention capacity (AWRC) is powerful method for the evaluation of potential cookie baking quality in soft wheat and highly significant correlations have been obtained between AWRC and cookie diameter (Yamazaki, 1953). For assessing the cookie quality of early-generation, micro AWRC test was introduced by Kitterman & Rubenthaler (1971). As a simple and fast method for the evaluation of starch swelling properties, starch swelling volume (SSV) and power (SSP) were introduced to determine potential noodle quality (Crosbie, 1991). Flour swelling volume (FSV), highly correlation with noodle eating quality test, is proposed by Crosbie *et al.* (1992) and whole-wheat flour swelling volume (WSV), significantly correlated with eating quality of cooked noodles, is introduced by Crosbie & Lambe (1993).

Korean wheat cultivars generally have high grain yield, early maturation, semi-dwarf and moderate vernalization. Quality improvement has been recently received more attention by wheat breeders than ever in Korea. Although the accurate evaluation of seed characterisrtics in early-generations is very important to improve end-use quality of wheat, there is little information about seed characteristics related to end-use quality in Korean wheat. This study is aimed at the evaluation of biochemical marker with a single kernel and whole-wheat flours with small-sample methods for rapid screening of wheat lines for potential use in quality improvement in Korean wheat breeding programs.

MATERIALS AND METHODS

Seed samples used in this study include 22 culitivars (No 1-22 in Table I), 8 local breeding lines (No 23-30) and 44 experimental lines (No 31-74) were selected from Suwon and Milyang. Seeds were ground on a Udy cyclone mill (Udy Co., Fort Collins, CO, U.S.A.) fitted with a perforated screen with 0.5 mm openings. Protein content (N × 5.7) was determined by boric acid modification of Micro-Kjeldahal method. Apparent and total amylose content was determined by the procedure of Morrison & Bernard (1983) with a primary starch. Apparent amylose content was measured in the presence of lipids which complex with amylose to reduce its iodine-biding capacity and total amylose content was measured with lipid-free preparations. The primary starch was prepared by the procedure of South & Morrison (1990). MST and AWRC were performed according to the procedure by Dick & Quick (1983) and Kitterman & Rubenthaler (1971), respectively. WSV was followed the procedure by Crosbie & Lambe (1993).

Electrophoresis results, HMW-GS and friabilin using single kernel, were provided by our previous reports (Hong & Park, 1998, 1999). To determine GBSS isoforms, the purification of starch granules from mature single kernel was performed as previously described in friabilin (Hong & Park, 1999). The preparation of GBSS from isolated starch granules was based on the procedure described by Seo *et al.* (1998). One-dimensional SDS-PAGE analysis of GBSS was performed by 17% resolve gel and detected with silver stain.

For the analysis of the HMW-GS and friabilin with a single kernel, one-fourth of single kernel, excluding the embryo, was excised to determine the HMW-GS and the extraction of total proteins was performed as previous study (Hong & Park, 1998). The remnant of kernel was used for analysis of friabilin. The procedure of purification of starch granule and the extraction of protein was performed as previous (Hong & Park, 1999). The analysis of friabilin was performed using non-gradient, discontinuous SDS-PAGE and resolving gels were 13.5% with 2.6% PDA cross-linker and included 10% (v/v) glycerol replacement of distilled water to reduce diffusion.

Statistical analysis was performed by the SAS package (SAS, 1995). The principal component analysis was used for the classification.

RESULTS AND DISCUSSION

Characteristics of Whole-Wheat Flour

Characteristics of whole-wheat flours of 74 Korean wheat cultivars and experimental lines are summarized in Table 1 and 2. Protein contents of wheat grain was ranged from 11.0 to 15.8%. In wheat cultivars, Keumkangmil (15.7%) and Joeunmil (15.2%), recently developed cultivars, showed higher protein content than other cultivars. Urimil, Namhaemil, Cheonggemil, Olmil, Alchanmil and Olgeurumil showed lower protein content (<12.0%) than other cultivars. Protein content of local breeding lines and experimental lines was 12.3 - 15.7% and 12.6 - 15.8%, respectively. Apparent and total amylose content of wheat cultivars was 21.4 - 24.3% and 26.1 - 32.4%, respectively. Apparent and total amylose content of local breeding lines (21.1 - 25.0% and 27.7 - 31.1%) and experimental lines (20.6 - 24.9% and 27.6 - 31.8%) was similar to that of cultivars. In characteristics of whole-wheat flour, WSV was 9.0 - 16.3 ml in wheat cultivars, 11.3 - 15.1 ml in local breeding lines and 9.0 -16.9 ml in experimental lines. MST volume was 3.1 - 26.2 ml in

Table 1. Protein and amylose contents in whole-wheat flours of 74 Korean wheats*.

No	Cultivar / Line	Protein (%)	Amylose		No	Cultivar / Line	Protein (%)	Amylose	
			Apparent (%)	Total (%)				Apparent (%)	Total (%)
1	Joeunmil	15.2	23.8	29.1	38	Suwon 225	14.4	21.5	27.9
2	Jinpummil	13.8	23.9	30.5	39	Suwon 229	14.3	21.8	27.8
3	Seodunmil	13.4	22.1	29.2	40	Suwon 230	14.4	22.3	29.0
4	Gobunmil	14.7	24.3	31.0	41	Suwon 234	15.8	22.9	29.9
5	Keumkangmil	15.7	21.4	32.4	42	Suwon 236	13.3	23.5	29.1
6	Urimil	11.0	22.8	27.2	43	Suwon 239	11.5	24.3	29.6
7	Tapdongmil	13.6	22.4	27.6	44	Suwon 241	12.9	24.2	30.1
8	Eunpamil	13.5	21.5	26.1	45	Suwon 243	13.9	23.4	29.4
9	Geurumil	13.2	23.9	30.0	46	Suwon 244	14.2	21.5	29.6
10	Chokwang	14.0	22.5	27.7	47	Suwon 245	14.5	22.2	29.3
11	Namhaemil	11.5	22.1	30.2	48	Suwon 246	14.1	22.7	27.9
12	Cheonggemil	11.7	22.7	28.0	49	Suwon 249	13.6	22.5	28.8
13	Dahongmil	12.8	23.6	29.0	50	Suwon 252	12.8	23.2	28.6
14	Olmil	11.9	21.9	27.4	51	Suwon 258	13.8	20.8	28.4
15	Alchanmil	11.6	22.7	28.0	52	Suwon 259	15.1	23.1	31.6
16	Olgeurumil	11.0	22.0	28.7	53	Suwon 260	14.3	22.6	31.3
17	Changkwang	14.3	22.0	28.8	54	Suwon 263	14.6	23.5	31.8
18	Jinpoong	14.3	24.4	29.7	55	Suwon 264	13.2	22.9	31.0
19	Shinkwang	12.6	22.5	29.2	56	Suwon 266	13.4	22.9	30.6
20	Naemil	14.5	22.1	26.7	57	Suwon 268	12.6	22.2	31.3
21	Yongkwang	14.3	23.0	28.0	58	Suwon 269	13.9	23.4	30.0
22	Kyongkwang	15.0	21.8	27.1	59	Suwon 270	13.5	22.4	30.3
23	Jaeraesomaek	12.3	22.8	27.8	60	Suwon 271	14.4	23.9	30.1
24	Jaeraejong 1	15.7	21.1	28.2	61	Suwon 272	14.3	21.7	27.7
25	Somaekjaerae	13.8	25.0	31.1	62	Suwon 273	14.5	23.5	29.0
26	Jaeraeneulmil	14.0	23.1	30.0	63	Suwon 274	13.8	22.4	29.3
27	Jaeraemil	15.6	22.8	29.2	64	Suwon 275	12.8	24.7	29.2
28	Chungnamjaerae	15.7	23.9	30.1	65	Suwon 276	14.0	22.8	29.8
29	Tongmil	15.1	23.4	30.0	66	Suwon 277	14.3	23.6	29.6
30	Jaeraejong	14.3	22.8	27.7	67	Suwon 278	13.8	22.8	29.3
31	Suwon 205	14.6	21.1	28.7	68	Suwon 280	13.4	23.2	29.7
32	Suwon 207	15.8	23.4	30.6	69	Milyang 10	14.4	24.9	30.0
33	Suwon 209	14.7	22.7	31.2	70	Milyang 11	14.9	23.9	30.0
34	Suwon 210	12.6	22.6	28.0	71	Milyang 12	15.2	23.2	30.1
35	Suwon 211	14.1	21.0	30.0	72	Milyang 14	15.0	23.4	29.1
36	Suwon 213	14.9	20.6	27.6	73	Milyang 15	14.6	23.7	29.2
37	Suwon 218	15.2	22.4	29.1	74	Milyang 27	13.6	23.8	29.7

*74 Korean wheats include 22 culitivars (No 1-22), 8 local breeding lines (No 23-30) and 44 experimental lines (No 31-74) selected Suwon and Milyang.

wheat cultivars and 3.1 - 25.1 ml in experimental lines. Local breeding lines showed narrow and lower MST volume (4.2 - 9.0 ml) than other Korean wheats. AWRC was 78.3 - 109.7% in wheat cultivars, 75.8 - 91.0% in local

Table 2. Whole-wheat flour swelling volume, modified-SDS-sedimentation volume, and micro-alkaline water retention capacity of 74 Korean wheats.

No	Cultivar / Line	WSV † (ml)	MST (ml)	AWRC (%)	No	Cultivar / Line	WSV (ml)	MST (ml)	AWRC (%)
1	Joeunmil	9.0	16.8	109.7	38	Suwon 225	11.4	8.6	92.2
2	Jinpummil	11.3	18.5	96.3	39	Suwon 229	12.8	9.4	93.9
3	Seodunmil	9.7	13.7	104.0	40	Suwon 230	14.1	12.0	77.6
4	Gobunmil	10.7	26.2	97.7	41	Suwon 234	12.9	12.0	95.7
5	Keumkangmil	9.5	23.9	92.1	42	Suwon 236	13.5	13.4	88.6
6	Urimil	14.8	12.1	82.9	43	Suwon 239	13.9	6.8	86.2
7	Tapdongmil	12.5	21.9	89.3	44	Suwon 241	14.5	3.1	83.4
8	Eunpamil	10.3	19.5	97.3	45	Suwon 243	12.3	13.4	95.3
9	Geurumil	10.6	16.4	96.6	46	Suwon 244	14.8	3.8	77.3
10	Chokwang	14.1	6.2	80.3	47	Suwon 245	15.2	6.0	78.3
11	Namhaemil	16.3	5.9	78.3	48	Suwon 246	15.9	4.5	75.2
12	Cheonggemil	14.0	4.1	82.7	49	Suwon 249	14.7	3.3	80.3
13	Dahongmil	12.4	6.0	92.9	50	Suwon 252	15.8	2.3	100.0
14	Olmil	15.1	3.1	84.2	51	Suwon 258	11.6	16.4	82.5
15	Alchanmil	13.7	21.0	91.3	52	Suwon 259	9.9	15.2	84.7
16	Olgeurumil	14.5	12.1	84.6	53	Suwon 260	12.3	14.7	90.0
17	Changkwang	12.3	15.3	78.7	54	Suwon 263	15.3	9.3	74.3
18	Jinpoong	15.0	7.0	79.2	55	Suwon 264	12.8	16.6	89.1
19	Shinkwang	14.5	7.7	82.2	56	Suwon 266	16.0	8.4	76.7
20	Naemil	11.1	13.4	81.0	57	Suwon 268	16.9	8.2	76.6
21	Yongkwang	11.9	13.1	96.6	58	Suwon 269	13.7	13.0	80.7
22	Kyongkwang	12.9	4.9	84.1	59	Suwon 270	12.6	13.0	80.7
23	Jaeraesomaek	11.3	8.2	91.0	60	Suwon 271	13.9	13.0	77.0
24	Jaeraejong 1	14.2	4.2	79.1	61	Suwon 272	14.1	6.7	77.3
25	Somaekjaerae	14.2	6.7	77.9	62	Suwon 273	12.4	14.6	95.1
26	Jaeraeneulmil	13.6	7.3	78.6	63	Suwon 274	13.9	12.8	87.3
27	Jaeraemil	13.3	5.9	82.1	64	Suwon 275	13.9	9.1	84.4
28	Chungnamjaerae	15.1	9.0	77.0	65	Suwon 276	13.4	13.7	95.0
29	Tongmil	14.9	8.9	75.8	66	Suwon 277	13.9	12.9	93.0
30	Jaeraejong	13.5	4.7	89.9	67	Suwon 278	9.4	25.1	102.0
31	Suwon 205	12.9	14.2	92.5	68	Suwon 280	9.0	15.2	106.7
32	Suwon 207	13.3	13.7	83.6	69	Milyang 10	15.6	2.7	72.7
33	Suwon 209	13.0	13.2	84.5	70	Milyang 11	15.7	4.7	76.3
34	Suwon 210	10.3	21.1	100.7	71	Milyang 12	16.2	4.7	71.9
35	Suwon 211	14.4	5.6	80.7	72	Milyang 14	15.9	5.0	75.2
36	Suwon 213	13.5	6.8	83.1	73	Milyang 15	13.7	9.7	85.4
37	Suwon 218	10.8	17.6	95.3	74	Milyang 27	15.0	9.5	76.9

†WSV = Whole-wheat Flour Swelling Volume, MST = Modified-SDS-sedimentation volume, AWRC = Micro-Alkaline Water Retention Capacity.

breeding lines and 71.9 - 106.7% in experimental lines.

Corsbie & Lambe (1993) showed that higher swelling volume was strongly correlated with good eating quality of cooked noodles. Dick & Quick (1983) reported that MST volume was superior to predict cooked spaghetti firmness using durum wheat and accounted for 53% of the variation in cooked firmness. There was highly negative correlations between AWRC and cookie diameter (Kitterman & Rubenthaler, 1971). Therefore, cultivars and lines with higher WSV could be used as genetic resource to improve noodle quality. Lines with higher protein content and MST could be also used for improving bread-baking quality. Lines with lower protein content and AWRC could produce larger cookie diameter. Namhaemil, Olmil, Olgeurumil and Urimil could be proper to noodle-making because these cultivars have lower protein content (<12.0%) and higher WSV (>14.8 ml) than other cultivars. Although several lines of local and experimental lines, including Chungnamjaerae, Suwon268 and Milyang12, showed high WSV (>15.1 ml), protein contents of these lines were higher than above Korean wheat cultivars. Gobunmil, Keumkangmil and Tapdongmil can be used for bread-baking because these cultivars showed higher protein content (>13.6%) and MST (>21.9 ml) than other cultivars and lines. Milyang 10 showed lower AWRC (<72.7%) than other cultivars and lines but protein content was 15.2%.

Biochemical Markers in Korean wheats

The classification of HMW-GS for the 74 Korean wheats is shown in Table 3. The Korean wheats were divided into 15 different groups on the basis of the HMW-GS composition. Three alleles were identified at the *Glu-A1,* five at the *Glu-B1* loci and three at *Glu-D1* loci. The high frequency (85.1%) of null allele on *Glu-A1* loci was found. The subunits of 1Bx7+1By8 were present at high frequency (74.3%) in *Glu-B1* loci. Low frequency (10.8%) of 1Dx5 + 1Dy10 subunits and the high frequency (31.1%) of 1Dx2 + 1Dy12 subunits were found. Especially, Korean wheats showed the high frequency (58.1%) of 1Dx2.2 + 1Dy12 subunits.

Table 3. Classification of 74 Korean wheats with respect to high molecular weight glutenin subunits (HMW-GS) compositions.

HMW-GS Composition †			Cultivar / Line				
Glu-A1	*Glu-B1*	*Glu-D1*					
Null ‡	1Bx7 + 1By8	1Dx2.2 + 1Dy12	Jinpummil	Seodunmil	Urimil	Geurumil	Chokwang
			Namhaemil	Cheonggemil	Olmil	Suwon 225	Suwon 229
			Suwon 239	Suwon 243	Suwon 244	Suwon 246	Suwon 249
			Suwon 258	Suwon 260	Suwon 264	Suwon 269	Suwon 271
			Suwon 273	Suwon 275	Suwon 280	Milyang 10	Milyang 11
			Milyang 12	Milyang 14			
Null	1Bx7 + 1By8	1Dx2 + 1Dy12	Shinkwang	Jaeraesomaek	Jaeraejong 1	Somaekjaerae	Jaeraeneulmil
			Jaeraemil	Chungnamjaerae	Tongmil	Jaeraejong	Suwon 211
			Suwon 234	Suwon 259	Suwon 272	Milyang 27	
Null	1Bx7 + 1By8	1Dx5 + 1Dy10	Tapdongmil	Alchanmil	Suwon 236	Keumkangmil	Suwon 277
Null	1Bx7 + 1By9	1Dx2.2 + 1Dy12	Eunpamil	Suwon 245	Suwon 252	Suwon 263	Suwon 268
			Suwon 270	Suwon 274	Suwon 276	Milyang 15	
Null	1Bx7 + 1By9	1Dx2 + 1Dy12	Dahongmil	Suwon 209	Suwon 218		
Null	1Bx7 + 1By9	1Dx5 + 1Dy10	Suwon 210				
Null	1Bx7	1Dx5 + 1Dy10	Suwon 205				
Null	1Bx13 + 1By16	1Dx2 + 1Dy12	Suwon 266				
Null	1Bx13 + 1By16	1Dx2.2 + 1Dy12	Joeunmil	Suwon 278			
1Ax1	1Bx17 + 1By18	1Dx2.2 + 1Dy12	Naemil				
1Ax1	1Bx17 + 1By18	1Dx2 + 1Dy12	Jinpoong				
1Ax1	1Bx7 + 1By8	1Dx2 + 1Dy12	Changkwang	Yungkwang	Suwon 241		
1Ax1	1Bx7 + 1By9	1Dx2 + 1Dy12	Gobunmil				
1Ax1	1Bx7 + 1By9	1Dx5 + 1Dy10	Suwon 207				
1Ax2*	1Bx7 + 1By8	1Dx2.2 + 1Dy12	Olgeurumil	Kyungkwang	Suwon 213	Suwon 230	

†Nomenclature according to Payne and Lawrence (1983).
‡Null is Null Allele

Shewry *et al.* (1992) reported that the allelic subunits of 1Dx5 + 1Dy10 were associated with better bread quality than the other subunits followed by the 1Ax subunits 1Ax1 or 1Ax2*, and the 1B pairs 1Bx17 + 1By18 or 1Bx7 + 1By8. They also proposed that wheat varieties with a good bread-making quality might require allelic subunits 1Ax1 or

Table 4. Identification of friabilin and granule bound starch synthase (GBSS) in 74 Korean wheats.

No	Cultivar / Line	Friabilin	GBSS‡			No	Cultivar / Line	Friabilin	GBSS		
			Wx-A1	*Wx-B1*	*Wx-D1*				*Wx-A1*	*Wx-B1*	*Wx-D1*
1	Joeunmil	A†	a§	a	a	38	Suwon 225	P	a	a	a
2	Jinpummil	A	a	a	a	39	Suwon 229	A	a	a	a
3	Seodunmil	A	a	a	a	40	Suwon 230	A	a	a	a
4	Gobunmil	A	a	a	a	41	Suwon 234	P	a	a	a
5	Keumkangmil	A	a	a	a	42	Suwon 236	A	a	a	a
6	Urimil	P	a	a	a	43	Suwon 239	P	a	a	a
7	Tapdongmil	A	a	a	a	44	Suwon 241	P	a	a	a
8	Eunpamil	A	a	a	a	45	Suwon 243	P	a	a	a
9	Geurumil	A	a	a	a	46	Suwon 244	P	a	a	a
10	Chokwang	P	a	a	a	47	Suwon 245	P	a	a	a
11	Namhaemil	P	a	a	a	48	Suwon 246	P	a	a	a
12	Cheonggemil	P	a	a	a	49	Suwon 249	A	a	a	a
13	Dahongmil	P	a	a	a	50	Suwon 252	P	b	a	a
14	Olmil	P	a	a	a	51	Suwon 258	A	a	a	a
15	Alchanmil	A	a	a	a	52	Suwon 259	P	a	a	a
16	Olgeurumil	P	a	a	a	53	Suwon 260	P	a	a	a
17	Changkwang	P	a	a	a	54	Suwon 263	P	a	a	a
18	Jinpoong	P	a	a	a	55	Suwon 264	P	a	a	a
19	Shinkwang	P	a	a	a	56	Suwon 266	A	a	a	a
20	Naemil	P	a	a	a	57	Suwon 268	P	a	a	a
21	Yungkwang	P	a	a	a	58	Suwon 269	P	a	a	a
22	Kyungkwang	A	a	a	a	59	Suwon 270	P	a	a	a
23	Jaeraesomaek	A	a	a	a	60	Suwon 271	P	a	a	a
24	Jaeraejong 1	P	a	a	a	61	Suwon 272	A	a	a	a
25	Somaekjaerae	P	a	a	a	62	Suwon 273	P	a	a	a
26	Jaeraeneulmil	P	a	a	a	63	Suwon 274	P	a	a	a
27	Jaeraemil	P	a	a	a	64	Suwon 275	P	a	a	a
28	Chungnamjaerae	P	a	a	a	65	Suwon 276	A	a	a	a
29	Tongmil	P	a	a	a	66	Suwon 277	P	a	a	a
30	Jaeraejong	P	a	a	a	67	Suwon 278	A	a	a	a
31	Suwon 205	A	a	a	a	68	Suwon 280	A	a	a	a
32	Suwon 207	P	a	a	a	69	Milyang 10	P	a	a	a
33	Suwon 209	P	a	a	a	70	Milyang 11	P	a	a	a
34	Suwon 210	A	a	a	a	71	Milyang 12	P	a	a	a
35	Suwon 211	P	a	a	a	72	Milyang 14	P	a	a	a
36	Suwon 213	P	a	a	a	73	Milyang 15	A	a	a	a
37	Suwon 218	A	a	a	a	74	Milyang 27	P	a	a	a

§a = Wild Type or functional *Wx* allele, b = Mutant or Null allele.
†P = Presence, A = Absence
‡Allelic designation according to Yamamori *et al.* (1994).

1Ax2* on *Glu-A1* locus, 1Bx17 + 1By18 or 1Bx7 + 1By8 on *Glu-B1* locus, and 1Dx5 + 1Dy10 on *Glu-D1* locus. However, no Korean wheat cultivars and lines have all these allelic combinations. Glutenin rank sum (GRS) that was calculated according to a modified scoring system proposed by Payne *et al.* (1987) was negatively correlated with cookie diameter (Souza *et al.*, 1994). Soft wheat cultivars with good quality score of HMW-GS with the increase of protein content increased gluten strength, but the cultivars with low quality score of HMW-GS may not increase this problem, even the increase of protein content (Czuchjowska *et al.*, 1996). Some cultivars with 1Dx5 + 1Dy10 subunit of HMW-GS yielded significantly lower damaged starch, longer dough mixing times and larger cookie diameter with superior top grain compared to some cultivars with higher with 1Dx2 + 1Dy12 subunit of HMW-GS (Finney & Bains, 1999). Therefore, introduction of 1Ax subunits, 1Ax1 or 1Ax2*, and 1Dx5 + 1Dy10 subunits should be highly considered in Korean wheat breeding programs for better bread-baking quality.

The results of friabilin and GBSS analysis are shown in Table 4. Fig. 1 shows electrophoration patterns of GBSS of several Korean wheat cultivars and lines. It was possible to have good resolution of GBSS with single kernel analysis system. Since single kernel analysis system was required to only 3/4 of single kernel, this single kernel analysis system can be utilized effectively for small samples selection in wheat quality breeding programs. Friabilin band was present in 46 lines (62.2%). In this study, Suwon 252 was the only line that had a null allele on the *Wx-A1* locus while other lines contained all three GBSS isoforms in Korean wheat cultivars and lines.

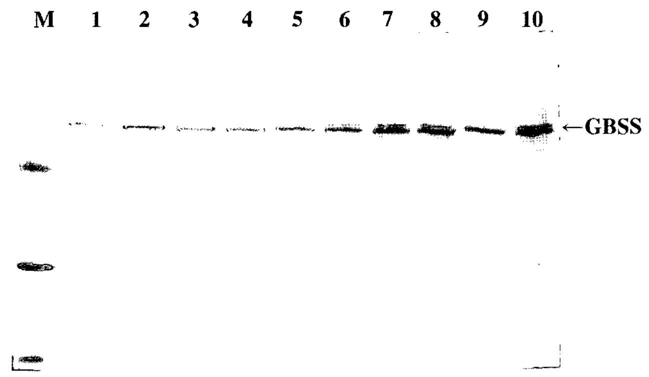

Fig. 1. One-dimensional SDS-PAGE (17%) patterns of GBSS isolated from single kernel of Korean wheats. Cultivars corresponding to each lane were: lane 1; Urimil, lane 2; Tapdongmil, lane 3; Eunpamil, lane 4; Geurumil, lane 5; Chokwang, lane 6; Namhaemil, lane 7; Cheonggemil, lane 8; Dahongmil, lane 9; Suwon 252, lane 10; Alchanmil. M contains molecular weight markers of 97.4, 66.2, 45, 31 and 21.5 kDa. Arrow indicates GBSS band.

Fig. 2 showed electrophoration patterns of friabilin and HMW-GS with a single kernel in Korean wheat varieties and lines. The result of friabilin with three-fourth single kernel was the same result of that from our previous result with single kernel (Hong & Park, 1999). Bettege *et al.* (1995) reported that a single kernel method used acetone precipitation to reduce the amount of samples could detect friabilin with samples of 0.2 mg of starch. This study was able to detect the friabilin with 5mg of starch that was easily isolated by the method provided by South & Morrison (1990) using centrifuging macerated single kernel through cesium chloride gradient. The result of HMW-GS compositions from one-fourth single kernel was some poor resolution compared with that of our previous result (Hong & Park, 1998), but there were no problem to evaluate the HMW-GS compositions. Therefore, to utilize this method in Korean wheat breeding programs, the improvement of the analytic method of HMW-GS and the method of GBSS and friabilin

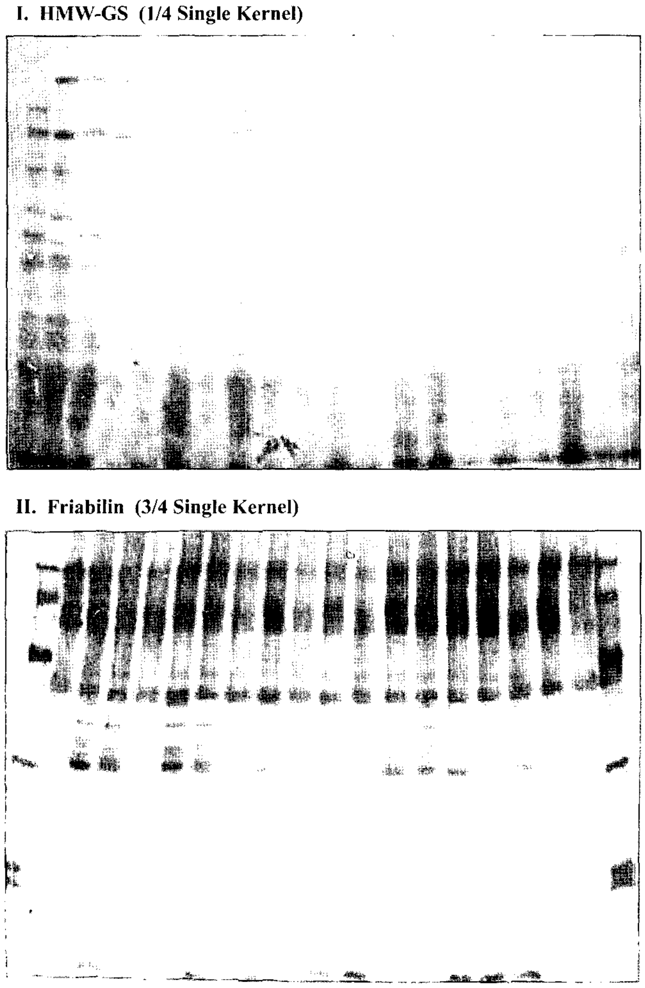

Fig. 2. One-dimensional SDS-PAGE patterns of HMW-GS (I) and friabilin (II) isolated from single kernel of Korean wheats.

with a single kernel will be accomplished in our laboratory.

Because both amylose-free and reduced-amylose wheat starch would have potentiality of commercial utilities, breeders recently focused on the availability of these starches for improvement of noodle quality. The higher eating quality of noodles can be achieved by the genetic manipulation of the ratio of amylose to amylopectin or the amylose content that can be controlled by GBSS (Miura & Tanii, 1994; Miura *et al.*, 1994). High flour swelling volume and low final viscosity of starch were associated with lines containing a null of *Wx-B1* (Ross *et al.*, 1997). Lines with null of *Wx-B1* might affect a subtle change in starch structure and showed the high starch viscosity with high flour swelling volume (Zhao *et al.*, 1998). However, the patterns of GBSS isoforms of Korean wheats in this study showed different patterns compared with Japanese and Australian cultivars in which the abundance of either single or both null at the *Wx-A1* and *Wx-B1* alleles (Miura & Tanii, 1994). For genetic diversity in relation to amylose content and improvement of noodle quality in Korean wheat, it is necessary primarily to obtain the amylose-free and reduced-amylose wheat lines with genetic manipulation through recombination between Korean wheats lines and null lines on *Wx* loci.

Relationships Between Whole-Wheat Flour Properties and Biochemical Markers

The correlation coefficients among whole-wheat flour characteristics are summarized in Table 5. There were highly significant correlations between MST and AWRC ($r = 0.592$, $P < 0.01$), but these characteristics had no significant correlations with protein content in this study. Bassett *et al.* (1989) reported that AWRC gave low and usually no significant correlations with all quality parameters including protein content except cookie diameter in soft white wheats. SDS sedimentation volume seemed to be unable to differentiate between Canadian wheats when they have high protein content, and was thus not suitable for use in predicting dough strength (Ayoub *et al.*, 1993). Gaines (1991) proposed that as protein content increases, variability in quality parameters became increasingly unpredictable, especially among most soft white wheats cultivars. The relationships among protein content, MST and AWRC in this

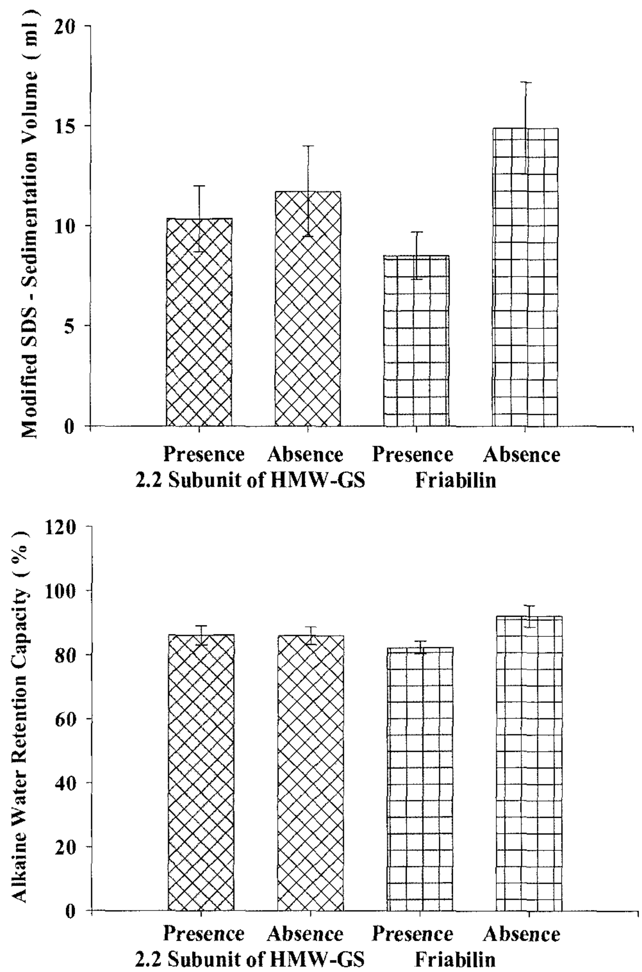

Fig. 3. The difference of modified SDS-sedimentation volume and micro-alkaline water retention capacity according to the presence and absence of 1Dx2.2 + 1Dy12 subunit of high molecular weight glutenin subunits (HMW-GS) and friabilin in 74 Korean wheats.

Table 5. Correlation coefficients among whole-wheat flour characteristics of Korean wheats.

Parameter†	Protein	Apparent Amylose	Total Amylose	WSV	MST
Apparent Amylose	-0.030				
Total Amylose	0.231*‡	0.402**			
WSV	-0.148	0.118	0.075		
MST	0.059	-0.032	0.132	-0.726**	
AWRC	-0.083	-0.012	-0.149	-0.766**	0.592**

†WSV = Whole-wheat Flour Swelling Volume, MST = Modified-SDS-sedimentation volume, AWRC = Micro-Alkaline Water Retention Capacity.
‡*Indicates significance at the 0.05 level and **at the 0.01 level.

study was in agreement with above the reports. These results might be influenced by high protein content of whole-wheat flours in Korean wheat cultivars and lines. Korean wheats also had the narrow variation in amylose content influenced by genetic variation of GBSS. This property of Korean wheats might be expressed no significant correlations between amylose content and WSV.

Fig. 3 shows that the difference of protein content, MST, and AWRC according to the presence and absence of 1Dx2.2 + 1Dy12 subunit of HMW-GS (2.2-presence and absence) and friabilin (friabilin-presence and absence) in Korean wheat cultivars and lines. There was no difference in the protein content between 2.2-presence and absence lines and friabilin-presence and absence lines. Friabilin-absence lines showed the higher MST value (14.9 ml) than friabilins-presence lines (8.5 ml), but there was no difference between 2.2-presence and absence lines. Difference in the AWRC values between 2.2-presence and absence lines was not found, but friabilin-absence lines showed the higher AWRC value (92.1%) than that of friabilin-presence lines (82.4%). Both 2.2-absence and friabilin-absence lines showed the higher MST and AWRC values (16.3 ml and 90.5%) than those of other lines. There was the high co-occurrence (36.5%) of 1Dx2.2 + 1Dy12 subunits of HMW-GS and friabilin. The concentrated researches on biochemical-genetic relationship, the high frequency of co-occurrence of 1Dx2.2 + 1Dy12 subunits of HMW-GS and friabilin in Korean wheat cultivars should be studied.

CONCLUSION

The results of biochemical markers with single kernel, HMW-GS, GBSS and friabilin, and small-sample methods, MST, AWRC and WSV, might provide useful tools to characterize wheat lines and contribute for identification and selection breeding lines in early generation of wheat breeding programs. The introduction of 1Dx5 + 1Dy10 subunits of HMW-GS, the increase of the flour protein content and overcome of narrow genetic diversity in amylose content with genetic recombination using other genetic resource carrying null GBSS isoforms should be considered for the future improvement of end-use qualities of Korean wheat cultivars.

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REFERENCES

Axford, D. W. E., E. E. McDermott, and D. G. Redman. 1979. Note on the sodium Dodecyl Sulfate test of breadmaking quality: Comparison with Pelschenke and Zeleny tests. Cereal Chem. 56 : 582-584.

Ayoub, M., J. F. Reid, and D. L. Smith. 1993. Evaluation of the SDS-sedimentation test for the assessment of eastern Canadian bread wheat quality. Can. J. Plant Sci. 73 : 995-999.

Bassett, L. M., R. E. Allan, and G. L. Rubenthaler. 1989. Genotype×environment interaction on soft wheat quality. Agronomy J. 81 : 955-960.

Bettege, A. D., C. F. Morris, and G. A. Greenblatt. 1995. Assessing genotypic softness in single wheat kernels using starch granule-associated friabilin as a biochemical marker. Euphytica 86 : 65-72.

Crosbie, G. B. 1991. The relationship between starch swelling properties, paste viscosity and boiled noodle quality in wheat flours. J. Cereal Sci. 13 : 145-150.

Crosbie, G. B. and W. J. Lambe. 1993. The application of the flour swelling volume test for potential noodle quality to wheat breeding lines. J. Cereal Sci. 18 : 267-276.

Crosbie, G. B., W. J. Lambe., H. Tsutsui, and R. F. Gilmour. 1992. Further evaluation of the flour swelling volume test for identifying wheats potentially suitable for Japanese noodles. J. Cereal Sci. 15 : 271-280.

Czuchjowska, Z., P. Y. Lin, and S. Smolinski. 1996. Role in dough rheology of high molecular weight glutenin subunits of soft white winter and club wheats. Cereal Chem. 73 : 338-345.

Dick, J. W. and J. S. Quick. 1983. A modified screening test for rapid estimation of gluten strength in early-generation durum wheat breeding lines. Cereal Chem. 60 : 315-318.

Finney, P. L. and G. S. Bains. 1999. Protein functionality differences in eastern U.S. soft wheat cultivars and interrelation with end-use quality tests. Lebensm-Wiss. U-Thechno. 32 : 406-415.

Gaines, C. S. 1991. Associations among quality attributes of red and white soft wheat cultivars across locations and crop years. Cereal chem. 68 : 56-59.

Hong, B. H. and C. S. Park. 1998. Genetic variation of high molecular weight glutenin (HMW-Glu) subunit in Korea wheat. Korean J. Crop Sci. 43 : 259-263.

Hong, B. H. and C. S. Park. 1999. Analysis of kernel hardness of Korean wheat cultivars. Korean J. Crop Sci. 44 : 78-85.

Jolly, C. J., S. Rahman, A. A. Kortt, and T. J. V. Higgins. 1993. Characterization of the wheat Mr 15000 "grain-softness protein" and analysis of the relationship between its accumulation in the whole seed and grain softness. Theor. Appl. Genet. 86 : 589-597.

Kitterman, J. S. and G. L. Rubenthaler. 1971. Assessing the quality of early generation wheat selections with the micro AWRC test, Cereal Sci. Today 16 : 313-314, 316, 328.

Miura, H. and S. Tanii. 1994. Endosperm starch properties in several wheat cultivars preferred for Japanese noodle. Euphytica 72 : 171-175.

Miura, H., S. Tanii, T. Nakamura, and N. Watanabe. 1994. Genetic control of amylose content in wheat endosperm starch and differential effects of three *Wx* genes. Theor. Appl. Genet. 89 : 276-280.

Morrison, W. R. and L. Bernard. 1983. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. J. Cereal Sci. 1 : 9-20.

Payne, P. I. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Ann. Rev. Plant Physiol. 38 : 141-153.

Payne, P. I. and G. J. Lawrence. 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1 and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res. Commun. 11 : 29-35.

Payne, P. I., M. A. Nightingale, A. F. Krattiger, and L. M. Holt. 1987. The relationship between HMW glutenin subunit composition and bread-making quality of British-grown wheat varieties. J. Sci. Food. Agric. 40 : 51-65.

Ross, A. S., K. J. Quail, and G. B. Crosbie. 1997. Physicochemical properties of Australian flours influencing the texture of yellow alkaline noodles. Cereal Chem. 74 : 814-820.

SAS. 1995. SAS User's Guide. The Institute: Cary, NC.

Seo, Y. W., B. H. Hong, and Y. W. Ha. 1998. Identification of granule bound starch synthase (GBSS) isoforms in wheat. Korean J. Crop Sci. 43 : 89-94.

Shewry, P. I., N. G. Halford, and A. S. Tatham. 1992. High molecular weight subunits of wheat glutenin J. Cereal. Sci. 15 : 105-120.

South, J. B. and W. R. Morrison. 1990. Isolation and analysis of starch from single kernels of wheat and barley. J. Cereal Sci. 12 : 43-51.

Souza, E., M. Kruk, and D. W. Sunderman. 1994. Association of sugar-snap cookie quality with high molecular weight glutenin alleles in soft white spring wheats. Cereal Chem. 71 : 601-605.

Yamazaki, W. L. 1953. An alkaline water retention capacity test for the evaluation of cookie baking potentialities of soft winter wheat flours. Cereal Chem. 30 : 242-246.

Zeng, M., C. F. Morris, I. L. Battey, and C. W. Wrigley. 1997. Source of variation for starch gelatinization, pasting, and gelation properties in wheat. Cereal Chem. 74 : 63-71.

Zhao, X. C., I. L. Battey, P. J. Sharp, G. Crosbie, I. Barclay, R. Wilson, M. K. More, and R. Appels. 1998. A single genetic locus associated with starch granule properties and noodle quality in wheat. J. Cereal Chem. 27 : 7-13.