한작지(Korean J. Crop Sci.), 54(2): 181~191(2009)

Allelic Variation of Glutenin, Granule-Bound Starch Synthase I and Puroindoline in Korean Wheat Cultivar

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ABSTRACT To investigate the genetic variation of high- and low-molecular-weight glutenin subunits (HMW-GS and LMW-GS), granule-bound starch synthase I (GBSSI) and puroindoline in 24 Korean wheat cultivars. At the HMW-GS compositions, three *Glu-A1* alleles, five *Glu-B1* alleles and three *Glu-D1* alleles were identified. The high frequency of alleles at each locus was *Glu-A1c* allele (15 cultivars), *Glu-B1b* allele (16 cultivars) and *Glu-D1f* allele (16 cultivars). Four alleles were identified at the *Glu-A3* and *Glu-B3* loci and three at *Glu-D3* locus and *Glu-A3d*, *Glu-B3d* and *Glu-D3a* were mainly found at each Glu-3 locus. *Glu-A3d, Glu-B3d, Glu-D3b* or *c* (4 cultivars, respectively) and *Glu-A3d, Glu-B3d, Glu-D3a* and *Glu-A3c, Glu-B3d* or *h, Glu-D3a* (3 cultivar, respectively) were predominantly found in Korean wheats. At the GBSS compositions, 2 waxy wheat cultivars, Shinmichal and Shinmichal1, showed null alleles on the *Wx* loci and other cultivars were wild type in GBSS compositions. At the puroindoline gene compositions, Korean wheat cultivars carried 3 genotypes, which 10 cultivars (41.7%) were *Pina-D1a* and *Pinb-D1a*, 11 cultivars (45.8%) had *Pina-D1a* and *Pinb-D1b* and 3 cultivars (12.5%) carried *Pina-D1b* and *Pinb-D1a*. These genetic variations could present the information to improve flour and end-use quality in Korean wheat breeding programs.

Keywords : wheat, high-molecular-weight glutenin subunits (HMW-GS), low-molecular-weight glutenin subunits (LMW-GS), granule-bound starch synthase I (GBSSI), puroindoline

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In wheat (*Triticum aestivum*) breeding programs, wheat breeders want to select lines with desirable qualities for specific end-uses in early generation screening. Numerous biochemical and DNA markers are now being used to characterize the alleles of wheat quality genes and their influence on end-use quality (D'Ovidio & Masci, 2004; Gale, 2005; Gianibelli *et al.*, 2001; Graybosch, 1998). Among biochemical and DNA markers related to quality components of wheat, the role of glutenins that contribute to strength and extensibility of wheat doughs, puroindolines that affect grain texture, and granule-bound starch synthase that produces starches with altered amylose content are of particular interest to wheat breeders.

Glutenins are major seed storage proteins and can be separated into high-molecular-weight glutenin subunits (HMW-GS, 80-130 and low-molecualr-weight glutenin subunits (LMW-GS, 30-50 kDa) by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Gianibelli *et al.*, 2001). HMW- GS comprise about 10% of wheat gluten but they are key factors in the process of bread-baking, being major determinants of gluten elasticity. HMW-GS are encoded by *Glu-A1, Glu-B1* and *Glu-D1* on the long arm of chromosome 1 (Payne & Lawrence, 1983). The *Glu-A1* loci codes for only one subunit or no subunit at all, the *Glu-B1* loci codes for one or two subunits and the *Glu-D1* loci codes for two subunits. Payne & Lawrence (1983) proposed a nomenclature system of HMW-GS and Payne (1987) assigned scores to each identified *Glu-1* allele, which made it possible to predict the approximate bread making quality of wheat cultivars. Allelic variations of *Glu-1* affecting bread-baking quality

account for about 50-70% in various wheat germplasms (Branlard *et al.*, 2003; Graybosch, 1992; Hong & Park, 1998; Payne & Lawrence, 1983; Payne *et al.*, 1983; Payne *et al.*, 1987b; Redaelli *et al.*, 1997).

LMW-GS represent approximately 40% of wheat gluten and play major role in determining dough resistance and extensibility, and to a lesser degree strength, both directly and via their interaction with the HMW-GS (Andrews *et al.*, 1994; Cornish *et al.*, 2001; Gupta *et al.*, 1989; 1994 Gupta & MacRitchie, 1994; Luo *et al.*, 2001; Metakovskii *et al.*, 1990; Payne *et al.*, 1987a). The LMW-GS are encoded by *Glu-A3*, *Glu-B3* and *Glu-D3* located on the short arm of chromosome 1 (Singh & Shepherd, 1988). The LMW-GS have not been studied as intensively as the HMW-GS due to the complexity of the banding patterns observed and the overlap of mobilities of the LMW-GS and the gliadins. For these reasons, the study of the functionality of the LMW-GS and effect of allelic variation of these proteins on the quality attributes of wheat has received far less attention than that of the HMW-GS. Gupta & Sheperd (1990) detected twenty banding patterns in LMW-GS by SDS-PAGE, six for *Glu-A3*, nine for *Glu-B3* and five for *Glu-D3*, with between zero and eight protein bands linked to each allele. Jackson *et al.* (1996) proposed combining the classification systems of alleles of *Glu-3* and *Gli-1* loci in bread wheats due to the *Glu-3* loci are genetically linked to the complex *Gli-1* loci encoding γ- and ω-gliadins. Several studies have been reported to characterize the *Glu-3* allelic variations in genetic germplasms (Branlard *et al.*, 2003; Gupta & Shepherd, 1990; Redaelli *et al.*, 1997; Shan *et al.*, 2007) and to evaluate their effects on bread-baking quality (Branlard *et al.*, 2001; Flaete & Uhlen, 2003; Killermann & Zimmermann, 2000; Payne *et al.*, 1987a). SDS-PAGE is still preferred for a definitive identification for *Glu-1* and *Glu-3* because PCR-based DNA makers are available to discriminate several *Glu-1* and *Glu-3* alleles.

Granule-bound starch synthase I (GBSS I, *waxy*, *Wx* protein) is responsible for amylose content of wheat starch (Nakamura *et al.*, 1993). *Wx-A1*, *Wx-B1* and *Wx-D1* are located on chromosome arms of 7AS, 4AL and 7DS, respectively (Murai *et al.*, 1999). *Wx-A1b* and *Wx-B1b* were much more commonly found than *Wx-D1b* through the surveys of cultivars from diverse origins (Demeke *et al.*, 1997; 2000 Graybosch *et al.*; 1998; Marcoz-Ragot *et al.*, 2000). Wheat cultivars with *Wx-B1b* loci might affect a subtle change in starch structure and showed the high starch viscosity with high flour swelling volume (Zhao *et al.*, 1998) and can be preferred to improve noodle quality, especially white salted noodles (Baik *et al.*, 2003; Miura & Tanii, 1994; Nakamura *et al.*,1993; Oda *et al.*, 1980; Zhao *et al.*, 1998), and bread-baking quality (Martin *et al.*, 2004; Park & Baik, 2007). Recently, Shariflou *et al.* (2001) reported the development of DNA marker for the specific detection of thedeletion mutant of the *Wx-D1* gene derived from a Chinese land-race. McLauchlan *et al.* (2001) compared five sets of PCR primers used in Australian wheat breeding programs for assaying the presence of each homeoallele. Nakamura *et al.* (2002) reported a set of primer pairs for the assay of each *Wx-1* allele in wheat, which could be preferable to evaluate *Wx-1* alleles compared to a laborious protein analysis method.

Grain hardness of wheat is a useful characteristic to determine the classification and end-use quality, because this characteristic affecting milling yield, flour particle size, degree of starch damage and subsequent water adsorption of flour during food processing (Pomeranz & Williams, 1990). Grain hardenss is controlled by the hardness locus (*Ha*) located on the short arm of chromosome 5D (Symes, 1965; Baker; 1977). Puroindolines (*Pina-D1a* and *Pinb-D1a*) are directly linked to variation in grain texture (Gautier *et al.*, 1994). Soft wheats contain both *Pina-D1a* and *Pinb-D1a* alleles, whilst hard wheats contain either *Pina-D1b* allele (null mutation of *Pina-D1b* allele) or six different single point mutations (*Pinb-D1b-g*) (Giroux & Morris, 1997; Giroux & Morris, 1998; Lillemo & Morris, 2000; Morris *et al.*, 2001).

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. We already reported the compositions of HMW-GS and GBSS using the protein markers in Korean wheats (Park *et al.*, 2005). But, there was no information of LMW-GS compositions and allelic variation of GBSS and puroindolines using DNA markers. The objectives of this study were (1) to determine the HMW-GS composition in Korean wheat cultivars developed since 2000 (2) to provide information about the LMW-GS and puroinolines of Korean wheat cultivars and (3) to determine allelic variaitions of GBSS using DNA markers.

Materials and Methods

Materials

Twenty four Korean wheat cultivars developed since 1976 in Rural Development Administration (RDA) were used in this study. Five seed of each cultivar were grown in a temperature-controlled greenhouse to analyze the genetic compositions of GBSS and puroindoline. Two weeks after germination, single leaves from individuals within cultivars were collected, bulked and snap-frozen in liquid nitrozen and stored at -80℃ until needed. All plants were kept in the greenhouse for analysis of glutenin compositions. Seeds harvested were ground on a Udy cyclone mill (Udy Co., Fort Collins, CO, USA.) fitted with a perforated screen with 0.5 mm openings to evaluate the HMW-/LMW-GS compositions and stored at -20℃ until needed. Pavon, Pitic and Opata were obtained from International Maize and Wheat Improvement Center (CIMMYT, Mexico) and used as HMW-/LMW-GS standards. Genomic DNA wasextracted from 100 mg of young leaf tissue using the Genomic DNA prep kit (Solgent Co., Ltd, Korea) according to the manufacturer's instructions.

HMW-/LMW-GS compositions

HMW-/LMW-GS were evaluated according to the protocol of Singh *et al.* (1991) with some modifications. Gliadins were extracted from 40 mg of wholemeal with 1500 μL of 50% propanol with incubation for 20 min at 65℃ followed by centrifugation for 5 min at 10,000 g. The supernatant was transferred to a new tube for analysis of gliadins and the residues were used for glutenin extraction. The supernatant was evaporated for 24 hr at 65℃ followed by mixing 400 μL of sample buffer [2% (w/v) SDS, 40% (v/v) glycerol, and 0.023% (w/v) bromophenol blue]. After 5 min incubation at 90℃ in heat block and centrifugation, 8 μL of supernatant were used for SDS-PAGE of gliadins. The residues were extracted with 100 μL of extraction buffer [50% (v/v) propanol, 0.08M Tris-HCl, pH 8.0] containing 2% (w/v) freshly added dithiothreitol. The samples were incubated for 30 min at 65℃. After 5 min centrifugation, 100 μL of extraction buffer containing 1.4% (v/v) freshly mixed 4-vinylpyridine. After incubation for 15 min at 65℃ and centrifugation for 5 min, the supernatant was transferred to a new tube and mixed one volume of sample buffer. After 5 min incubation at 90℃ in heating block and centrifugation, 8 μL of supernatant were used for SDS-PAGE of glutenin. The separating gel had a single concentration acrylamide (14.0% T) and was prepared using 1M Tris buffer with a pH 8.5. After running the SDS-PAGE for 20 hr at 15 mA/gel, the gel was stained overnight with a commassie blue R-250 and destained in 10% trichloroacetic acid. HMW-GS were classified using the nomenclature of Payne & Lawrence (1983). *Glu-A3* and *Glu-D3* alleles were evaluated according to the nomenclature of Singh *et al.* (1991). Allelic variations of *Glu-B3* were evaluated by the combining their corresponding allelic variations of gliadin according to the nomenclature of Jackson *et al.* (1996) and Peña *et al.* (2004) because *Glu-B3* alleles were associated with *Gli-B1* alleles.

Primers and PCR conditions for GBSS

The primer *Wx-A1* (Forward: 5'-TCGTGTTCGTCGGCG CCGAGATGG-3', Reverse: 5'-CCGCGCTTGTAGCAGTG GAAGTACC), *Wx-B1* (Forward: 5'-CTGGCCTGCTACCT CAAGAGCAACT-3', Reverse: 5'-CTGACGTCCATGCCG TTGACGA-3'), *Wx-D1* (5'-CTG GCCTGCTACCTCAAGA GCCAACT-3', Reverse: 5'-CTGTTTCACCATGATCGCT CCCCTT-3') was designed based on the sequences of mutant and non-mutant waxy alleles described in Nakamura *et al.* (2002) and Shariflou *et al.* (2001). PCR was performed in 200 μL microcentrifuge tubes containing 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer, 2.5 unit of Taq DNA polymerase (Takara, Japan) and 100 ng of genomic DNA. Total volume was 50 μL. The PCR cycle consisted of an initial 5 min denaturation at 95℃, followed by 33 cycles of 94℃ for 60 sec, 55℃ for 60 sec, and 72℃ for 1 min, and 1 cycle of 72℃ for 5 min. DNA amplification was performed in a MJ Research 200 thermal cycler (MJ Research Inc., USA).

Primers and PCR conditions for Puroindoline

The primer *Pina-D1* (Forward: 5'-ATG AAG GCC CTC TTC CTC A-3', Reverse: 5'- TCA CCA GTA ATA GCC AAT AGT G-3') and *Pinb-D1* (Forward: 5'-ATG AAG ACC TTA TTCCTC CTA-3', Reverse: 5'-TCA CCA GTA ATA GCC ACT AGG GAA -3') was designed based on the sequences of *Pina-D1* and *Pinb-D1* described in Gautier *et*

al. (1994). Each 50 μL reaction include 100 ng DNA, 1.5 mM $MgCl_2$, 10 pmol of each primer, 0.2 mM of each dNTP, and 0.5 μL (2.5) unit of Taq DNA polymerase (Takara, Japan). The PCR cycle consisted of an initial 4 min denaturation at 94℃, followed by 36 cycles of 94℃ for 60 sec, 58℃ for 90 sec, and 72℃ for 2 min, and 1 cycle of 72℃ for 10 min. DNA amplification was performed in a MJ Research 200 thermal cycler (MJ Research Inc., USA). PCR products of *Pinb-D1* was restricted with *BsrB*I at 37℃ for 1 hr followed by separation using 2.0% agarose gel.

Results and Discussion

Allelic variaitions of HMW-GS

Allelic compositions of HMW-GS in 24 Korean wheat cultivars are summarized in Table 1 and are shown in Figure 1 and 2. Three alleles were identified at the *Glu-A1*. Fifteen cultivars (62.5%) carried *Glu-A1c*, seven cultivars (29.2%) had *Glu-A1b* and two cultivars (8.3%) contained *Glu-A1a*. *Glu-B1* locus revealed three alleles : *Glu-B1b* (sixteen cultivars, 66.7%), *Glu-B1c* (five cultivars, 20.8%) and *Glu-B1f*(three cultivars, 12.5%). At the *Glu-D1* locus, 66.7% of Korean wheat cultivars carried allele *Glu-D1f*, 20.5% was *Glu-D1d* and 12.5% was *Glu-D1a*.

Compared to previous reports of various sets of wheat germplasms (Branlard *et al.*, 2003; Graybosch, 1992; Payne & Lawrence, 1983; Payne *et al.*, 1983; Payne *et al.*, 1987b; Redaelli *et al.*, 1997), Korean wheat cultivars showed the higher frequency of *Glu-A1c* and *Glu-D1f* and no difference in *Glu-B1* alleles. Japanese wheat cultivars also carried higher frequency of *Glu-A1c* and *Glu-D1f* and French wheat cultivars also had higher frequency of *Glu-A1c*. The *Glu-B1* locus was the most variable in wheat germplasms, but *Glu-B1b* and *Glu-B1c* were frequently found in Korean wheats. Payne (1987) proposed *Glu-B1b* was to have a positive effect on bread-baking quality, whereas *Glu-B1c* was unfavorable for gluten strength. However, Primard *et al.* (1991) reported there was no difference found between *Glu-B1b* and *Glu-B1c* comparing the bread-baking quality in the same genetic backgrounds of Great Plains germplasm.

Compared to US wheat flours with similar protein content, Korean wheats with *Glu-D1f* showed the higher SDS sedimentation volume, mixograph water absorption (Park *et al.*, 2006). Texture profiles of cooked noodles prepared from Korean wheats with *Glu-D1f* and around 10% protein content were comparable to those of commercial noodle flours. But,

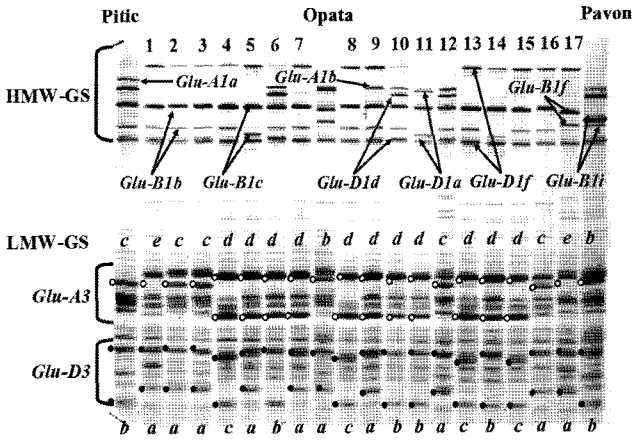

Fig. 1. One-dimensional SDS-PAGE patterns of reduced and alkylated glutenin subunits for allelic variations of HMW-GS and LMW-GS in Korean wheat cultivars. Arrows indicate identification alleles of *Glu-A1, Glu-B1* and *Glu-D1* of HMW-GS. Open circles indicate identification alleles of *Glu-A3* of LMW-GS. Closed circles indicate identification alleles of *Glu-D3* of LMW-GS. 1, Ol; 2, Geuru; 3, Dahong; 4, Chungkye; 5, Eunpa; 6, Tapdong; 7, Namhae; 8, Uri; 9, Olguru; 10, Alchan; 11, Gobun; 12, Keumkang; 13, Seodun; 14, Saeol; 15, Jinpoom; 16, Milseong; 17, Joeun.

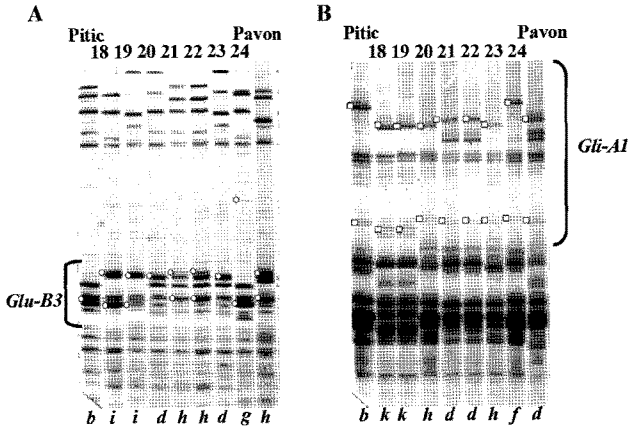

Fig. 2. One-dimensional SDS-PAGE patterns of reduced and alkylated glutenin subunits and gliadins for allelic variations of *Glu-B3* (A) and *Gli-B1* (B) in Korean wheat cultivars. Open circles and squares indicate identification alleles of *Glu-B3* and their linked *Gli-B1* alleleic variants in their respective gels. 18, Anbaek; 19, Jopoom; 20, Shinmichal; 21, Jonong; 22, Jokyung; 23, Younbaek; 24, Shinmichal1.

loaf volume baked from Korean wheat flours with *Glu-D1f* was lower than that of hard wheat flours of US, in spite of similar protein and quality. The relationships between *Glu-D1f* and grain hardness of wheat was also found in Korean and Japanese wheats (Oda *et al.*, 1992; Park *et al.*, 2005).

Shewry *et al.* (1992) proposed that wheat varieties with good bread-baking quality might require *Glu-A1a* or *b*, *Glu-B1b* or *i* and *Glu-D1d* on the *Glu-1* loci. Alchan, Jokyung, Jonong, Keumkang and Tapdong showed *Glu-A1b*, *Glu-B1b* and *Glu-D1d* and their *Glu-1* score are 10, according to the system of Payne (1987). Keumkang and Tapdong showed higher bread loaf volume and crumb grain score, and softer crumb firmness than other Korean wheat cultivars and lines, but lower than those of hard red spring wheat standard flours for bread-baking (Park *et al.*, 2002). Protein content of Keumkang and Tapdong was higher (> 11.5%) than that of Alchan (9.5%) and Jonong and Jokyung are developed since 2003. Therefore, the increased frequency of *Glu-A1a* or *b* and *Glu-D1d* should be importantly considered in Korean wheat breeding programs for better bread-baking quality.

Allelic variaitions of LMW-GS

Allelic compositions of LMW-GS in 24 Korean wheat cultivars are summarized in Table 1. Variations of *Glu-A3* and *Glu-D3* are shown in Figure 1 and *Glu-B3* is shown

Table 1. Allelic compositions of high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) in 24 Korean wheat cultivars.

Cultivar	High-molecular-weight glutenin subunits			Low-molecular-weight glutenin subunits		
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Alchan	*b*	b	*d*	*d*	d	b
Anbaek	*c*	c	*a*	*e*	i	a
Chungkye	*c*	*b*	*f*	*d*	d	c
Dahong	*c*	b	*f*	*c*	h	a
Eunpa	*c*	*c*	*f*	*d*	d	a
Geuru	*c*	*b*	*f*	*c*	i	a
Gobun	*c*	*c*	*a*	*d*	d	b
Jinpoom	*c*	*b*	*f*	*d*	d	c
Joeun	*c*	*f*	*f*	*e*	h	a
Jokyoung	*a*	*b*	*d*	*c*	h	a
Jonong	*a*	*b*	*d*	*c*	h	a
Jopoom	*c*	*f*	*f*	*d*	i	a
Keumkang	*b*	*b*	*d*	*c*	h	a
Milseong	*c*	*b*	*f*	*c*	d	a
Namhae	*c*	*b*	*f*	*d*	d	a
Ol	*c*	*b*	*f*	*e*	d	a
Olgeuru	*b*	*b*	*f*	*d*	d	a
Saeol	*c*	*c*	*f*	*d*	d	b
Seodun	*c*	*b*	*f*	*d*	d	c
Shinmichal	*b*	*b*	*f*	*c*	d	a
Shinmichal1	*b*	*c*	*a*	*a*	g	b
Tapdong	*b*	*b*	*d*	*d*	d	b
Uri	*c*	*b*	*f*	*d*	d	c
Younbaek	*b*	*f*	f	*c*	d	a

in Figure 2. At the *Glu-A3* locus, twelve cultivars (50.0%) carried *Glu-A3d*, eight cultivars (33.3%) had *Glu-A3c* and *Glu-A3a* was only found in Shinmichal1. Three cultivars, Anbaek, Joeun and Ol showed *Glu-A3e*, which is null allele (no protein in SDS-PAGE). Eagles *et al.* (2002) reported that *Glu-A3e* showed negative effects on dough rheological characteristics. Australian researchers proposed that *Glu-A3b* and *Glu-A3d* alleles showed stronger dough strength than other alleles (Gupta *et al.*, 1989; 1991; Metakovsky *et al.*, 1990; Vawser *et al.*, 2002). The rankings of *Glu-A3* alleles to dough strength were $a=d=f \geq e$ in French wheat cultivars and $d>c=e$ alleles in New Zealand wheats (Branlard *et*, 2001; Luo *et*, 2001). *Glu-A3b* allele was not found in Korean wheats. Among Korean wheat cultivars with *Glu-D1d*, Alchan and Tapdong had *Glu-A3d* and others had *Glu-A3c*. Therefore, the introduction of *Glu-A3b* and the increased frequency of *Glu-A3d* should be considered to increase dough strength for better bread- baking quality. He *et al.* (2005) reported that *Glu-A3d* was considered slightly better than others in dry white Chinese noodle quality.

Jackson *et al.* (1996) proposed the identification of *Glu-3* alleles in SDS-PAGE was much easier to combine the classification their corresponding *Gli-1* allles because of the linkage existing between the genes for *Gli-1* and the genes for *Glu-3* on the short arm of the chromosome 1. Peña *et al.* (2004) also showed *Glu-B3* alleles were associated to *Gli-B1* alleles, which are *Glu-B3b*:*Gli-B1b*, *Glu-B3d*:*Gli-B1h*, *Glu-B3f*:*Gli- B1g*, *Glu-B3g*:*Gli-B1f*, *Glu-B3h*:*Gli-B1d*, *Glu-B3i*:*Gli-B1k* and *Glu-B3j*:*Gli-B1l* in SDS-PAGE. Figure 2 shows *Glu-B3* alleles and their linked *Gli-B1* alleles of Korean wheat cultivars. Fifteen cultivars (50.0%) carried *Glu-B3d*, five cultivars (33.3%) had *Glu-B3h*, three cultivars (33.3%) had *Glu-B3i* and Shinmichal1 carried *Glu-B3g*. Wheat lines with *Glu-D1d* and *Glu-B3d* showed the strongest gluten strength, followed by groups possessing *Glu-D1d* combined *Glu-B3b, Glu-D3f* and *Glu-B3g* (Peña *et al.*, 2004). *Glu-B3i* and *Glu-B3h* alleles showed intermediate gluten strength. *Glu-B3j*, which indicates the presence of the 1BL/1RS translocation showed the lowest gluten strength. Shan *et al.* (2007) reported that *Glu-B3g* (41.0%) was the most common allele at the *Glu-B3* locus, followed by *Glu-B3f* (12.6%) and *Glu-B3b* (12.2%) in US hard wheats recently developed. Alchan and Tapdong had *Glu-D1d* and *Glu-B3d* and other Korean wheats with *Glu-D1d* had *Glu-B3h*. Therefore, the introduction of *Glu-B3b* and *Glu-B3f* and the increased frequency of *Glu-B3g* should be considered in Korean wheat breeding programs to increase dough strength for better bread-baking quality.

At the *Glu-D3* locus, the frequency of alleles *a* (fifteen cultivars, 62.5%) was higher than *b* (five cultivars, 20.8%) and *c* (four cultivars, 16.7%). The frequencies of alleles *a* (30.2%), *b* (27%), and *c* (26.6%) at the *Glu-D3* loci were approximately equal in US hard wheats (Shan *et al.*, 2007). Reports on correlations between *Glu-D3* and bread-baking quality are often contradictory. Branlard *et al.* (2001) reported that *Glu-D3a* positively affected on dough strength in French wheat, whereas *Glu-D3*c was unfavorable for bread-baking quality. *Glu-D3b* allele showed stronger dough strength than *Glu-Da* and *Glu-D3c* in Australian and New Zealand cultivars (Gupta *et al.*, 1989, 1991; Metakovsky *et al.*, 1990; Luo *et*, 2001). But, Vawser *et al.* (2002) reported that no differences were found in dough strength in *a*, *b* and *c* at *Glu-D3* loci. Cornish *et al.* (1993) reported *Glu-D3c* was good quality for bread-baking. They also proposed the best combinations of *Glu-3* for bread-baking are *b b b*, *b b c* and *c b c* (at loci *Glu-A3*, *Glu-B3* and *Glu-D3*, respectively). Four genotypes of *Glu-3* alleles were predominantly found in Korean wheats, *d d b* (4 cultivars) and *d d c* (4 cultivars) > *d d a* (3 cultivars), *c d a* (3 cultivars) and *c h a* (3 cultivar). But, *Glu-3 alleles* of Korean wheat cultivars with *Glu-D1d* were *c h a* (Jokyung, Jonong and Keumkang) and *d d b* (Alchan and Tapdong). In US hard wheats, the most common *Glu-3 alleles* were *c g b* and *c f c* and 85% of these wheats contained *Glu-A1a/b, Glu-B1b/c and Glu-D1d* (Shan *et al.*, 2007).

Allelic Variaitions of GBSS

Allelic compositions of GBSS from PCR-based DNA markers are summarized in Table 2. Shinmichal and Shinmichal1, waxy wheats showed *Wx-A1b*, *Wx-B1b* and *Wx-D1b*, other Korean wheat cultivars carried *Wx-A1a*, *Wx-B1a* and *Wx-D1a* (Figure 3). Single or two null alleles at *Wx-1*, called as partial waxy, were not found in Korean wheat cultivars. Suwon 252 was the only line that had *Wx-A1b* with SDS-PAGE in our previous study (Park *et al.*, 2005). Abundant of single-null partial waxy wheat cultivars were found in Japanese and Australian cultivars, and western Australian wheat cultivars preferred

Table 2. Allelic compositions of granule-bound starch synthase I (GBSS I) and puroindoline compositions in 24 Korean wheat cultivars.

Cultivar	Granule-bound starch synthase I [a]			Puroindoline [b]	
	Wx-A1	*Wx-B1*	*Wx-D1*	*Pina-D1*	*Pinb-D1*
Alchan	*a*	*a*	*a*	*a*	*b*
Anbaek	*a*	*a*	*a*	*a*	*b*
Chungkye	*a*	*a*	*a*	*a*	*a*
Dahong	*a*	*a*	*a*	*a*	*a*
Eunpa	*a*	*a*	*a*	*a*	*b*
Geuru	*a*	*a*	*a*	*a*	b
Gobun	*a*	*a*	*a*	*a*	b
Jinpoom	*a*	*a*	*a*	*a*	*b*
Joeun	*a*	*a*	*a*	*b*	*a*
Jokyoung	*a*	*a*	*a*	*a*	*b*
Jonong	*a*	*a*	*a*	*a*	*a*
Jopoom	*a*	*a*	*a*	*a*	*b*
Keumkang	*a*	*a*	*a*	*a*	*b*
Milseong	*a*	*a*	*a*	*a*	*a*
Namhae	*a*	*a*	*a*	*a*	*a*
Ol	*a*	*a*	*a*	*a*	*a*
Olgeuru	*a*	*a*	*a*	*a*	*a*
Saeol	*a*	*a*	*a*	*a*	*a*
Seodun	*a*	*a*	*a*	*a*	*b*
Shinmichal	*b*	*b*	*b*	*a*	*a*
Shinmichal1	*b*	*b*	*b*	*b*	*a*
Tapdong	*a*	*a*	*a*	*a*	*b*
Uri	*a*	*a*	*a*	*a*	*a*
Younbaek	*a*	*a*	*a*	*b*	*a*

[a] *Wx* allele desinations are *a* = wild type and *b* = null allele.
[b] *Pina-D1* allele desinations are *a* = wild type and *b* = null allele. *Pinb-D1* desinations are *a* = wild type and *b* = mutant of the glycine to serine in *Pinb-D1*.

for white salted noodles in Japan, which those cultivars contained *Wx-B1b* in GBSS (Miura & Tanii, 1994; Zhao *et al.*, 1998). Compared to wheat flours with wild type in *Wx* alleles, partial waxy wheats are more resistance to retrogradation during storage after cooking (Hayakawa *et al.*, 1997; Sasaki *et al.*, 2000; Seib 2000) and exhibited softer crumb texture of pan bread even after storage (Baik *et al.*, 2003; Morita *et al.*, 2002).

Double-null partial waxy wheats have a starch amylose content of 15.4-18.9%, produce softer and more elastic texture of white salted noodles and show comparable loaf volume and crumb structure of pan bread than wheat flours

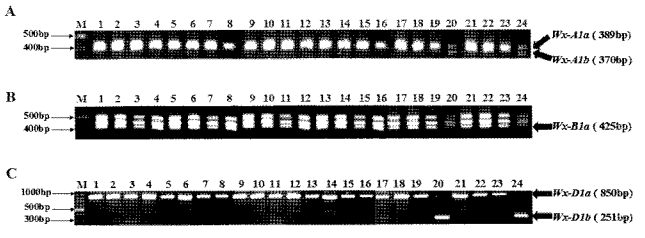

Fig. 3. Agarose gel electrophoresis of PCR amplified *Wx-A1* (A), *Wx-B1* (B) and *Wx-D1* (C) alleles of Korean wheat cultivars. M, molecular size marker 1, Ol; 2, Geuru; 3, Dahong; 4, Chungkye; 5, Eunpa; 6, Tapdong; 7, Namhae; 8, Uri; 9, Olguru; 10, Alchan; 11, Gobun; 12, Keumkang; 13, Seodun; 14, Saeol; 15, Jinpoom; 16, Milseong; 17, Joeun; 18, Anbaek; 19, Jopoom; 20, Shinmichal; 21, Jonong; 22, Jokyung; 23, Younbaek; 24, Shinmichal1.

with wild and single-null partial waxy wheat (Baik *et al.*, 2003). Double-null partial waxy wheat also have shown shorter cooking time of noodles than wild type and B null wheat flours, and have imparted desirable characteristics for making French bread with extended shelf life (Park & Baik, 2004, 2007). Therefore, it is necessary primarily to obtain single-null partial waxy, especially *Wx-B1b* and double-null partial waxy wheats to improve end-use quality of Korean wheats.

Allelic Variaitions of Puroindolines

Allelic compositions of puroindolines from PCR-based DNA markers are summarized in Table 2. Three Korean wheat cultivars (Joeun, Shinmichal and Younbaek) carried *Pina-D1b* allele, which is a deletion of the pina gene (Figure 4). Eleven cultivars (Alchan, Anbaek, Eunpa, Geuru, Gobun, Jinpoom, Jokyung, Jopoom, Keumkang, Seodun and Tapdong) had *Pina-D1b* allele, which contain a single mutation of glycine to serine at position 46 in pinb gene and detected by cleavage of the amplified pinb gene with *BsrB*I (Figure 2-B). Except for *Pinb-D1b*, not any mutant of pinB (*Pinb-D1c-g*) found in Korean wheat cultivars. Eleven cultivars (Chungkye, Dahong, Jonong, Milseong, Namhae, Ol, Olgeuru, Saeol, Shinmichal, Uri and Younbaek) showed *Pina-D1a* and *Pinb-D1a*. These cultivars can be included in soft wheats because soft wheats contain *Pina-D1a Pinb-D1b* alleles, whilst hard wheats contain either *Pina-D1b* allele or one of a number of mutants of *Pinb-D1b-g* alleles (Morris, 2002). Other Korean wheats could be called as hard wheats based on the variations of puroindolines.

Wheats with *Pina-D1b* allele showed decreased flour particle sizes and flour yield and increased water adsorption of dough mixing (Cane *et al.*; 2004; Giroux *et al.*, 2000 and Martin *et al.*, 2001). Hard wheats with *Pinb-D1b* allele had higher flour yields, lower flour ash content and particle sizes than hard wheats with index values of flour, decreased milling yield and increased water adsorption of dough mixing than hard wheats with *Pina-D1b* allele (Cane *et al.*, 2004 and Martin *et al.*, 2001). Hard wheats with *Pinb-D1b* allele also improved crumb grain score and larger loaf volumes compared to with wheats carrying *Pina-D1b* allele (Martin *et al.*, 2001). Therefore, the effects of genetic variations of puroindolines on flour and end-use qualities of Korean wheats should be accomplished to improve flour yied and bread-baking quality in breeding programs.

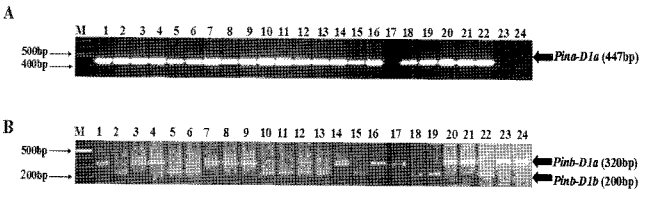

Fig. 4. Agarose gel electrophoresis of PCR amplified *Pina-D1* allele (A) and *Pinb-D1* allele (B) cut with *BsBrI* of Korean wheat cultivars. M, molecular size marker; 1, Ol; 2, Geuru; 3, Dahong; 4, Chungkye; 5, Eunpa; 6, Tapdong; 7, Namhae; 8, Uri; 9, Olgeuru; 10, Alchan; 11, Gobun; 12, Keumkang; 13, Seodun; 14, Saeol; 15, Jinpoom 16, Milseong; 17, Joeun 18, Anbaek; 19, Jopoom; 20, Shinmichal; 21, Jonong; 22, Jokyung; 23, Younbaek; 24, Shinmichal1.

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