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Association of Puroindolines Genotypes and Grain Properties, Milling Performances and Physical Properties of Flour in Korean Wheats

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ABSTRACT Puroindoline alleles, grain characteristics, milling performances and physical properties of flour of 22 Korean wheat cultivars were evaluated to determine the influence of puroindolines genotypes on grain and flour characteristics and to provide useful information for improving milling and end-use quality in Korean wheat breeding programs. Nine Korean wheat cultivars carried with *Pina-D1a/Pinb-D1a*, 11 cultivars had *Pina-D1a/Pinb-D1b* and 2 cultivars were *Pina-D1b/Pinb-D1a*. Korean wheats carrying with *Pina-D1a/Pinb-D1a* genotypes showed lower test weight and thousand kernel weight, area and roundness of grain and hardness index in grain characteristics, lower flour yield and higher proportion of break flour in milling performances and lower average particle size of flour, ash and damaged starch content, water retention capacity, yellowness-blueness and higher lightness of flour than wheats with *Pina-D1a*/*Pinb-D1b* or *Pina-D1b*/*Pina-D1a* genotypes. *Pina-D1a*/*Pinb-D1b* genotypes showed lower 1000-kernel weight, grain area, higher average of particle size of flour, higher ash and damaged starch content than *Pina-D1b*/*Pina-D1a* genotypes. There was no difference in hardness index of grain, milling performances, flour color between *Pina-D1a*/*Pinb-D1b* and *Pina-D1b*/*Pina-D1a* genotypes. These results could present the information to improve milling quality and physical properties of flour in Korean wheat breeding programs.

Keywords : wheat, puroindolines, hardness, milling

Hardness of wheat grain, either soft or hard, is an important index in differentiating wheat classes because hardness influences on milling performances and flour qualities and has been often used as a key determinant of the end-use properties. Hard wheats generally produce coarser particles size of flour and greater number of mechanically damaged starch than soft wheats. Therefore, hard wheat flours are generally used for breads and other yeast-leavened foods, soft wheats are preferred for pastries, cakes and cookies. Wheat grain hardness is controlled by the hardness locus (*Ha*) located on the short arm of chromosome 5D (Symes, 1965). It was elucidated that the major gene must control the production of proteins which surrounded starch granules (Barlow *et al*., 1973; Simmonds *et al*., 1973). Friabilin, as water-soluble 15-kDa protein found in surface of the starch and strongly associated with grain hardness was identified in soft wheat starch granules (Greenwell and Schofield, 1986). Friabilin was abundant in all soft wheat starch granules, whereas rare on hard wheat starches and was absent in durum wheat (Jolly *et al*., 1993).

Two major polypeptides, termed as puroindoline a (*Pina*) and b (*Pinb*), were identified as the major components of friabilin (Blochet *et al*., 1993; Gautier *et al*., 1994; Rahman *et al*., 1994). Genes coding for puroindolines are tightly linked to the *Ha* locus on chromosome 5D and probably functions together as the *Ha* locus (Sourdille *et*, 1996; Giroux and Morris, 1997; Giroux and Morris, 1998). Soft wheats contain both *Pina-D1a* and *Pinb-D1a* alleles, designated as wild type in puroindolines. Hard wheats contain either 4 allelic variations at *Pina-D1* locus (*Pina-D1b* and *Pina-D1l-n*) or 12 allelic variations at *Pinb-D1* locus, including 6 single nucleotide mutation (*Pinb-D1b* to *Pinb-D1g*) and another frame shift mutant (allele Pinb-D1p to *Pinb-D1t*). (Giroux and Morris, 1997; Lillemo and Morris, 2000; Morris *et al*., 2001; Ram *et al*., 2005; Chen *et al*., 2005; Ikeda *et al*., 2005; Xia *et al*., 2005; Chen *et al*., 2006; Chen

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et al., 2007).

Pina-D1b or *Pinb-D1b* alleles were prevalently found in hard wheat germplams. Hard wheats carrying the *Pina-D1b* allele showed different effects on milling performances to *Pinb-D1a* genotypes. *Pina-D1b* genotypes showed harder grain hardness, higher flour yield, lower lightness and ash content of flour and higher water absorption than hard wheats with *Pinb-D1b* (Giroux *et al.*, 2000; Martin *et al.*, 2001; Nagamine *et al.*, 2003; Cane *et al.*, 2004). There was no information in association of puroindolines genotypes and milling performance and physical properties of flour in Korean wheats. In this study, the objectives were to elucidate the relationships among allelic variation of puroindolines, grain properties and milling performance, to determine the influence of puroindolines genotypes on physical properties of flour and to provide useful information for improving wheat quality in Korean wheat breeding programs.

MATERIALS AND METHODS

Materials

Twenty two Korean wheat cultivars were harvested Iksan (Upland Crop Experimental Farm of National Institute of Crop Science, RDA) in 2007. The seed was sawn on October 20 cultivated with the standard method of wheat in National Institute Crop Science. Mean temperature was higher (9.7℃) than that of average year (8.7℃) and average precipitation was lower (387.6 mm) than that of average year (492.0 mm).

PCR Conditions for Puroindoline Genotypes

Five seed of each cultivar were grown in a temperature-controlled greenhouse to analyze the genetic compositions of puroindolines. Two weeks after germination, single leaves from individuals within cultivars were collected, bulked and snap-frozen in liquid nitrogen and stored at -80℃ until needed. All plants were kept in the greenhouse for analysis of glutenin compositions. Genomic DNA was extracted from 100 mg of young leaf tissue using the Genomic DNA prep kit (Solgent Co., Ltd, Korea) according to the manufacturer's instructions.

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* were restricted with *BsrB*I at 37℃ for 1 hr followed by separation using 2.0% agarose gel.

Grain Characteristics

Test weight and 1000-kernel weight were measured by Grain Scale (Seedburo Equipment Co., USA) and Seed Counter (Pfeuffer GmbH, Germany), respectively. Grain properties, including length, width, area and roundness, were measured by digital image analysis system for grain (SeedCount Australasia Pty Ltd., Australia). Roundness of grain, a three dimensional assessment of a seed's sphericity, means endosperm percentage of a kernel influenced by kernel size and shape. Roundness is calculated by ((length/width) + (length/thickness) + (width/thickness))/3. Hardness index, kernel weight and diameter were measured by the single kernel characterization system (SKCS) 4100 (Perten Instruments AB, Sweden) according to the Approved Method 55-31 (AACC, 2000).

Milling Performances and Flour Characteristics

Wheat was milled using a Bühler experimental mill, according to AACC Approved Methods 26-10. Two kilograms of wheat was conditioned overnight to reach 15% moisture content and then milled with feed rate of 100 g/min and with roll settings of 8 and 5 in break rolls and 4 and 2 in reduction rolls. After milling, flour yield was calculated as the proportion of break and reduction flours to total products and break flour yield was calculated by the proportion of break flours to flour yield. Milling score was calculated according to the procedure described by Martin *et al.* (2001).

Particle size distribution of flour was measured by the multi-wavelength laser particle size analyzer LS13320 (Beckman Coulter, Inc. USA). The color of flour was measured by Minolta CM-2002 (Minolta Camera Co., Ltd, Japan) with an 11 mm measurement aperture. The color differences were recorded as CIE-LAB L* (lightness), a* (redness-greenness) and b* (yellowness-blueness) values. Moisture, ash content and water retention capacity of wheat flour were determined according to Approved Methods 44-15A, 08-01 and 56-11. The determination of starch damage content was followed as the procedure described by Gibson et al. (1992) using an enzymatic assay kits (MegaZyme Pty., Ltd., North Rocks, Australia).

Statistical Analysis

Statistical analysis of data was performed by the SAS software (SAS Institute, Cary, NC) using Fisher's least significant difference procedure (LSD), analysis of variance (ANOVA) and Pearson correlation coefficient. All data were determined at least in duplicate and all were averaged.

RESULTS AND DISCUSSION

Puroindoline Genotypes of Korean Wheats

In agarose gel electrophoresis of PCR amplified *Pina-D1* allele, size of PCR products was 447bp in *Pina-D1a* allele and no products was found in *Pina-D1b* (Fig. 1-A). After restriction with *BsrB*I of PCR products (447bp) of *Pinb-D1* allele, *Pinb-D1a and Pinb-D1b* showed 320bp and 200bp, respectively (Fig. 1-B) (Table 1). This is a single mutation of glycine to serine at position 46 in *Pin b-D1* gene and detected by cleavage of the amplified *Pin b-D1* gene with *BsrB*I (Giroux and Morris. 1998).

Nine Korean wheat cultivars (Chungkye, Dahong, Jonong,

Table 1. List of Puroindoline genotypes of 22 Korean wheat cultivars.

Cultivar	Puroindoline[a]	
	Pina-D1	*Pinb-D1*
Alchan	a	b
Anbaek	a	b
Chungkye	a	a
Dahong	a	a
Eunpa	a	b
Geuru	a	b
Gobun	a	b
Jinpoom	a	b
Joeun	b	a
Jokyoung	a	b
Jonong	a	a
Jopoom	a	b
Keumkang	a	b
Milseoung	a	a
Namhae	a	a
Ol	a	a
Olgeuru	a	a
Saeol	a	a
Seodun	a	b
Tapdong	a	b
Uri	a	a
Younbaek	b	a

[a]a means wild type, b means null mutant in *Pina-D1* and a single mutation of glycine to serine at position 46 in *Pinb-D1*.
[b]Least significant difference ($P < 0.05$). Differences between two means exceeding this value are significant.

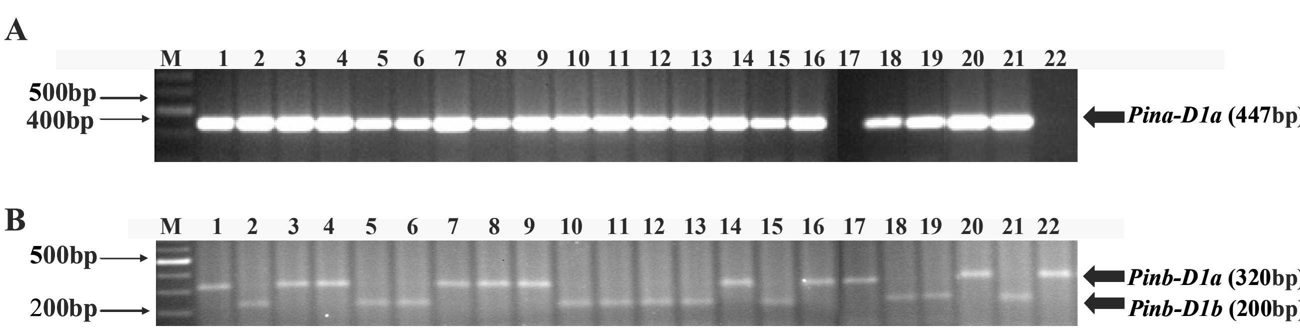

Fig. 1. Agarose gel electrophoresis of PCR amplified *Pina-D1* allele (A) and *Pinb-D1* allele (B) cut with *BsBrI* of Korean wheat cultivars. M, molecular size marker; 1, Ol; 2, Geuru; 3, Dahong; 4, Chungkye; 5, Eunpa; 6, Tapdong; 7, Namhae; 8, Uri; 9, Olgeuru; 10, Alchan; 11, Gobun; 12, Keumkang; 13,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

Table 2. Grain characteristics of 22Korean wheat cultivars.

Cultivar	Test weight (g)	1000-kernel weight (g)	Grain properties by SeedCount				SKCS[a]		
			Length (mm)	Width (mm)	Area (mm^2)	Roundness (Ratio)	Kernel weight (mg)	Kernel diameter (mm)	Hardness index
Alchan	828.00	38.04	5.89	3.36	17.35	1.87	33.93	2.54	71.92
Anbaek	831.00	47.64	6.46	3.62	14.65	1.93	49.23	2.94	67.35
Chungkye	828.50	37.33	6.11	3.26	12.90	1.76	30.43	2.32	39.23
Dahong	816.00	33.45	5.97	3.16	13.70	1.75	27.46	2.19	33.11
Eunpa	839.00	37.48	5.99	3.25	13.45	1.71	32.49	2.45	74.73
Geuru	797.50	51.15	6.91	3.58	16.40	1.75	49.91	3.07	59.38
Gobun	842.00	42.40	6.43	3.34	16.25	1.82	33.80	2.45	68.38
Jinpoom	819.50	39.69	6.23	3.34	13.85	1.73	40.59	2.80	64.59
Joeun	844.50	38.76	6.25	3.28	15.10	1.79	39.56	2.80	81.05
Jokyoung	814.00	48.93	7.02	3.51	14.75	1.90	48.88	2.95	60.54
Jonong	825.00	45.51	6.89	3.45	14.15	1.71	47.00	2.84	26.99
Jopoom	818.50	38.02	6.89	3.19	14.30	1.71	41.90	2.61	54.48
Keumkang	831.50	47.46	6.84	3.49	15.00	1.99	46.82	2.85	52.12
Milseoung	815.50	36.14	6.03	3.20	13.20	1.72	37.16	2.54	25.38
Namhae	787.00	37.59	6.16	3.34	14.85	1.78	32.56	2.45	39.93
Ol	795.00	39.60	6.32	3.40	13.75	1.69	37.23	2.60	34.70
Olgeuru	821.00	41.52	6.46	3.37	13.95	1.76	44.38	2.76	35.29
Saeol	825.00	38.60	6.75	3.20	13.20	1.73	33.84	2.25	22.70
Seodun	823.50	39.74	6.15	3.38	16.80	1.84	31.44	2.36	76.20
Tapdong	830.50	38.87	6.11	3.32	17.40	1.81	41.15	2.78	62.90
Uri	792.00	39.72	6.56	3.20	14.15	1.73	40.33	2.57	29.52
Younbaek	820.00	38.67	6.54	3.25	14.50	1.83	42.36	2.72	58.32
LSD[b]	4.12	0.64	0.05	0.04	0.20	0.02	1.24	0.07	1.72

[a]Single kernel characterization system.
[b]Least significant difference ($P < 0.05$). Differences between two means exceeding this value are significant.

Milseong, Namhae, Ol, Olgeuru, Saeol, Uri and Younbaek) showed *Pina-D1a* and *Pinb-D1a*. Two Korean wheat cultivars (Joeun and Younbaek) carried *Pina-D1b* allele, which is a deletion of the pinA gene. Eleven cultivars (Alchan, Anbaek, Eunpa, Geuru, Gobun, Jinpoom, Jokyung, Jopoom, Keumkang, Seodun and Tapdong) had *Pina-D1b* allele. Except for *Pinb-D1b,* not any mutant of pinB was found in Korean wheat cultivars. The prevalence of *Pinb-D1b* in Korean wheats was similar to the genetic variations in U.S. and Australian wheat germplams (Morris *et al.,* 2001; Cane *et al.*, 2004; Pickering and Bhave, 2007).

Association of Puroindoline Genotypes and Grain Characteristics

Test weight and thousand kernel weight of grain ranged from 787.00 g in Namhae to 844.50 g in Joeun and from 33.45 g in Dahong to 51.15 g in Geuru, respectively. Length and width of grain measured by SeedCount ranged from 5.89 mm in Alchan to 7.02 mm in Jokyung and from 3.16 mm in Dahong to 3.62 mm in Anbaek, respectively. Area and roundness of grain by SeedCount ranged from 12.90 mm^2 in Chungkye to 17.40 mm^2 in Tapdong and from 1.69 in Ol to 1.99 in Keumkang, respectively. Kernel weight and diameter measured by SKCS ranged from 27.46 mg in Dahong to 49.91 mg in Geuru and from 2.19 mm in Dahong to 3.07 mm in Geuru, respectively. Hardness index of grain by SKCS ranged from 22.70 in Saeol to 81.05 in Joeun. Kernel weight by SKCS increased as the increase of kernel diameter and also showed positive relationships with thousand kernel weight and grain length and width measured by SeedCount (Table 2).

Thousand kernel weight was positively correlated with length, width and roundness of grain by SeedCount (r = 0.723**, r = 0.889*** and r = 0.478*, respectively) and

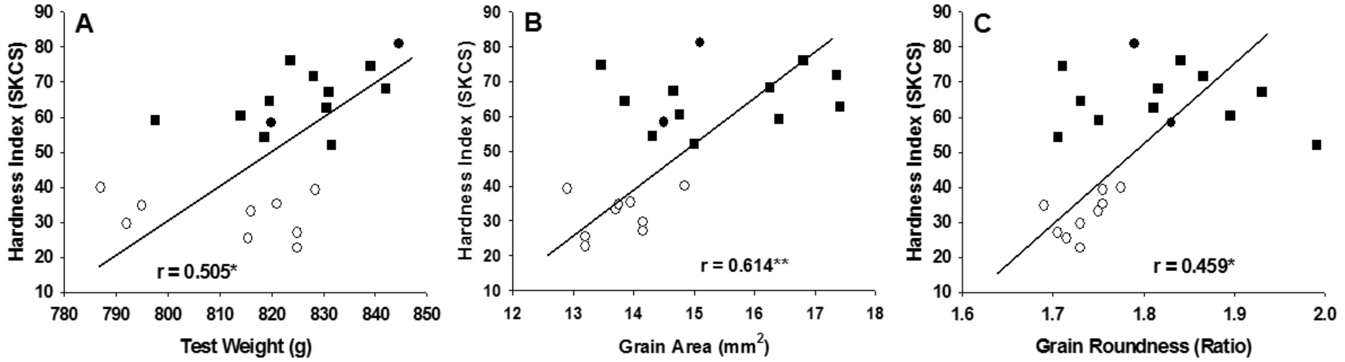

Fig. 2. Relationships between test weight (A), grain area (B), grain roundness (C) and hardness index of single kernel characterization system (SKCS). ○; Korean wheats with *Pina-D1a/Pinb-D1a*, ●; Korean wheats with *Pina-D1b/Pinb-D1a* and ■; Korean wheats with *Pina-D1a/Pinb-D1b*.

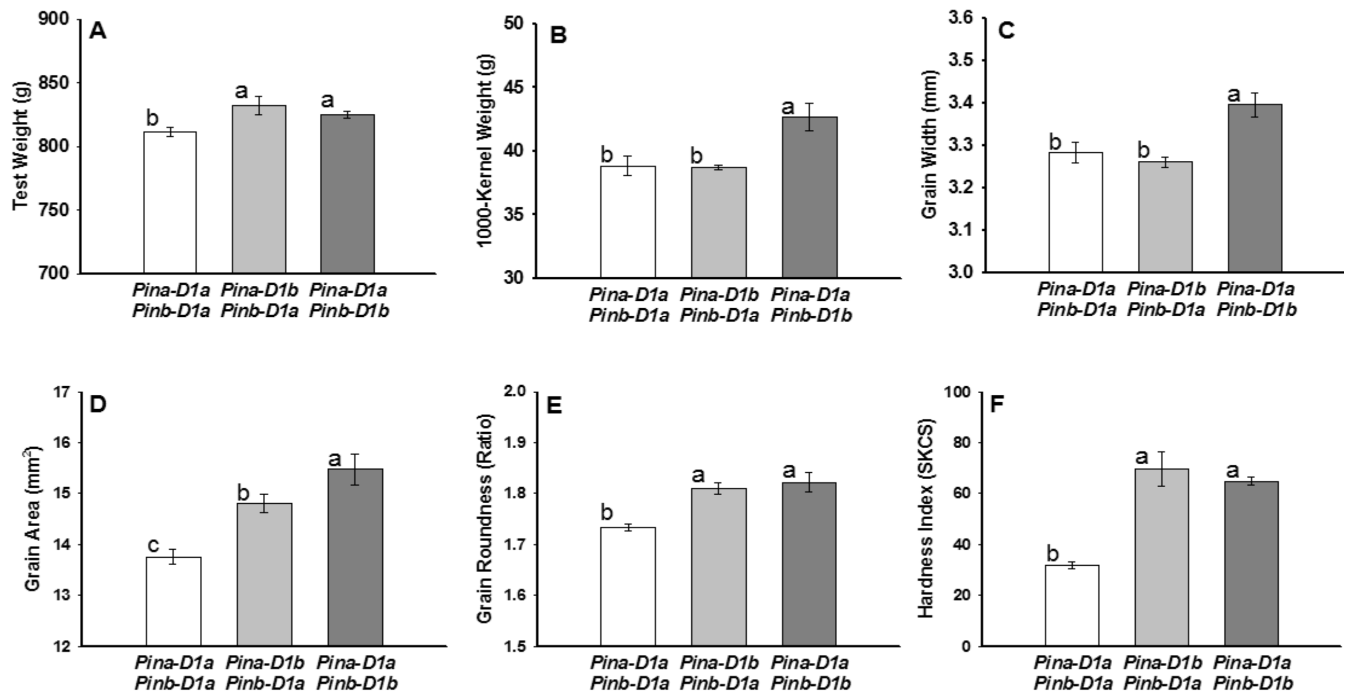

Fig. 3. The difference of test weight (A), 1000-kernel weight (B), grain width (C), grain area (D), grain roundness (E) and hardness index (F) of Korean wheat cultivars according to the allelic compositions of puroindolines. Each bar represents mean ± standard error and values on the bars with different letters are significantly different at $p < 0.05$.

also was positively correlated with kernel weight and kernel diameter measured by SKCS (r = 0.823*** and r = 0.780***, respectively). However, there was no relationships was not found in test weight and grain properties in spite of test weight is generally influenced by kernel shape.

Hardness index was positively correlated with test weight, area and roundness of grain by SeedCount (r = 0.505*, r = 0.614** and r = 0.459*, respectively). However, there were no significant relationships between hardness index and other grain properties, including length and width of grain and weight and diameter of kernel (Table 2). However, there was no different between hardness index and test weight in 139 recombinant inbred lines from *Pina-D1b* × *Pinb-D1a* (Martin *et al.*, 2001). Hardness index was significantly correlated with kernel weight and diameter in U.S. hard wheat populations (Giroux *et al.*, 2000; Martin *et*

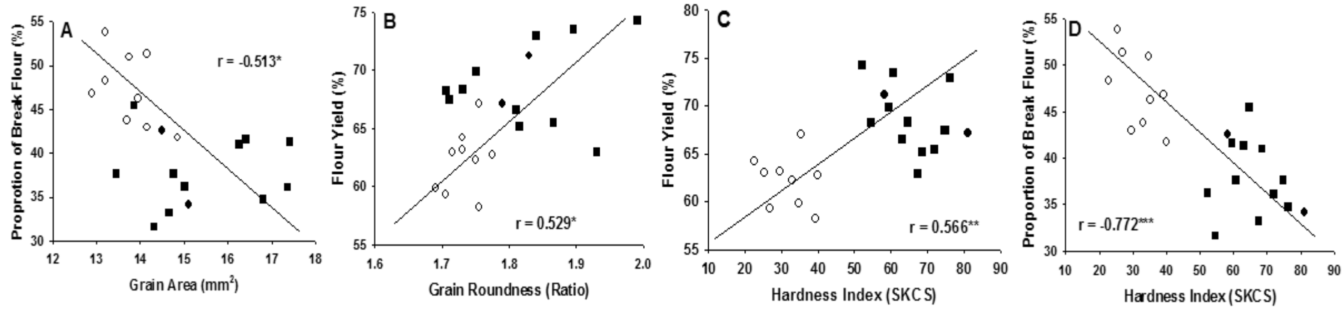

Fig. 4. Relationships between proportion of break flour and grain area measured by SeedCount (A), flour yield and grain roundness (B), flour yield and hardness index of single kernel characterization system (SKCS) (C) and proportion of break flour and hardness index (D). ○; Korean wheats with *Pina-D1a/Pinb-D1a*, ●; Korean wheats with *Pina-D1b/Pinb-D1a* and ■; Korean wheats with *Pina-D1a/Pinb-D1b*.

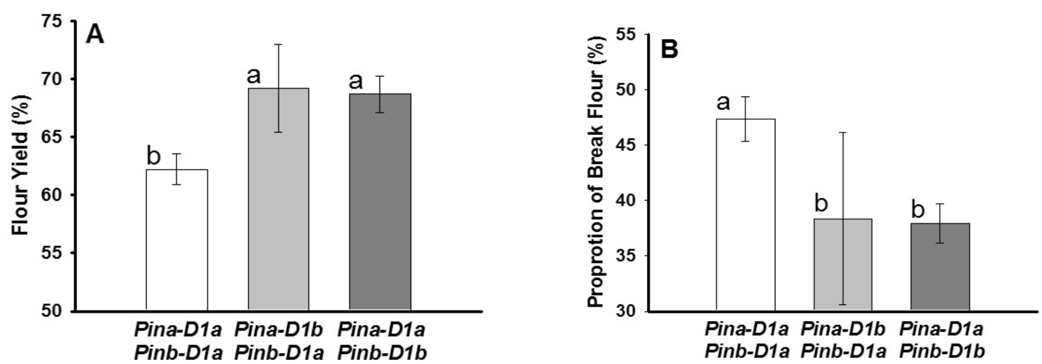

Fig. 5. The difference of flour yield (A) and proportion of break flour in flour (B) of Korean wheat cultivars according to the allelic compositions of puroindolines. Each bar represents mean ± standard error and values on the bars with different letters are significantly different at $p < 0.05$.

al., 2001) and F_4 populations of Japanese wheats derived from *Pina-D1b* × *Pinb-D1a* (Nishio *et al.*, 2005).

Korean wheats carrying with *Pina-D1a*/*Pinb-D1a* showed lighter test weight and thousand kernel weight (811.67 g and 38.83 g, respectively) than wheats with *Pina-D1b*/*Pinb-D1a* (832.25 g and 38.72 g, respectively) and *Pina-D1a*/*Pinb-D1b* (825.00 g and 42.67 g, respectively). Grain width measured by SeedCount was longer in *Pina-D1a*/*Pinb-D1b* genotype than that of other genotypes, but no difference was found in grain length. In area and roundness of grain by SeedCount, *Pina-D1a*/*Pinb-D1a* showed lower than *Pina-D1b*/*Pinb-D1a* or *Pina-D1a*/*Pinb-D1b*. *Pina-D1a*/*Pinb-D1a* showed lighter kernel weight, smaller kernel diameter and hardness index than *Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b* (Fig 3). There was no difference in kernel weight, diameter and hardness index by SKCS between *Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b*.

Hardness index of SKCS is widely accepted as the standard method to determine the single wheat kernel texture and is the best (most discriminating) measure of the material properties of the wheat endosperm manifested by the action of the *Ha* locus (Moriss *et al.*, 1999; Osborne and Anderssen, 2003). Korean wheats showed different grain properties according to the allelic variations of puroindolines in this study, but no different was found in test weight and kernel weight and diameter by SKCS among *Pina-D1b* and *Pinb-D1b* of grain of recombinant inbred lines from hard red spring wheats (Giroux *et al.*, 2000; Martin *et al.*, 2001). There was also no difference in thousand kernel weight between *Pina-D1b*/*Pinb-D1a* and *Pinb-D1a*/*Pinb-D1b* from doubled haploid lines (Nagamine *et al.*, 2003). *Pina-D1b*/*Pinb-D1a* showed higher hardness index of grain than *Pina-D1a*/*Pinb-D1b* in many previous study of hard wheats (Giroux *et al.*, 2000; Lillemo and

Table 3. Milling and Physical Properties of Flour in 22 Korean wheat cultivars.

	Milling properties			Physical properties of flour						
	Flour yield (%)	Proportion of break flour (%)	Milling score	Average of particle size (μm)	Ash (%)	Damaged starch (%)	Water retention capacity (%)	Color[a] L*	Color[a] a*	Color[a] b*
Alchan	65.53	36.15	71.42	80.63	0.49	3.29	70.67	89.92	-1.20	8.67
Anbaek	63.03	33.28	65.56	90.65	0.56	3.38	71.41	89.07	-1.58	10.47
Chungkye	58.23	46.80	63.37	63.61	0.51	2.92	62.87	89.98	-1.51	8.64
Dahong	62.29	43.78	69.16	57.97	0.48	2.24	62.70	90.69	-1.56	8.31
Eunpa	67.49	37.69	71.94	90.13	0.52	4.07	75.32	88.38	-1.42	10.39
Geuru	69.94	41.67	65.63	82.52	0.69	4.18	73.07	87.70	-1.18	9.89
Gobun	65.23	41.04	66.18	86.12	0.59	3.32	74.05	89.31	-1.36	10.03
Jinpoom	68.37	45.51	74.00	60.62	0.50	3.61	76.88	89.48	-1.71	10.78
Joeun	67.12	34.13	68.07	96.47	0.59	4.37	78.25	88.92	-1.10	9.57
Jokyoung	73.51	37.71	75.76	86.67	0.56	3.54	65.22	90.56	-1.58	10.54
Jonong	59.29	51.29	60.67	75.61	0.58	2.01	65.07	91.02	-1.09	7.78
Jopoom	68.30	31.69	72.91	78.96	0.52	3.02	68.13	89.69	-1.35	9.32
Keumkang	74.30	36.29	82.38	86.19	0.45	3.06	58.07	89.89	-1.40	9.60
Milseoung	62.97	53.82	69.66	63.21	0.48	2.19	61.84	90.64	-1.55	8.96
Namhae	62.71	41.76	68.35	58.50	0.50	2.25	67.36	90.79	-1.35	7.92
Ol	59.87	50.96	71.42	62.63	0.39	2.84	66.80	90.32	-1.57	9.32
Olgeuru	67.10	46.19	78.56	66.99	0.39	2.12	64.53	90.71	-1.36	7.85
Saeol	64.20	48.28	73.06	60.98	0.44	2.13	67.40	90.80	-1.35	7.94
Seodun	72.95	34.81	77.37	89.46	0.52	4.03	77.10	89.30	-1.58	11.08
Tapdong	66.63	41.34	70.95	81.36	0.52	3.42	71.16	89.12	-1.28	8.61
Uri	63.18	42.91	73.09	57.80	0.42	2.22	62.67	91.61	-1.47	8.00
Younbaek	71.24	42.56	72.24	91.32	0.59	3.47	73.52	89.83	-1.44	10.77
LSD[b]	0.92	1.52	2.54	1.72	0.04	0.41	0.70	2.27	0.05	0.11

[a]L* = lightness; a* = redness-greenness; b* = yellowness-blueness.
[b]Least significant difference ($P < 0.05$). Differences between two means exceeding this value are significant.

Morris, 2000; Martin *et al.*, 2001; Nagamine *et al.*, 2003; Cane *et al.*, 2004; Chen *et al.*, 2007). There was no significant difference in hardness index between *Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b* due to the less number of cultivars with *Pina-D1b*/*Pinb-D1a* in Korean wheats.

Association of Puroindolines Genotypes and Milling Performances

Flour yield and proportion of break flour ranged from 58.23% in Chungye to 74.30% in Keumkang and from 31.69% in Jopoom to 53.82% in Milseoung, respectively. Milling score ranged from 60.67 in Jonong to 82.38 in Keumkang. As the increase of flour yield, milling score increased and proportion of break flour decreased (Table 3).

The proportion of break flour was negatively correlated with area of grain measured by SeedCount (r = -0.503*). Flour yield and proportion of break flour were significantly correlated with roundness of grain measured by SeedCount (r = -0.576** and r = 0.529*). Hardness index of SKCS was positively correlated with flour yield (r = 0.566**) and was negatively correlated with proportion of break flour (r = -0.772***). However, there were no significant relationships among milling performances, test weight, 1000-kernel weight, length and width of grain (Fig. 4).

Grain characteristics may influence milling performances but relationships are conflicting. Kernel size and volume were positively correlated with flour yield within genotypes (Marshall *et al.*, 1986; Berman *et al.*, 1996), but kernel shriveling, not size, was primarily responsible for differences in milling properties (Gaines *et al.*, 1997). No relationship between kernel morphology and flour yield was found in the soft×hard wheat population (Bergman *et al.*, 2000).

Table 4. Correlation Coefficients Among Grain Characteristics, Milling Performances and Physical Properties of Flour.

Parameters	*Physical properties of flour*						
	Avg. particle sSize	Ash	Damaged starch	Water retention capacity	Color[a]		
					L*	a*	b*
Grain characteristics							
Test weight	0.514*[b]	0.227	0.378	0.330	−0.396	0.204	0.274
1000-kernel weight	0.427*	0.437*	0.268	−0.023	−0.274	0.159	0.300
Grain length	0.186	0.248	−0.062	−0.225	0.088	0.206	0.050
Grain width	0.414	0.364	0.322	0.086	−0.343	0.072	0.337
Grain area	0.520*	0.398	0.490*	0.428*	−0.445*	0.389	0.221
Grain roundness	0.561**	0.167	0.311	−0.035	−0.169	−0.062	0.375
Kernel weight	0.361	0.317	0.147	−0.073	−0.145	0.188	0.195
Kernel diameter	0.440*	0.420	0.355	0.121	−0.326	0.227	0.303
Hardness index	0.800***	0.520*	0.915***	0.774***	−0.777***	0.085	0.735***
Milling performances							
Flour yield	0.590**	0.252	0.576**	0.291	−0.393	−0.057	0.623**
Proportion of break flour	−0.687***	−0.321	−0.625**	−0.400	0.495*	−0.114	−0.468*
Milling score	0.053	−0.525*	0.058	−0.135	0.128	−0.332	0.208

[a] L* = lightness; a* = redness-greenness; b* = yellowness-blueness.
[b]*indicates significance at the 0.05 level, ** at the 0.01 level, and *** at the 0.001 level.

Milling yield was significantly correlated with test weight in Chinese wheats (Zhou *et al*., 2003) but no relationship was found in soft red winter wheats (Steve *et al*., 1995). Flour yield increased through recurrent selection for kernel weight (Wiersma *et al*., 2001). Hardness index of SKCS was negatively correlated with flour yield in hard wheats (Ohm *et al*., 1998; Martin *et al*., 2001) and Chinese winter wheats (Zhang *et al*., 2005) and also negatively correlated with break flour yield in U.S. wheats with various puroindoline genotypes (Bettge and Morris, 2000).

Korean wheats carrying with *Pina-D1a*/*Pinb-D1a* showed lower flour yield (62.20%) and higher proportion of break flour (47.31%) than wheats with *Pina-D1a*/*Pinb-D1b* (68.66% and 37.93%, respectively (Fig. 5). *Pinb-D1b* genotypes in doubled haploid population had higher milling yield than the *Pinb-D1a* soft lines (Nagamine *et al*., 2003). Hard wheats with *Pina-D1a*/*Pinb-D1b* had significantly higher flour yield, break flour yield and milling score than wheats with *Pina-D1b*/*Pinb-D1a* in recombinant inbred lines of hard red spring wheats (Martin *et al*., 2001) and Australian wheats (Eagles *et al*., 2006). However, there was no significant difference in flour yield and proportion of break flour between *Pina-D1a*/*Pinb-D1b* and *Pina-D1b*/*Pinb-D1a*. Only two Korean wheat cultivars, Joeun and Younbaek, contained *Pina-D1b*/*Pinb-D1a* and there were significant differences in milling performances between these wheat cultivars

Association of Puroindoline Genotypes and Physical Properties of Flour

Average of particle size of wheat flour ranged from 57.80 µL in Uri to 96.47 µL in Joeun. Ash and damaged starch content ranged from 0.39% in Olgeuru to 0.69% in Geuru and from 2.01% in Jonong to 4.37% in Joeun, respectively. Water retention capacity ranged from 58.07% in Keumkang to 78.25% in Joeun. Color of wheat flour was 87.70 − 91.61 in lightness, -1.71 − -1.09 in redness-greenness and 7.78 − 11.08 in yellowness-blueness, respectively (Table 3). As the increase of average of particle size of flour, ash content, damaged starch content and water retention capacity of flour were increased. Ash content also was increased as the increase of the damaged starch content and water retention capacity. Water retention capacity was influenced by particle size of flour and damaged starch content. Harder texture would tend to give rise to greater starch damage on milling processing and starch damage could have an effect on water absorption of rheological properties and end-use properties of flour (Evers and

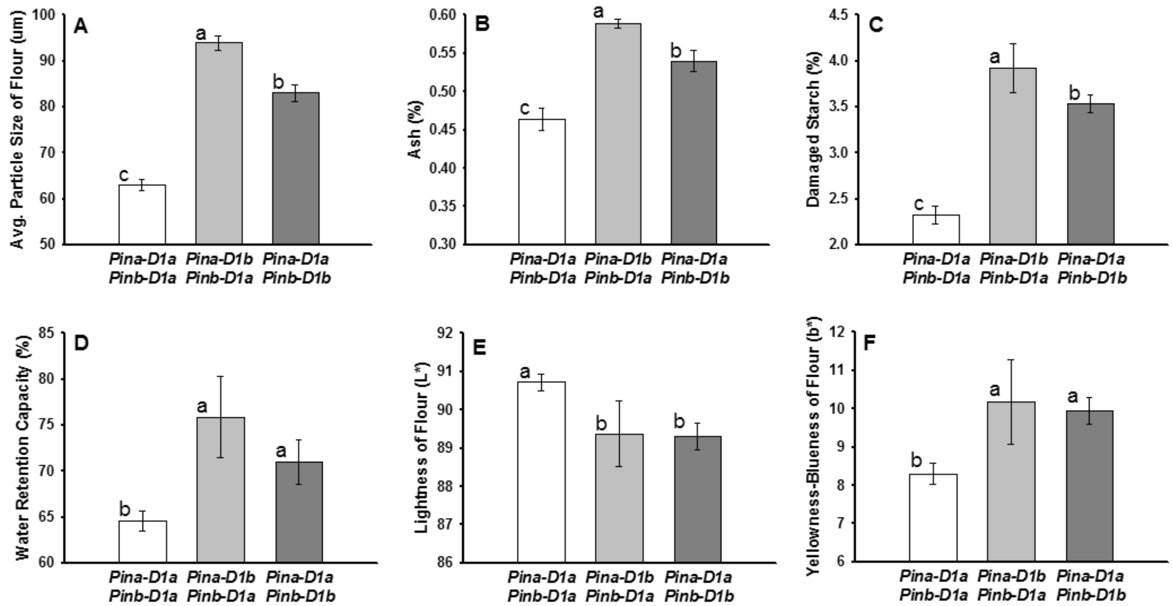

Fig. 6. The difference of average of particle size of flour (A), ash content (B), damaged starch content (C), water retention capacity (D), lightness of flour (E) and yellowness-bluenessof flour (F) of Korean wheat cultivars according to the allelic compositions of puroindolines. Each bar represents mean ± standard error andvalues on the bars with different letters are significantly different at $p < 0.05$.

Stevens, 1985). Lightness and yellowness-blueness of flour were significantly correlated with average of particle size of flour, ash and damaged starch and water retention capacity. Highly significant correlations were found between ash content and flour color because higher extraction produces darker flours with higher ash content (Kruger and Reed, 1988).

Grain characteristics, including test weight, 1000-kernel weight, area and roundness of grain were positively correlated with physical properties of flour in Korean wheats. Hardness index also positively correlated with average of particle size, ash, damaged starch and water retention capacity. Average of particle size and damaged starch were positively correlatedwith flour yield and were negatively correlated with proportion of break flour. Lightness and yellowness-blueness of flour was significantly correlated with hardness index and proportion of break flour but no significant relationship was found in redness-greenness (Table 4). These results are in good agreement with previous results (Bettege and Morris, 2000; Morgan *et al*., 2000; Martin *et al*., 2001; Nagamine *et al*., 2003; Zhang *et al*., 2005; Chen *et al*., 2007). Korean wheat cultivars with large grain, which lager grain area and 1000-kernel weight, could have hard texture in grain hardness, these properties could produce high flour yield and low proportion break flour and these flours could have coarse particle size, high ash and damaged starch content and low brightness of flour color.

Korean wheats carrying with *Pina-D1a*/*Pina-D1a* showed lower average particle size of flour, ash and damaged starch content, water retention capacity, yellowness-blueness and higher lightness of flour (63.03 μm, 0.46%, 2.33%, 64.58%, 8.30 and 90.73, respectively) than wheats with *Pina-D1a*/*Pinb-D1b* (83.03 μm, 0.54%, 3.54%, 71.01%, 9.94 and 89.31, respectively) or *Pina-D1b*/*Pina-D1a* (93.89 μm, 0.59%, 3.92%, 75.89%, 10.17 and 89.37, respectively). Korean wheats carrying with *Pina-D1b*/*Pina-D1a* showed higher average of particle size of flour, ash and damaged starch content (93.89 μm, 0.59% and 3.92%, respectively) than wheats with *Pina-D1a*/*Pinb-D1b* (83.03 μm, 0.54% and 3.54%, respectively), but no difference was found in water retention capacity and flour color (Fig. 6).

Soft wheats showed lower average particle size of flour, ash and damaged starch content than hard wheats in doubled haploid lines from Japan and U.S. wheats with different puroindolines genotypes (Bettege and Morris, 2000;

Nagamine *et al*., 2003). Hard wheats with *Pina-D1a/ Pinb-D1b* allele had lower ash content and flour particle sizes than hard wheats with *Pina-D1b/Pinb-D1a* allele in hard red spring wheats and Chinese wheats (Martin *et al.*, 2001; Chen *et al*., 2007). Chinese wheats with *Pina-D1a/ Pina-D1b* showed higher lightness of flour and lower yellowness-blueness than those of *Pina-D1b/Pinb-D1a* genotypes (Chen *et al*., 2007). Water absorption of *Pina-D1b/ Pina-D1a* was higher than that of *Pina-D1a/Pina-D1b* in Austarlian and Chinese wheats (Cane *et al*., 2004; Chen *et al*., 2007), but no significant difference in hard red spring wheats (Martin *et al*., 2001).

These significant differences in physical properties of Korean wheat flours based on the puroindoline genotypes could be resulted from the notable associations of puroindolines, grain characteristics and milling performances. Therefore, the evaluation of puroindolines genotypes of experimental lines in breeding programs and the further study to elucidate the effects of genetic variations of puroindolines on end-use qualities in Korean wheats should be accomplished to improve milling and flour quality of Korean wheats.

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