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Effect of allelic variations at the *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pinb-D1* loci on flour characteristics and bread loaf volume

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Abstract

Doubled haploid wheat lines developed from a cross between Keumkang, a hard white winter wheat, and Olgeuru, soft red winter wheat were used to determine the effects of allelic variation in *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pinb-D1* loci on physiochemical properties of flour and bread loaf volume. Variations in flour yield, average of particle size and damaged starch content were heavily affected by allelic composition on *Pinb-D1* loci and its contribution was estimated to be 78.8, 83.5 and 86.6%, respectively. *Glu-D1* and *Glu-A3* alleles were also responsible for variation in those properties, but no significant influence of *Glu-B3* alleles was observed. Variation in SDS-sedimentation volume was significantly affected by the allelic composition on *Glu-A3* and *Pinb-D1* loci, but allelic variations in glutenin and puroindoline exhibited little influence on protein content in DH lines. *Glu-D1* allele showed biggest influence on mixing time and mixing tolerance of dough and its contributed 51.0 and 10.8% variations, respectively. *Glu-B3* and *Pinb-D1* alleles also affected mixing time and mixing tolerance of dough and *Pinb-D1* allele variation influenced on water absorption of dough. *Glu-D1*, *Glu-B3* and *Pinb-D1* alleles were responsible for 17.9, 4.9 and 8.4% variation in bread loaf volume, respectively.

Introduction

Wheat protein, content and composition, is a primary factor in determining quality of bread and noodles because protein content and composition influence dough rheology, baking properties (Carson and Edwards, 2009). Wheat flour for bread with high loaf volume typically requires 12% or high protein content, while wheat flour with about 10% or low protein content is acceptable for use in the preparation of white salted noodles and cookie baking, respectively (Carson and Edwards, 2009). Glutenins, mainly related to protein quality in wheat, are divided in two groups, high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) (Gianibelli *et al*., 2001). HMW-GS are the key factors in bread-baking process as the major determinants of dough elasticity and LMW-GS play a major role in determining dough resistance and extensibility (D'Ovidio and Masci, 2004; Anjum *et al.*, 2007). LMW-GS and the HMW-GS can be linked via both inter- and intra-molecular disulfide bonds forming very large polymeric proteins (Gianibelli *et al*., 2001; D'Ovidio and Masci, 2004; Anjum *et al.*, 2007). HMW-GS are encoded by the *Glu-1* loci on the long arms of group 1 chromosomes and LMW-GS are encoded by the *Glu-3* loci on the short arm of these same chromosomes (Singh *et al*., 1988). The effects of *Glu-1* and/or *Glu-3* allelic variations on dough properties and bread quality have been extensively studied and are known to be significant because the allelic variations of *Glu-3* also play an important role in dough properties and bread quality although *Glu-1* alleles mainly determined the bread baking characteristics of dough (Gianibelli *et al*., 2001; D'Ovidio and Masci, 2004; Anjum *et al*., 2007).

Grain hardness is genetically controlled traits and has also influenced on the end-use quality of wheat (Morris, 2002; Bhave and Morris, 2008; Pasha *et al.*, 2010). Grain hardness is mainly determined by the variations in puroindoline a (*Pina-D1*) and puroindoline b (*Pinb-D1*) genes, which located in the Hardness locus (*Ha*) on the short arms of chromosome 5D. (Morris 2002). Soft wheats are designated as the wild type of puroindolines, which contain both *Pina-D1a* and *Pinb-D1a* genotypes (Bhave and Morris, 2008; Pasha *et al*., 2010). The mutations in *Pina-D1* and *Pinb-D1a* are present in hard wheats (Bhave and Morris, 2008; Pasha *et al.*, 2010). Hard wheats generally contain either one of the 8 allelic variants at *Pina-D1* locus (*Pina-D1b*, *f*, *k-n*, *p* and

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q) or 17 allelic variants at *Pinb-D1* locus, including 13 nucleotide mutation (*Pinb-D1b-g*, *l*, *q*, *t*, *v*, *w*, *aa* and *ab*) and another frame shift mutant (*Pinb-D1p*, *r*, *s* and *u*) (Bhave and Morris, 2008). Hard wheats prevalently contained *Pina-D1b* or *Pinb-D1b* alleles (Morris, 2002; Bhave and Morris, 2008; Pasha *et al.*, 2010). Allelic variations of *Pin-D1* in hard wheats influence physicochemical properties of flour, including particle size of flour, flour yield, damaged starch, protein and ash content, water absorption and bread loaf volume (Martin *et al.*, 2001; Cane *et al*., 2004; Chen *et al*., 2007; Park *et al*., 2010).

Most Korean wheat cultivars showed inferior bread quality to U.S. wheat cultivars, in spite of the similar protein content, mainly because Korean wheat breeding program has long focused on improving grain yield and early maturation. Allelic variations in glutenins and puroindolines have been identified and utilized for selection of elite breeding lines in many wheat breeding programs. Doubled haploid lines were developed through genetic recombination by crossing two Korean wheat cultivars with different allelic compositions in glutenins and puroindolines. The developed doubled haploid lines provide an opportunity to study the relationship between allelic variation in those loci and bread quality. The objective of this study was to determine the influence of allelic variations in glutenins and puroindolines on flour characteristics and bread loaf volume and to provide useful information for improving bread quality in wheat breeding programs.

Materials and Methods

Materials

Doubled haploid lines were derived from a Keumkang/Olgeuru cross. Keumkang, a hard white winter wheat, is the most commonly grown cultivar in Korea and known to possess a good milling quality and medium dough strength. Olgeuru is a soft red winter wheat. A set of 96 doubled haploid lines were developed from F1 plants of the Keumkang and Olgeuru cross using the wheat × maize system of Inagaki and Mujeeb-Kazi (1995) at CIMMYT (El Batan, Mexico). Doubled haploid lines were grown at the Upland Crop Experimental Farm of National Institute of Crop Science, RDA (Iksan, Korea) for seed multiplication in 2009. Field trials of this doubled haploid population were sown in randomized complete blocks with 3 replicates in 2010/2011 and 2011/2012 on 50% of clay loam soil. The seeds were sown in late October and each plot consisted of three 4-m rows spaced 25 cm apart and plots were combine-harvested in mid June in both years. Fertilizer was applied at 5: 7: 5kg/10a (N: P: K) before sowing and weeds, insects and disease were stringently controlled. No supplemental irrigation was applied. Mean temperature of these two years (10.3ºC) was higher than that of an average year (0.2ºC), and average precipitation (608 mm) was lower than that of an average year (576 mm). Grain from each plot was dried using forced air driers and bulked from replications to provide grain for quality analysis.

Allelic variations

Glutenin compositions were evaluated according to the protocol of Singh *et al.* (1991) with some modifications adopted by Peña *et al.* (2004). Gliadins were extracted from 40 mg of whole grain meal with 1500 µL of 50% propanol with incubation for 20 min at 65ºC 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 65ºC 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 90ºC 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 65ºC. After a 5 min centrifugation, 100 µL of extraction buffer containing 1.4% (v/v) freshly mixed 4-vinylpyridine. After incubation for 15 min at 65ºC 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 90ºC in heat 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 and Lawrence (1983). *Glu-A3* allele was 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. Genomic DNA was extracted from young leaf tissue (100 mg) using the Genomic DNA prep kit (Solgent Co., Seoul, Korea) according to the manufacturer's instructions. The allelic variations of puroindolines

were determined by the procedure described by Gautier *et al.* (1994).

Analytical methods

Wheat was milled using a Bühler experimental mill, according to AACCI Approved Methods 26-31.01. (AACCI, 2010). Two kilograms of wheat were conditioned overnight to reach 15% moisture content and then milled with a 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 grain weight fed to the mill. Distribution of flour particle size was measured by the multi-wavelength laser particle size analyzer LS13320 (Beckman Coulter, Inc., USA). Moisture and protein contents of wheat flour were determined according to AACCI Approved Methods 44-15.02 and 46-30.01, respectively (AACCI, 2010). The determination of damage starch was carried out following the procedure described by Gibson *et al.* (1992) using an enzymatic assay kits (MegaZyme Pty., Ltd., Australia). A SDS sedimentation test was performed according to AACCI Approved Methods 56-70.01 (AACCI, 2010) with a modification in flour weight to 3 g. Optimum water absorption, mixing time and mixing tolerance of wheat flour were determined using a 10-g mixograph (National Mfg. Co., USA) according to AACCI Approved Methods 54-40.02 (2010).

Bread baking

Bread was baked according to the optimized straight-dough bread-making method according to AACCI Approved Methods 10-10.03 (AACCI, 2010). The ingredients of baking formula consisted of 100 g (14% moisture basis) of flour, 6 g of sugar, 3 g of shortening, 1.5 g of salt, 5.0 g of fresh yeast, 50 mg of ascorbic acid, and 0.25 g of barley malt (about 50 DU/g, 20ºC). The optimum water absorption and mixing time were determined by the feel and appearance of the dough during the mixing. The dough was fermented in a cabinet at 30ºC and 85% relative humidity for 70 minutes with two punches and a proof period of 60 minutes, and then baked at 210ºC for 18 minutes. After cooling for 2 hrs at room temperature, a slice 2.0 cm thick was cut from the center portion of the bread. Loaf volume was measured immediately by rapeseed displacement and weighted after the bread was taken out of the oven. Firmness of the bread crumb was evaluated with a compression test using TA-XT2 Texture Analyser (Stable Micro Systems, England). The slice was placed on a flat metal plate and compressed to 25% of its thickness at the speed of 1.0 mm/sec using a plastic plunger with a flat surface of 2.0 cm diameter.

Statistical analysis

Statistical analysis of the data was performed by SAS software (SAS Institute, NC, USA) using Fisher's least significant difference test (LSD), analysis of variance (ANOVA) and pair-wise t-test. Analysis of variance was conducted using the general linear model procedure, and the genotype × year component was used as the error term. Sources of variation in the model were considered to be fixed effects. PROC GLM was used to estimate the relative contribution of tested loci for measured traits of 98 DH lines. The mean of each marker allele type was compared by one-way ANOVA, and differences were considered significant at $P < 0.05$, unless otherwise specified. The pair-wise t test at $P = 0.05$ was conducted to compare means when F tests were significant. The ratio of phenotypic variation explained (R^2) was then estimated for the declared loci. All determinations were performed at least in triplicates and all were averaged.

Results and Discussion

Allelic compositions and analysis of variance in DH lines

Allelic variation in *HMW-GS*, *LMW-GS* and puroindoline loci in the parental cultivars and selected doubled haploid wheat lines are shown in Figure 1. Parental cultivars, cvs. Keumkang and Olgeuru, showed different allelic compositions in *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pinb-D1*. Keumkang carried *Glu-D1d*, *Glu-A3c*, *Glu-B3h* and *Pinb-D1b*, and Olgeuru had *Glu-D1f*, *Glu-A3d*, *Glu-B3d* and *Pinb-D1a*. Keumkang and Olgeuru shared the same allelic compositions at *Glu-A1*, *Glu-B1* and *Pina-D1* and carried *Glu-A1b*, *Glu-B1b* and *Pina-D1a* alleles. Among 96 doubled haploid wheat lines, 47 lines carried *Glu-D1d* and 49 lines *Glu-D1f* at *Glu-D1* loci. Fifty lines carried *Glu-A3c* and 46 lines *Glu-A3d* at *Glu-A3* loci. Fifty-one lines carried *Glu-B3d* and 45 lines *Glu-B3h* at *Glu-B3* loci. Forty-four lines carried *Pinb-D1a* and 52 lines *Pinb-D1b* at *Pinb-D1 loci*. Fourteen types of allelic composition were identified in 96 doubled haploid lines. Seven and eight lines exhibited the same allelic compositions to parental cultivars, Keumkang and Olgeuru, respectively.

Genotype, year and their interactions significantly influenced flour characteristics, mixing properties and bread volume, except flour yield (Table 1). Flour compositions, mixing properties and bread loaf volume were influenced by environments as well as

Table 1. Analysis of variance for flour characteristics, mixing properties and loaf volume of bread in 96 doubled haploid (DH) wheat lines grown for two years

Source	df	*F*-value[a]				
		Flour yield	Physicochemical properties of flour			
			Average of particle size	Damaged starch	Protein	SDS-sedimentation
Genotype (G)	95	51.1***	2308.8***	3082.3***	520.2***	1223.9***
Year (Y)	1	1.7	5062.5***	460.5***	226.7***	4293.0***
G × E	95	2.2***	105.7***	117.2***	230.0***	453.4***

Source	df	*F*-value			
		Mixograph			Bread loaf volume
		Water absorption	Mixing time	Mixing tolerance	
Genotype (G)	95	5.3***	213.9***	62.8***	80.7***
Year (Y)	1	184.6***	9.8**	15.4***	218.8***
G × E	95	3.7***	35.2***	35.2***	10.8***

a** and *** are significant at P = 0.01 and P = 0.001, respectively.

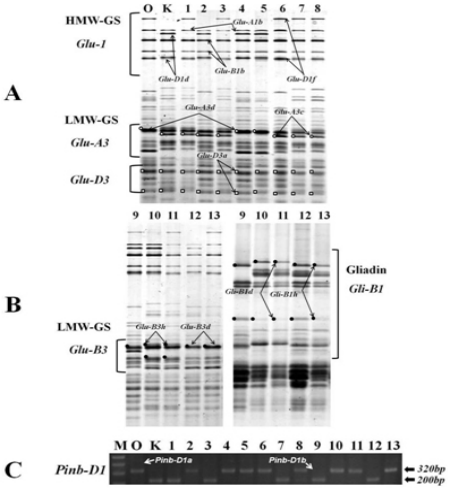

Figure 1. Allelic variations of *Glu-1*, *Glu-A3* and *Glu-D3* (A), and *Glu-B3* and *Gli-B1* (B) with SDS-PAGE of reduced and alkylated glutenin subunits and *Pinb-D1* (C) by PCR amplification cut with BsBrI of doubled haploid lines derived from Keumkang/Ogeuru cross. Open circles, closed circles and squares indicate identification alleles of *Glu-3*. O, Olgeuru; K, Keumkang; M, molecular size marker; 1-13; doubled haploid lines.

cultivars, as reported previously (Park *et al*., 2012). Genotype accounted for the largest proportion of the variation among flour yield and damaged starch content and mixing time in flour characteristics (89, 96 and 85%, respectively). The variation of average of particle size of flour, SDS-sedimentation volume and water absorption of mixograph were significantly influenced by production environment rather than genotype. The variation of bread loaf volume was also significantly influenced by different cultivated years. The variation of genotype by environment interaction was less significant than that of genotype and environment for all traits.

Effects of allelic variation on flour characteristics and mixing properties

DH lines with allelic variation in *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pinb-D1* loci exhibited significant differences in flour yield and flour characteristics (Table 2). Keumkang, a hard wheat carrying *Pinb-D1b*, showed higher flour yield, average particle size and damaged starch content than Olgeuru, a soft wheat with *Pinb-D1a* (Table 3). Keumkang also was higher in protein content and SDS-sedimentation volume than Olgeuru. Keumkang exhibited higher water absorption and mixing tolerance and longer mixing time of mixograph than Olgeuru. Variation in flour yield, average particle size and damaged starch content were predominantly affected by the allelic composition on *Pinb-D1* loci, which is estimated to have contributed 78.8, 83.5 and 86.6% variations, respectively. *Glu-D1* allele was also responsible for 5.4, 4.5 and 4.2% variations in flour yield, average of particle size and damaged starch content, respectively. *Glu-A3* allele explained 3.5 and 4.6% variations in average of particle size and damaged starch content, respectively. No significant differences in flour yield, average of particle size and damaged starch content were found among wheat carrying *Glu-B3* alleles.

DH lines carrying Pinb-D1b exhibited higher flour yield (72.7%), average particle size of flour (76.6 μm) and damaged starch content (5.3%) than those with *Pinb-D1a* (67.2%, 57.6 μm and 2.4%, respectively). DH lines carrying *Glu-D1f* showed higher flour yield (70.9%) than those with *Glu-D1d* (69.5%). DH lines carrying *Glu-D1f* and *Glu-A3c* showed higher average particle size and damaged starch content than those with *Glu-D1d* and *Glu-A3d.*

Table 2. Analysis of variance for flour characteristics, mixing properties and loaf volume of bread in 96 doubled haploid (DH) wheat lines grouped into those with allele for of *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pinb-D1* loci

Locus	*F*-value (R^2)[a]				
	Flour yield (%)	Physicochemical properties of flour			
		Average of particle Size (μm)	Damaged starch (%)	Protein (%)	SDS sedimentation (mL)
Glu-D1	5.4**	4.5**	4.2**	0.1	0.8
Glu-A3	1.2	3.5**	4.6**	0.1	5.7***
Glu-B3	0.7	0.1	0.4	0.2	0.6
Pinb-D1	78.8***	83.5***	86.6***	0.5	5.6***

Locus	*F*-value (R^2)			
	Mixograph			Bread loaf volume (cc)
	Water absorption (%)	Mixing time (min)	Mixing tolerance (mm)	
Glu-D1	0.1	51.0***	10.8***	17.9***
Glu-A3	0.1	0.3	0.1	1.3
Glu-B3	0.1	6.2***	2.0*	4.9**
Pinb-D1	5.3**	2.6*	4.1**	8.4***

[a]The mean of each allele type was compared by one-way ANOVA, where * and ** are levels of significance at P ≤ 0.05 and P ≤ 0.01, respectively. The ratio of phenotypic variation explained (R^2) was estimated only for the significant loci.

Table 3. Flour characteristics of doubled haploid (DH) wheat lines possessing different alleles in *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pinb-D1* loci

Locus	Allele	No of DH lines	Flour yield (%)	Average of particle size (μm)	Damaged starch (%)	Protein (%)	SDS sedimentation (mL)
Parental Lines							
Keumkang			73.7a[a]	71.0a	4.5a	15.6a	71.3a
Olgeuru			66.4b	57.8b	2.9b	12.0b	47.0b
Glu-D1	*d*	47	69.5b	65.7b	3.7b	14.3a	58.8a
	f	49	70.9a	70.0a	4.3a	14.3a	57.4b
Glu-A3	*c*	50	70.5a	69.7a	4.3a	14.3a	50.3b
	d	46	69.9b	65.9b	3.7b	14.4a	60.0a
Glu-B3	*d*	51	70.5a	68.3a	4.1a	14.4a	58.6a
	h	45	69.9b	67.5a	3.9a	14.3a	57.5a
Pinb-D1	*a*	44	67.2b	57.6b	2.4b	14.4a	56.1b
	b	52	72.7a	76.6a	5.3a	14.2a	59.8a

[a]Means followed by different letters are different at P < 0.05 within each locus.

Table 4. Difference in flour characteristics of 96 doubled haploid (DH) wheat lines carrying different allelic variation in *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pinb-D1* loci

Allelic variation				No of DH lines	Flour yield (%)	Average of particle size (μm)	Damaged starch (%)	Protein (%)	SDS sedimentation (mL)
Glu-D1	*Glu-A3*	*Glu-B3*	*Pinb-D1*						
d	*c*	*d*	*a*	2	67.5b[a]	52.9c	2.3c	14.5a	51.1c
d	*c*	*d*	*b*	5	72.7a	76.2a	5.4ab	14.8a	58.6abc
d	*c*	*h*	*a*	10	67.1b	58.0b	2.5c	13.8a	54.3bc
d	*c*	*h*	*b*	7	72.1a	77.6a	5.5a	14.2a	63.2a
d	*d*	*d*	*a*	11	67.3b	58.1b	2.5c	14.6a	60.0ab
d	*d*	*d*	*b*	8	72.8a	75.9a	5.2ab	14.2a	62.1ab
f	*d*	*d*	*a*	8	67.0b	55.8bc	2.3c	14.2a	56.8abc
f	*d*	*d*	*b*	8	72.8a	74.2a	4.8b	14.3a	60.1ab
f	*d*	*h*	*a*	5	67.6b	58.6b	2.4c	14.4a	57.0abc
f	*d*	*h*	*b*	6	72.4a	75.2a	5.2ab	14.2a	64.0a
f	*c*	*d*	*a*	4	66.7b	57.9b	2.7c	14.0a	55.7abc
f	*c*	*d*	*b*	9	72.9a	78.4a	5.6a	14.3a	56.4abc
f	*c*	*h*	*a*	4	67.5b	59.7b	2.4c	14.0a	50.8c
f	*c*	*h*	*b*	9	73.3a	77.8a	5.5a	14.8a	55.9abc

[a]Values followed by the same letter are not significantly different at $P < 0.05$.

Table 5. Mixing properties and loaf volume of bread of 96 doubled haploid (DH) wheat linescarrying different allelic variation in *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pinb-D1* loci

Locus	Allele	No of DH lines	Mixograph			Bread loaf volume (cc)
			Water absorption (%)	Mixing time (min)	Mixing tolerance (mm)	
Parental Lines						
Keumkang			66.3a[a]	3.8a	16.3a	888.3a
Olgeuru			62.0b	2.3b	12.5b	666.7b
Glu-D1	*d*	47	64.7a	3.9a	13.7a	831.1a
	f	49	64.7a	2.3b	11.7b	765.3b
Glu-A3	*c*	50	64.7a	3.0a	12.7a	788.7b
	d	46	64.7a	3.1a	12.7a	806.9a
Glu-B3	*d*	51	64.6a	3.3a	13.1a	813.7a
	h	45	64.8a	2.8b	12.3b	779.2b
Pinb-D1	*a*	44	63.9b	3.3a	12.1b	773.0b
	b	52	65.4a	2.9b	13.3a	818.2a

[a]Means followed by different letters are different within each locus at $P < 0.05$.

DH lines carrying with the *Glu-D1f*, *Glu-A3c* and *Glu-B3d* alleles also had higher frequency of *Pina-D1b* (32, 30 and 30 lines, respectively) than those with *Glu-D1d*, *Glu-A3d* and *Glu-B3h* (20, 22 and 22 lines, respectively). Nagamine *et al.* (2003) also reported that wheat lines carrying *Pina-D1b* allele exhibited higher flour yield, average particle size of flour and damaged starch content than lines with *Pina-D1a* allele in 110 DH lines derived from the cross between soft and hard wheat cultivars. Korean wheat cultivars carrying *Glu-B3h* exhibited higher flour yield and average particle size of flour than those with *Glu-B3d* because cultivars with *Glu-B3h* also carried the *Pina-D1b* or *Pinb-D1b* allele (Shin *et al*., 2012). Regardless allelic variations at *Glu-D1*, *Glu-A3* and *Glu-B3* loci, DH lines carrying *Pina-D1b* showed higher flour yield, average of particle size and damaged starch content than those with *Pina-D1a* (Table 4). These results indicate that flour yield and physical properties of flour are influenced more by puroindolines than glutenin compositions in Korean wheat genotypes.

Variation in SDS-sedimentation volume was significantly affected by the allelic composition on *Glu-A3* and *Pinb-D1* loci and their contributions were estimated to be 5.7 and 5.6%, respectively. DH lines carrying *Glu-A3d* and *Pinb-D1b* exhibited higher SDS-sedimentation volume (60.0 and 59.8 mL, respectively) than those with *Glu-A3c* and *Pinb-D1a* (50.3 and 56.1 mL, respectively). Shin *et al.* (2012) reported that Korean wheat cultivars carrying *Glu-D1d* exhibited higher SDS-sedimentation volume than those with *Glu-D1f*, but *Glu-B3h* produced a higher SDS-sedimentation volume than Glu-B1d due to the high protein content. Japanese wheat cultivars carrying *Glu-D1d* allele also showed higher SDS-sedimentation volume than those with *Glu-D1f* allele (Yanaka *et al.*, 2007). CIMMYT lines carrying *Glu-A3d* showed higher SDS-sedimentation volume than other alleles, but no significant difference was found between *Glu-B3d* and *Glu-B3h* alleles (Maucher *et al*., 2009). Allelic variations in glutenin and puroindoline did not affect protein content of DH lines, although protein content related to allelic variations at *Glu-D1* and *Glu-B3* loci in Korean wheat cultivars (Shin *et al*., 2012). He *et al.* (2005) reported that protein content was not influenced by glutenin compositions in Chinese wheats, except *Glu-B3j* allele related to the presence of 1BL/1RS wheat-rye translocation. DH lines carrying all of *Glu-D1d* or *f*, *Glu-A3c* or *d*, *Glu-B3h* and *Pinb-D1b* alleles exhibited higher SDS-sedimentation volume (> 63.2 mL) than those combined with other alleles (Table 4). DH lines carrying all of *Glu-D1d* or *f*, *Glu-A3c*, *Glu-B3d* or *h* and *Pinb-D1a* alleles exhibited lower SDS-sedimentation volume (< 51.1 mL) than those combined with other alleles. These results mean SDS-sedimentation volume influenced by allelic variation of glutenins and *Pinb-D1* alleles in DH populations.

Allelic composition on *Glu-D1* showed a much greater influence on mixing time and mixing tolerance of dough than that of *Glu-A3*, *Glu-B3* and *Pinb-D1* and the contribution of *Glu-D1* was estimated to be 51.0 and 10.8%, respectively (Table 2). *Pinb-D1* allele was responsible for 5.3% in water absorption

Table 6. Difference in mixing properties and bread loaf volume of 96 doubled haploid (DH) wheat lines carrying different allelic variation of in *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pinb-D1* loci

Allelic variation				No of DH lines	Mixograph			Bread loaf volume (cc)
					Water absorption (%)	Mixing time (min)	Mixing tolerance (mm)	
Glu-D1	*Glu-A3*	*Glu-B3*	*Pinb-D1*					
d	*c*	*d*	*a*	2	63.3b[a]	5.0a	13.3abcde	769.2cd
d	*c*	*d*	*b*	5	66.6a	4.2b	15.5a	875.5ab
d	*c*	*h*	*a*	10	63.2b	3.5bc	12.0bcdef	782.2cd
d	*c*	*h*	*b*	7	65.9ab	3.6bc	14.8ab	871.0ab
d	*d*	*d*	*a*	11	64.4ab	4.1b	13.9abcd	813.5bc
d	*d*	*d*	*b*	8	65.2ab	4.0b	14.7abc	905.7a
f	*d*	*d*	*a*	8	63.6ab	2.8cd	10.7ef	766.7cd
f	*d*	*d*	*b*	8	65.7ab	2.6d	12.0cdef	782.0cd
f	*d*	*h*	*a*	5	64.2ab	2.3d	11.6def	781.6cd
f	*d*	*h*	*b*	6	65.2ab	2.1d	13.0abcdef	770.9cd
f	*c*	*d*	*a*	4	63.8ab	2.6d	11.6def	724.6de
f	*c*	*d*	*b*	9	63.8ab	2.4d	12.7abcdef	792.4cd
f	*c*	*h*	*a*	4	64.7ab	2.3d	10.3f	690.8e
f	*c*	*h*	*b*	9	66.0ab	2.1d	11.4def	757.3cde

[a]Values followed by the same letter are not significantly different at $P < 0.05$.

of mixograph. *Glu-B3* and *Pinb-D1* alleles were also responsible for 6.2 and 2.6% variations in mixing time and 2.0 and 4.1% variation in mixing tolerance, respectively. Shin *et al.* (2012) also reported the significant influences of *Glu-D1*, *Glu-A3* and *Glu-B3* alleles on variations in mixing time of Korean wheat cultivars. Liu *et al.* (2005) reported that the contributions of *Glu-D1* alleles to mixograph mixing time and mixing tolerance were significantly higher than those of *Glu-A3* and *Glu-B3* alleles in Chinese wheat genotypes. DH lines carrying *Pinb-D1b* allele exhibited higher water absorption of mixograph (65.4%) than those with *Pinb-D1a* allele (63.9%) (Table 5). Allelic variation in the *Glu-D1*, *Glu-A3* and *Glu-B3* loci exhibited no significant influence on water absorption of mixograph. Water absorption of mixograph positively correlated with damaged starch content in Korean wheat cultivars (Park *et al*., 2010). DH lines carrying *Pinb-D1b* allele probably had greater starch damage during milling than those with *Pinb-D1a* allele (Table 3), resulting in increased water absorption of dough (Table 5). DH lines carrying *Pinb-D1b* allele exhibited longer mixing tolerance of mixograph (13.3 mm) than those with *Pinb-D1a* allele (12.1 mm), which agrees with the previous report on Korean wheat cultivars by Shin *et al.* (2012). DH lines carrying *Glu-D1d* and *Glu-B3d* alleles exhibited longer mixing time and higher mixing tolerance than those with *Glu-D1f* and *Glu-B3h* alleles (Table 5). *Glu-A3* alleles showed no significant influence on mixing time and mixing tolerance of dough. DH lines carrying *Pinb-D1b* allele showed shorter mixing time and higher mixing tolerance (3.3 min and 12.1 mm, respectively) than those with *Pinb-D1a* allele (2.9 min and 13.3 mm, respectively). This result agrees with the report that Korean wheats carrying with *Glu-D1d* and *Glu-A3d* showed longer mixing time of mixograph than those with *Glu-D1f* and *Glu-A3c* (Shin *et al*., 2012). Yanaka *et al.* (2007) proposed that the more pronounced effects of the *Glu-D1d* allele on dough strength compared to *Glu-D1f* allele came from the higher capacity of the former to form larger-sized polymer than the latter. Liu *et al.* (2005) also observed the longer mixing time and higher mixing tolerance of *Glu-D1d*, *Glu-A3d* and *Glu-B3d* than other alleles in Chinese wheat cultivars. Maucher *et al*. (2009) also reported CIMMYT lines carrying with *Glu-B3d* and *Glu-D3d* showed longer mixing time of mixograph than those with other alleles at *Glu-B3* and *Glu-D3* loci, no significant differences in mixing time among *Glu-A3* alleles.

DH lines carrying all of *Glu-D1d*, *Glu-A3c*, *Glu-B3d* and *Pinb-D1a* exhibited higher mixing time of mixograph (5.0 min) than those with other alleles (Table 6). DH lines carrying *Glu-D1f* showed shorter mixograph mixing time (< 2.8 min) than those with *Glu-D1d* allele (> 3.5 min), regardless of allelic composition on *Glu-A3* and *Pinb-D1* loci. This result indicates that mixograph mixing time is largely determined by glutenin composition and little affected by puroindolines, and agrees with the report that dough mixing properties of flours are mainly controlled by quantity and quality of protein (Finney and Shogren, 1972). DH lines carrying all of *Glu-D1d*, *Glu-A3c*, *Glu-B3d* and *Pinb-D1b* alleles exhibited higher mixing tolerance (15.5 mm) than those with other alleles. DH lines carrying all of *Glu-D1f*, *Glu-A3c*, *Glu-B3h* and *Pinb-D1a* alleles exhibited lower mixing tolerance (10.3 mm) than those with other alleles. Regardless of *Glu-A3*, *Glu-B3* and *Pinb-D1* alleles, DH lines carrying *Glu-D1f* showed lower mixing tolerance of mixograph (< 13.0 mm) than those with other alleles, except DH lines carrying all of *Glu-D1d*, *Glu-A3c*, *Glu-B3h* and *Pinb-D1a* alleles (12.0 mm). These results indicate that dough stability during mixing mainly influenced by

the allelic variations on *Glu-D1* rather than *Glu-A3* and *Glu-B3* alleles. Liu *et al.* (2005) proposed that the effects of the glutenin loci on mixing tolerance of mixograph could be ranked as *Glu-D1* > *Glu-B1* = *Glu-B3* > *Glu-A3* > *Glu-A1* in Chinese wheats. Peña *et al*. (2004) proposed that the effects of the *Glu-B3* locus on gluten strength could be ranked as *Glu-B3d* > *Glu-B3b* = *Glu-D3f* = *Glu-B3g* > *Glu-B3i* = *Glu-D3h* in wheat genotypes carrying *Glu-D1d* allele.

Effects of allelic variation on bread loaf volume

Glu-D1, *Glu-B3* and *Pinb-D1* alleles were responsible for 17.9, 4.9 and 8.4% variation in loaf volume of bread, respectively (Table 2). Keumkang, contained *Glu-D1d*, *Glu-B3h* and *Pinb-D1b* alleles, showed higher bread loaf volume (888.3 mL) than Olgeuru, contained *Glu-D1f*, *Glu-B3d* and *Pinb-D1a* alleles (666.7 mL) (Table 3). DH lines carrying *Glu-D1d*, *Glu-A3d*, *Glu-B3d* and *Pinb-D1b* alleles exhibited higher bread loaf volume (831.1, 806.9, 813.7 and 818.2 mL, respectively) than those with *Glu-D1f*, *Glu-A3c*, *Glu-B3h* and *Pinb-D1a* alleles (765.3, 788.7, 779.2 and 773.0 mL, respectively) (Table 5). DH lines carrying all of *Glu-D1d*, *Glu-A3d*, *Glu-B3d* and *Pinb-D1b* alleles exhibited higher loaf volume of bread (905.7 mL) than those with other alleles (< 875.5mL, Table 6). DH lines carrying all of *Glu-D1f*, *Glu-A3c*, *Glu-B3h* and *Pinb-D1a* alleles exhibited lower bread volume (690.8 mL) than those with other alleles. Shewry *et al.* (1992) suggested that *Glu-A1a* or *b*, *Glu-B1b* or *i* and *Glu-D1d* were required for baking bread because these alleles have stronger influences on gluten strength than other alleles at the *Glu-1* loci. Zhang *et al*. (2012) reported that *Glu-A3e* and *Glu-B3c* represented inferior alleles for bread-making quality, whereas *Glu-A3d*, *Glu-B3b*, *Glu-B3g* and *Glu-B3i* were related to superior bread-making quality in 16 near-isogenic lines. Martin *et al.* (2001) reported that *Pinb-D1b* allele was more desirable for bread baking and could be used to select wheat lines with superior milling and bread baking quality within hard wheat class. Loaf volume of bread positively correlated with flour yield, physicochemical properties of flour and mixing properties of dough. Loaf volume of bread positively correlated with SDS-sedimentation volume and mixograph properties in Korean wheat cultivars and CIMMYT wheat lines (Park *et al*., 2010, 2012). High protein contents could be usually desirable for bread wheats because increased protein content is typically associated with higher loaf volumes and increased water absorption is desirable for hard wheat because loaf volume increase (Pomeranz, 1988). Graybosch *et al.* (1993) also reported that protein content was found to be the primary factor contributing to the variation in bread loaf volume in hard wheats.

Conclusion

Allelic composition of *Pinb-D1* loci dominantly flour yield, average particle size of flour and damaged starch content in 96 doubled haploid wheat lines developed from a cross between a hard white winter wheat variety and a soft red winter wheat variety. SDS-sedimentation volume was affected by the allelic composition of *Glu-A3* and *Pinb-D1* loci. *Glu-D1* loci predominantly affected mixing time and mixing tolerance of mixograph and bread loaf volume. *Glu-B3* and *Pinb-D1* alleles also have significant contribution to the variation in mixing properties and bread loaf volume. Among 14 types of allelic composition in 96 doubled haploid lines, DH lines carrying all of *Glu-D1d*, *Glu-A3d*, *Glu-B3d* and *Pinb-D1b* alleles exhibited higher loaf volume of bread than those combined with other alleles. These results indicate that bread loaf volume mainly influenced by the allelic variations on *Glu-D1* and *Pinb-D1* rather than *Glu-A3* and *Glu-B3* alleles. Therefore, glutenin and puroindoline compositions also need to be considered in quality improvement of bread.

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