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Effect of allelic variation in three glutenin loci on dough properties and bread-making qualities of winter wheat

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We investigated the relationships between allelic variations on three *Glu-* loci, *Glu-D1*, *Glu-A3* and *Glu-B3*, and processing qualities (dough strength and bread-making qualities) by using doubled haploid (DH) lines. The genotypic group of *Glu-D1d* had a longer mixing peak time (PT), a parameter of strong dough, than that of *Glu-D1a*. The group carrying *Glu-B3g* had a longer PT than that of the group carrying *Glu-B3b* when it was accompanied by *Glu-D1d*, and the group carrying *Glu-A3d* had a longer PT than that of the group carrying *Glu-A3f* when it was accompanied by *Glu-D1d* and *Glu-B3g*. Regarding the extent of the effects on dough strength, each of the two alleles on each of the three *Glu-* loci could be ranked as $d > a$ on *Glu-D1*, $d > f$ on *Glu-A3* and $g > b$ on *Glu-B3*. The wheat with compositions of *d-f-b* (allele on *Glu-D1*, *Glu-A3* and *Glu-B3*, respectively) had the highest specific loaf volume (SLV), and *d-d-g* had lower SLV than those of other three combinations carrying *Glu-D1d*, although the dough strengths (PTs) of the *d-f-b* group was secondarily high and that of the *d-d-g* group was very high. It is considered that the *d-d-g* combination group has excessively strong dough and that the poor contribution of *d-d-g* combination to loaf volumes may be due to the extra-strong dough properties.

Key Words: *Triticum aestivum*, glutenin gene, high-molecular-weight glutenin subunit (HMW-GS), low-molecular-weight glutenin subunit (LMW-GS), dough property, bread-making quality, doubled haploid line.

Introduction

Hard wheat with strong dough properties is preferable for bread-making and, thus, it is important to develop an efficient selection strategy for hard wheat with strong dough properties and good bread-making qualities. The protein content (Finny and Yamazaki 1946) and protein composition (Branlard and Dardevet 1985, Gupta *et al.* 1989, Gupta and Shepherd 1990, Payne *et al.* 1979, 1981, 1987) are the major determinants of strong dough and bread-making qualities of wheat. Most wheat protein corresponds to the 'gluten' protein consisting of glutenin and gliadin. Glutenin proteins, consisting of high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight glutenin subunits (LMW-GSs), are closely associated with dough strength and, accordingly, with bread-making qualities (Branlard *et al.* 2001). It has been reported that HMW-GSs are encoded by three loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, and that there are three alleles (*a*~*c*) on *Glu-A1*, eleven alleles (*a*~*k*) on *Glu-B1*, and six alleles (*a*~*f*) on *Glu-D1* (Payne and Lawrence 1983). LMW-GSs are encoded by three loci, *Glu-A3*, *Glu-B3* and *Glu-D3* and there are six alleles (*a*~*f*) on *Glu-A3*, nine alleles (*a*~*i*) on *Glu-B3*, and five alleles (*a*~*e*) on *Glu-D3* (Gupta and Shepherd 1990).

Many previous reports indicated that alleles at each locus have different effects on dough properties and bread-making qualities. The HMW-GS pair 5 + 10 encoded by *Glu-D1d* on the 1D chromosome contributes to strong dough and good bread-making qualities (Campbell *et al.* 1987, Cressy *et al.* 1987, Lagudah *et al.* 1987, Payne *et al.* 1981). HMW-GS 1 and 2* encoded by *Glu-A1a* and *Glu-A1b*, respectively, on the 1A chromosome and 7 + 8, 7 + 9 and 17 + 18 encoded by *Glu-B1b*, *Glu-B1c* and *Glu-B1i*, respectively, on the 1B chromosome also contribute to strong dough and good bread-making qualities (Campbell *et al.* 1987, Cressy *et al.* 1987, He *et al.* 2005, Lagudah *et al.* 1987, Lawrence *et al.* 1984, Moonen *et al.* 1982, Wrigley 2003).

The contribution of alleles encoding LMW-GSs to a

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strong dough quality also varies. Gupta *et al.* (1991) reported that LMW-GS alleles can be ranked with respect to their effect on maximum dough resistance (Rmax) as follows: *Glu-A3*, $b > d = e > c$; *Glu-B3*, $b = i >> g = h >> c$; and *Glu-D3*, $b = a >> c$. Eagles *et al.* (2002) reported that, at the *Glu-A3* locus, the null allele *e*: produced lower dough extensibility than did other alleles, which is in agreement with the findings of Gupta and MacRitchie (1994). They also reported that *Glu-B3b* produced a significantly higher Rmax than did *Glu-B3c* at the *Glu-B3* locus and that *Glu-B3j* produced low Rmax and extensibility values.

In a ranking of six *Glu-* loci with respect to dough properties, Gupta *et al.* (1994) reported that the loci could be ranked as *Glu-D1* > *Glu-B1* > *Glu-B3* > *Glu-A3* > *Glu-D3* = *Glu-A1* with respect to Rmax. Zhang *et al.* (2009) also reported that *Glu-D1*, together with *Glu-B3*, played the most important role in determining dough properties. It is suggested from the previous reports that *Glu-D1*, *Glu-B1*, *Glu-B3* and *Glu-A3* have great effects on strength of dough, while the analyzed alleles are limited in each report.

Interaction among loci, Eagles *et al.* (2002) showed that combinations of *Glu-B1* × *Glu-A3* and *Glu-B1* × *Glu-B3* showed particularly large effects on Rmax and extensibility. Interaction and additive effects between *Glu-1* and *Glu-3* alleles on dough properties have been previously shown (Eagles *et al.* 2002, Gupta *et al.* 1989, 1994, Khelifi and Branlard 1992, Nagamine *et al.* 2000, Tabiki *et al.* 2006). It is noteworthy that the cumulative effect of *Glu-D1d* and *Glu-B3b* on a strong dough quality is relatively large (Gupta *et al.* 1989). However, it is difficult to conclusively evaluate the contribution ranking of *Glu-* alleles, since most the materials used in previous studies have different genetic backgrounds.

In the present study, the relationships between allelic variations on *Glu-* loci and dough strength and between the compositions of alleles and bread-making qualities were investigated to accelerate the breeding of hard wheat varieties with good bread-making qualities. We focused on three loci, *Glu-D1*, *Glu-A3* and *Glu-B3*, which have been considered to play important roles in dough properties, and we used doubled haploid (DH) lines derived from a cross between two varieties carrying the same genotypes on the other loci to minimize the influence of difference in genetic background.

Materials and Methods

Plant materials

We used 252 doubled haploid (DH) lines derived from a cross between two winter wheat varieties, Kitami 81 (Kitahonami; soft wheat) and Kachikei 63 (Yumechikara; hard wheat). Both varieties are suitable for cultivation in Hokkaido, the northernmost island of Japan. These DH lines were produced by the wheat × maize method (Ushiyama *et al.* 2007). The seeds of DH lines were sown in late September 2006 in a research field at the National Agricultural Research Center for Hokkaido Region, Memuro, Hokkaido.

Table 1. Genotypes on all six glutenin loci in parents of DH lines

parents	HMW-GS			LMW-GS		
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Kachikei63	*a*	*b*	*d*	*f*	*b*	*a*
Kitami81	*a*	*b*	*a*	*d*	*g*	*a*

These lines were grown in an experimental plot consisting of 8-m rows with 72-cm widths under standard field management conditions. The parental varieties had allelic variation at three *Glu-* loci, *Glu-D1*, *Glu-A3* and *Glu-B3*, as shown in Table 1.

Flour quality tests

Wheat samples were milled with a Brabender Jr. test mill (Brabender Inc., Duisburg, Germany). The protein content was measured using a near-infrared reflectance instrument (Inframatic 8120, Percon Co., Hamburg, Germany). To evaluate dough properties, sodium dodecyl sulfate (SDS)-sedimentation volume (SMV) in a small-scale test was measured as described by Takata *et al.* (1999), and mixing peak time (PT) was measured using a 2 g-Mixograph (National Manufacturing Division of TMCO, Lincoln, NE, USA). The longer PT corresponds to stronger dough, as PT indicates strength (elasticity) of dough. SMV is an indicator of good bread-making quality, as it reflects difference in quality and quantity of gluten.

Glutenin preparation

The extraction solution (ES) consisted of 50% (v/v) 1-propanol and 0.08 M Tris-HCl (pH 8.0). Albumin, globulin and gliadin fractions were extracted from the ground flour (30 mg) in 600 µl of ES by incubating at 60°C for 30 min with gentle shaking (36 rpm). After a brief centrifugation (18,000 × g, 20°C), the supernatant was removed. This extraction was repeated three times. The glutenin fraction was extracted by resuspending the pellet in 150 µl of ES containing 60 mM dithiothreitol (DTT) at 65°C for 1 hr. After centrifugation, the supernatant (glutenin fractions) were alkylated by adding an equal volume of ES containing 1.4% vinylpyridine (v/v) to the supernatant. After incubation at 65°C for 30 min, 1.2 ml of acetone was added to the alkylated fractions. After centrifugation, the pellet was dried at 60°C.

Determination of Glu-1 and Glu-3 genotypes

The genotypes (alleles) of *Glu-A1*, *Glu-B1* and *Glu-D1* (HMW-GSs) in each variety were determined by performing SDS polyacrylamide gel electrophoresis (SDS-PAGE) and comparing the resolution patterns to those shown in the catalogue by Payne and Lawrence (1983). The glutenin pellet was dissolved in a 100 µl sample buffer containing 62.5 mM Tris (pH 6.8), 20% (v/v) glycerol, 2% (w/v) SDS and 0.002% (w/v) bromophenol blue (BPB). The glutenin solution was subjected to 12.5% SDS-PAGE. After

electrophoresis, the gel was stained using a coomassie brilliant blue (CBB) solution (0.25% (w/v) CBB, 45% (w/w) methanol, and 10% (w/w) acetic acid).

The genotypes of *Glu-A3*, *Glu-B3* and *Glu-D3 w*ere determined by performing SDS-PAGE and 2-dimensional (2D)-PAGE and by comparing the resolution patterns to that of each allele shown by Gupta and Shepherd (1990), Ikeda *et al.* (2006) and Jackson *et al.* (1996). The 2D-PAGE analysis was performed following the method described by Ikeda *et al.* (2006).

Bread-making tests

Of the 252 DH lines, the bread-making qualities of 144 hard-kernel DH lines were evaluated. The hard-kernel wheat lines were selected by using the Single Kernel Characterization System (SKCS) (SKCS4100, Perten Instruments). The hardness index values of all the lines were measured by using the SKCS and 144 lines with over 70 of value were selected as hard-kernel wheat. It has been checked that all the lines had *Pinb-D1d* associated with the hard endosperm by PCR as described by Ikeda *et al.* (2005). The bread-making tests were performed following the 'straight-dough method' modified from the Japan Yeast Industry Association method (1991) and the American Association of Cereal Chamists (AACC) method (Method 10-09, 1995). The ingredients (100 g flour, 5 g sucrose, 2 g salt, 5 g shortening, 2 g yeast, 30 ppm ascorbic acid solution, and an adequate volume of distilled water) were mixed in a '100 g Micro-Mixer' (National Manufacturing Division of TMCO, Lincoln, NE, USA). The previously analyzed 'peak time' in the 2 g-Mixograph was adopted as the mixing time in bread-making. After mixing, the dough was rounded and allowed to rest for 50 min in a fermentation cabinet at 30°C (first fermentation), then sheeted, rounded, and allowed to rest for 30 min (second fermentation). Then, dough was sheeted and allowed to rest for 15 min (bench time). The dough was panned and proofed at 38°C and 85% humidity for 55 min (final proof) and then baked at 200°C for 25 min. The bread was weighted after baking, and the loaf volume was measured by the rapeseed-replacement method after cooling at room temperature for 1 hr. The specific loaf volume (SLV) was obtained by dividing loaf volume (ml) by the bread weight (g).

Statistical analysis

Data were submitted to an analysis of variance (ANOVA) and an analysis of covariance (ANCOVA) to examine the effects of allelic variation with individual *Glu-* locus and the effects of interactions of alleles on three *Glu-* loci with using a software 'Excel-Toukei 2008' (Social Survey Research Information Co., Ltd.). SLV of each genotypic group was corrected by removing the effect of flour protein content. The corrected values were calculated from the following formula. Corrected SLV = SLV – a (FP – AFP) a: regression coefficient between SLV and flour protein content, FP: flour protein content, AFP: average of flour protein content.

Table 2. Numbers of DH lines in eight groups with different allelic combinations of three loci

Loci	genotype	number of lines	expected ratio	χ^2	P
D1-A3-B3	*a-d-b*	33	1 : 1 : 1 : 1 : 1 : 1 : 1 : 1	6.41	0.49
	a-d-g	25			
	a-f-b	30			
	a-f-g	28			
	d-d-b	42			
	d-d-g	29			
	d-f-b	29			
	d-f-g	36			

Results

Glutenin genotype compositions of DH lines

It was revealed that Kachikei 63 and Kitami 81, the parents of the DH lines had different genotypes on three *Glu-* loci, *Glu-D1*, *Glu-A3* and *Glu-B3* by SDS-PAGE and 2D-PAGE in this study (Table 1). Accordingly, these lines have eight allelic combinations of three loci, and the numbers of lines having each combination ranged from 25 to 42, as shown in Table 2. A χ^2 test showed that the numbers of DH lines with eight allelic combinations were similar (Table 2).

Relationships between dough strength and genotypes of Glu-D1, Glu-A3 and Glu-B3

The *F*-values of the flour qualities from ANOVA (analysis of variance) are shown in Table 3. There were not significant effects of allelic variation on flour protein contents except for interaction of *Glu-A3* × *Glu-B3*. The flour protein contents of eight genotypic combinations are shown in Fig. 1. The lines with a genotypic combination of *d-d-b* (the three characters indicating the alleles on three loci, *Glu-D1*, *Glu-A3* and *GluB3*, respectively) had higher protein contents, while the lines with a combination of *d-f-b* had lower protein contents.

The *F*-values of the PTs from ANOVA are shown in Table 3. There was highly significant effect of allelic variation on *Glu-D1* and the interaction of *Glu-D1* × *Glu-B3* . The effect of allelic variation on *Glu-B3* was also significant. The PTs of eight groups with different genotypic combinations are shown in Fig. 2. Four combinations carrying *Glu-D1d* (*d-d-b*, *d-d-g*, *d-f-b* and *d-f-g*), had longer PTs than those carrying *Glu-D1a* (*a-d-b*, *a-d-g*, *a-f-b* and *a-f-g*). The results showed that genotypic groups carrying *Glu-D1d* exerted stronger dough than those of *Glu-D1a*. In combinations carring *Glu-D1d*, two combinations carrying *Glu-B3g* (*d-d-g* and *d-f-g*), had longer PTs than those carrying *Glu-B3b* (*d-d-b* and *d-f-b*). On the other hand, four combinations carrying *Glu-D1a*, *a-d-b*, *a-d-g*, *a-f-b* and *a-f-g*, had similar PTs. This result indicated that the group carrying *Glu-B3g* had stronger dough properties than the group carrying *Glu-B3b* only when it was accompanied by *Glu-D1d*. The group with the '*d-d-g*' combination had the longest PT.

Table 3. *F*-values of flour qualities from ANOVA for alleles on three loci (n = 252)

Locus	d.f.	FP	PT	SMV
Glu-D1	1	0.73	59.95**	9.60**
Glu-A3	1	0.75	3.16	14.20**
Glu-B3	1	0.48	4.69*	1.55
Glu-D1 × *Glu-A3*	1	1.33	0.29	0.17
Glu-D1 × *Glu-B3*	1	1.28	10.66**	0.56
Glu-A3 × *Glu-B3*	1	3.99*	0.13	1.82
Glu-D1 × *Glu-A3* × *Glu-B3*	1	3.29	1.74	0.09

FP: flour protein content, PT: mixing peak time, SMV: SDS-sedimetation value
* $P < 0.05$, ** $P < 0.01$

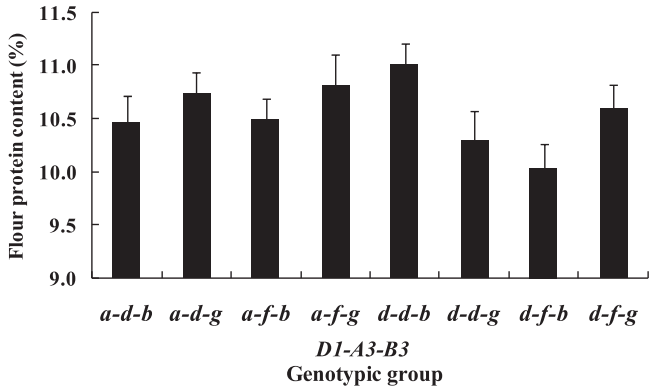

Fig. 1. Mean flour protein contents of the 252 DH lines grouped by genotypes of the three glutenin loci, *Glu-D1*, *Glu-A3* and *Glu-B3*. Bars indicate standard errors.

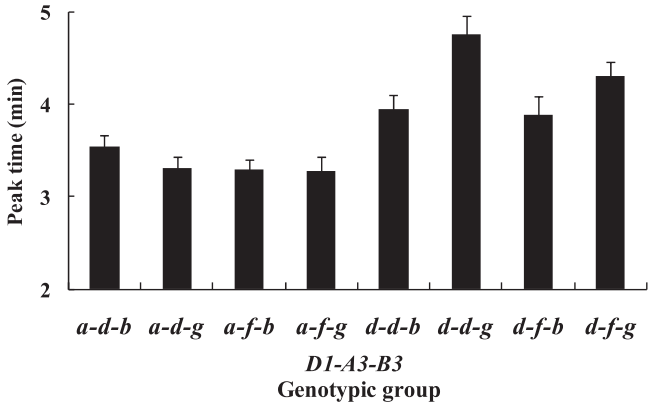

Fig. 2. Mean peak time of the 252 DH lines grouped by the genotypes of the three glutenin loci, *Glu-D1*, *Glu-A3* and *Glu-B3*. Bars indicate standard errors.

The *F*-values of the SMVs from ANOVA are shown in Table 3. There were significant effects of allelic variation on *Glu-D1* and *Glu-A3*. The SMVs of eight groups with different genotypic combinations are shown in Fig. 3. When the SMVs of two groups carrying different genotype on *Glu-D1* and same genotypes on *Glu-A3* and *Glu-B3* (for example *a-d-b* and *d-d-b*) are compared, the groups carrying *Glu-D1d* had higher SMVs than the groups carrying *Glu-D1a*. When

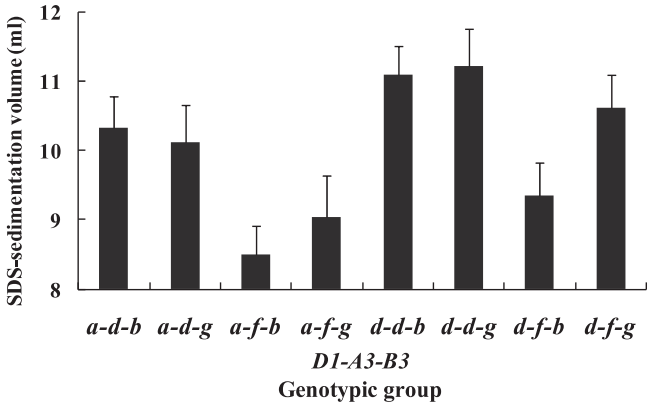

Fig. 3. Mean SDS-sedimentation volume of the 252 DH lines grouped by genotypes of the three glutenin loci, *Glu-D1*, *Glu-A3* and *Glu-B3*. Bars indicate standard errors.

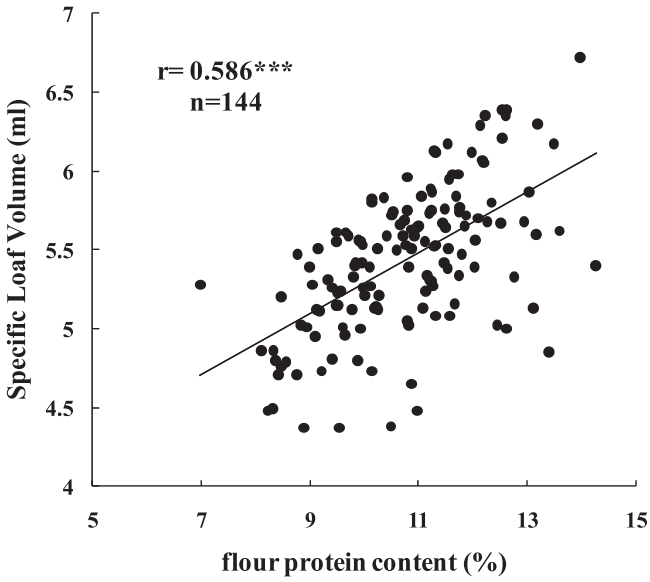

Fig. 4. Relationship between flour protein content and specific loaf volume in 144 DH lines. ***; significant at 0.01% level.

the SMVs of two groups carrying different genotype on *Glu-A3* and same genotype on *Glu-D1* and *Glu-B3* (for example *a-d-b* and *a-f-b*) are compared, the group carrying *Glu-A3d* had a higher SMV than group carrying *Glu-A3f*. The combinations of *d-d-b*, *d-d-g* and *d-f-g* showed higher SMVs, and the combinations of *a-f-b* and *a-f-g* showed lower SMVs.

Relationships between bread-making quality and genotypes of Glu-D1, Glu-A3 and Glu-B3

Of the 252 DH lines, the SLVs of 144 hard-kernel lines were measured to evaluate the bread-making qualities. The relationship between protein content and bread-making quality was analyzed by calculating the correlation coefficient between SLVs and protein content. SLV showed a high correlation with flour protein content (r = 0.586***) (Fig. 4). This result indicated that bread-making quality was greatly affected by flour protein content. Thus we analyzed lines

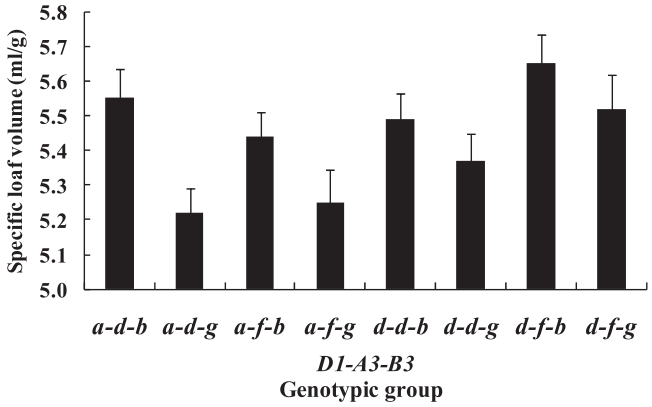

Fig. 5. Mean specific loaf volume removed effect of flour protein content in the 144 DH lines grouped by genotypes of the three glutenin loci, *Glu-D1*, *Glu-A3* and *Glu-B3*. Bars indicate standard errors.

Table 4. *F*-values of SLV from ANCOVA for alleles on three loci (n = 144)

Locus	d.f.	SLV
Glu-D1	1	5.27*
Glu-A3	1	1.04
Glu-B3	1	10.05**
flour protein content	1	79.68**
Glu-D1 × *Glu-A3*	1	2.46
Glu-D1 × *Glu-B3*	1	1.19
Glu-A3 × *Glu-B3*	1	0.27
Glu-D1 × *Glu-A3* × *Glu-B3*	1	0.39

SLV: specific loaf volume
* $P < 0.05$, ** $P < 0.01$

using flour protein content as a covariate by ANCOVA for removing effect of protein content. At first, we analyzed interactions between factors (allelic variation) and covariate (flour protein content) to test parallecity of regression on each genotypic group. The ANOVA showed that there were not significant interactions between factors and covariate. Therefore, the regression coefficients of each genotypic group were considered to be similar.

The *F*-values of the SLVs from ANCOVA using 144 DH lines are shown in Table 4. There were significant effects of allelic variation on *Glu-D1* and *Glu-B3*. On the other hand, there were not significant effects of allelic variation on *Glu-A3* and interactions of *Glu-D1* × *Glu-A3*, *Glu-D1* × *Glu-B3*, *Glu-A3* × *Glu-B3* and *Glu-D1* × *Glu-A3* × *Glu-B3*.

The corrected SLVs of eight groups with different genotypic combinations are shown in Fig. 5. When the SLVs of two groups carrying different genotype on *Glu-D1* and same genotypes on *Glu-A3* and *Glu-B3* are compared, the groups carrying *Glu-D1d* had higher SLVs than the groups carrying *Glu-D1a* except for *a-d-b* and *d-d-b*. When the SLVs of two groups carrying different genotype on *Glu-B3* and same genotypes on *Glu-D1* and *Glu-A3* (for example *a-d-b* and *a-d-g*) are compared, the groups carrying *Glu-B3b* had higher SLVs than the groups carrying *Glu-B3g*. The combinations of *d-f-b* had highest SLVs, and *a-d-g*, *a-f-g* and *d-d-g* had lower SLVs.

Discussion

Interaction and additive effects between *Glu-1* and *Glu-3* alleles on strong dough properties have been reported (Eagles *et al.* 2002, Gupta *et al.* 1989, 1994, Khelifi and Branlard 1992, Nagamine *et al.* 2000, Tabiki *et al.* 2006). In present study, the effect of *Glu-D1* alleles on PT was highly significant. The groups carrying *Glu-D1d* produced stronger dough (i.e., longer PT) than did groups carrying *Glu-D1a*. Therefore, we confirmed that *Glu-D1d* had a large effect on dough strength.

The effect of *Glu-B3* alleles on PT was significant, and the interaction of *Glu-D1* × *Glu-B3* was also highly significant. The group carrying *Glu-B3g* had stronger dough properties than the group carrying *Glu-B3b* only when it was accompanied by *Glu-D1d*.

Some previous reports ranked $b > g$ or $b = g$ on *Glu-B3* with respect to the effects on dough strength (Branlard *et al.* 2001, Gupta *et al.* 1991). The result in present study was inconsistent with these previous reports. The materials used in these studies had allelic variation on all *Glu-* loci. On the other hand, the materials used in present study had same alleles on *Glu-A1*, *Glu-B1* and *Glu-D3*. It was thought that the different genetic background of these materials caused inconsistency of results. Funatsuki *et al.* (2007) compared the effects of *Glu-B3b* and *Glu-B3g* on strong dough by measuring PTs of lines derived from a cross between two winter varieties, and showed that dough with *Glu-B3g* was stronger than that with *Glu-B3b*. The result was consistent with the result in present study.

On the other hand, the effect of *Glu-A3* alleles on PT was no significant, but in the genotypic combinations of *Glu-D1*, *Glu-A3* and *Glu-B3* loci, the group of *d-d-g* produced stronger dough than did the group of *d-f-g*, indicating that the group carrying *Glu-A3d* had stronger dough properties than the group carrying *Glu-A3f* when it was accompanied by *Glu-D1d* and *Glu-B3g*. Furthermore, the effect on of *Glu-A3* alleles on SMV was highly significant, and the genotypic group of *Glu-A3d* had a higher SMV than that of *Glu-A3f*. Regarding the extent of the effects on dough strength, each of the two genotypes on each of the three *Glu-* loci could be ranked as $d > a$ on *Glu-D1*, $d > f$ on *Glu-A3* and $g > b$ on *Glu-B3*.

Of the eight combinations on three loci, the *d-d-g* group had the highest PT and SMV. The PT value of the *d-d-g* group is much higher than those of commercial strong flour and strong flour from Japanese strong wheat varieties that we have been analyzing regularly by a 2 g-Mixograph. It is considered that the excessively strong dough with the *d-d-g* group is due to additive effect of the alleles contributing to strong dough. Funatsuki *et al.* (2007) suggested that wheat carrying a combination of *Glu-D1d* and *Glu-B3g* result in extra-strong wheat by using two segregating populations,

which is considered to be consistent with the dough property of *d-d-g* group in the present study.

According to many previous reports, *Glu-D1d* has a positive effect on loaf volume (Campbell *et al.* 1987, Cressey *et al.* 1987, Lagudah *et al.* 1987, Lawrence *et al.* 1984, Moonen *et al.* 1982). In our results using 144 DH lines with a wide range of flour protein contents, it was revealed that the genotypic group of *Glu-D1d* had better SLV than that of *Glu-D1a*. This result is in agreement with findings in previous studies (Campbell *et al.* 1987, Cressey *et al.* 1987, Lagudah *et al.* 1987, Lawrence *et al.* 1984, Moonen *et al.* 1982). In addition, the genotypic group of *Glu-B3b* had better SLV than that of *Glu-B3g*. The *Glu-B3g* contributed to increase in dough strength but not to improvement of SLV.

It is known that bread-making quality is greatly affected by flour protein content. In this study, there was a positive correlation between SLV and flour protein content. The result of ANCOVA, removing effect of flour protein content, indicated that the allelic variation on *Glu-D1* and *Glu-B3* alone could have significant effects on bread-making qualities.

Of the eight combinations on three loci, the wheat with compositions of *d-f-b* had the highest SLV and *d-d-g* had lower SLV than those of other three combinations carrying *Glu-D1d*, although the dough strengths (PTs) of the *d-f-b* groups was secondarily high and that of the *d-d-g* group was very high. Bushuk (1980) showed that the volume of a loaf made of 100% excessively strong flour of a CWES (Canadian Western Extra-Strong) wheat was lower than that of a loaf made of blended flour with weak flour. It is therefore considered that the *d-d-g* combination group has excessively strong dough similar to the extra-strong wheat. The poor contribution of *d-d-g* combination to loaf volume may therefore be due to the extra-strong dough properties.

The results suggest that wheat with compositions of *d-f-b* is useful for processing bread by using unblended flour and that wheat with the extra-strong composition of *d-d-g* is useful for processing bread by using blended flour with weak flour. On the other hand, fresh pasta made of common wheat flour needs to have a very elastic texture which the strong dough of wheat is correlated with. Wheat with *d-d-g* composition is considered to be useful for processing fresh-pasta, as the composition *d-d-g* exerted the very strong dough.

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