Difference in Combination between Glu-B1 and Glu-D1 Alleles in Bread-Making Quality Using Near-Isogenic Lines

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The difference of combination between Glu-B1 and Glu-D1 alleles in bread-making quality was investigated using the near-isogenic lines (NILs) of Glu-I alleles. We found that composition of the HMWG subunits has different effects on bread-making quality. The SDS-sedimentation volume, physical dough strength, and specific loaf volume of Harunoakebono (7+9/5+10) and NIL 17/18/5+10 were significantly higher than those of the other NILs. NIL 20/2.2+12 showed the least value among NILs. The results were a sticky dough and a poor-quality loaf. NIL 20/5+10 or NIL 20/2.2+12 was found to have a large negative effect compared to subunits 7+9/5+10 or NIL 7+9/2.2+12. The functional properties of NIL 20/2+12 were not significantly different from those of NIL 7+9/2+12. Subunit 20 had different effects on the functional properties when coupled with other subunits at Glu-D1 alleles.

Keywords: wheat, high-molecular-weight glutenin, near-isogenic line, bread-making quality

In an effort to become more agriculturally self-sufficient, Japan has increased its wheat production. At the same time, better wheat quality has been aggressively sought for better food processing, and bread-making quality has been improved in addition to the development of new cultivars for good Udon noodle quality.

High-molecular-weight glutenin (HMWG) subunits, which are encoded by the Glu-I loci on the long arms of group 1 chromosomes, play an important role in bread-making quality (Payne et al., 1980). HMWG subunits 5+10 coded by Glu-D1 had a greater positive effect on this quality than subunits 2+12 at the same locus (Payne et al., 1981, Moonen et al., 1982). HMWG subunits produced from three Glu-I loci affected the functional properties differently. Other flour components of low-molecular-weight glutenin (LMWG), gliadin, lipids, and pentosans also affected quality (Pogna et al., 1982, Gupta et al., 1989, Morrison et al., 1989, Roels et al., 1993). Near-isogenic lines (NILs) are useful in analyzing the effects of gluten protein because they have the same genetic background except for some specific characteristics (Payne et al., 1987, Lawrence et al., 1988, Wesley et al., 1999). The additive effects of a couple of HMWG subunits have been reported in inbred lines (Carrillo et al., 1990, Gupta et al., 1994).

We reported the quality of bread-making in eight kinds of near isogenic lines substituted by single HMWG subunits (2000). The HMWG subunits 2.2+12 is a major subunit at the Glu-D1 locus in Japan, and subunit 20 at the Glu-B1 locus has been observed in the leading Japanese cultivar, Hokushin. Subunits 2.2+12 and subunit 20 showed a negative effect on bread-making quality. The purpose of this study was to evaluate the differences in combination between Glu-B1 and Glu-D1 alleles on this quality using NILs. NILs were developed among HMWG subunits 17+18, 20, or 7+9 at the Glu-B1 locus and 5+10, 2.2+12, or 2+12 at the Glu-D1 locus.

Materials and Methods

Plant materials NILs substituted for single HMWG subunits were developed by more successive backcrossing from the lines presented in a previous report (Takata et al., 2000). The recurrent parent Harunoakebono (2*, 7+9, 5+10) was crossed with donor parents, Haruyutaka, Norin 61, and Chiakukomugi, whose HMWG subunits 17+18, 20, 2.2+12, and 2+12 were introduced to Harunoakebono (Table 1). The progenies were backcrossed eight times by Harunoakebono, and their HMWG subunits were confirmed by SDS-PAGE using 5% and 10% of separation gel. In B,F2 seeds, the homozygous genotype of NILs substituted by single HMWG subunits (20, 17+18, 2.2+12, 2+12) was checked by SDS-PAGE. NILs substituted by double HMWG subunits (20/2.2+12, 17+18/2.2+12, 20/2+12) were developed by crossing NILs substituted by single HMWG subunits at Glu-B1 and Glu-D1 (Table 2). Four kinds of NILs substituted for single HMWG subunits, three kinds of NILs substituted for double HMWG subunits, and Harunoakebono were cultivated with three replicates, except for NILs that were substituted by double HMWG subunits NIL 20/2+12, in which two replicates were obtained. This was done at the National Agriculture Research Center for the Hokkaido Region in 2001; rows in each 6 m plot were 72 cm apart and 10 g/m2 of nitrogen was applied to each plot.

Flour quality Samples of grain with a 16% moisture content were milled with a Brabender Quadrumat Jr. test mill (Brabender Inc., Duisburg, Germany). Flour protein content was...
measured by a near-infrared spectroscope with an Inframatic 8120 (PerCon Co., Hamburg, Germany). An SDS-sedimentation test was performed with 2.5 g of flour. A flour sample was soaked for 24 h in 100 ml of an SDS-lactic acid solution, and the sedimentation volume was then read at 40 min after being inverted ten times, as reported previously (Takata et al., 1999).

Physical dough property  
Flour-mixing properties were estimated by an improved 35 g Swanson head pin-type mixer (National Mfg., Lincoln, NE.) with a motor-head revolving speed of 110 rpm/min; the electric current was recorded during mixing. Water absorption was followed by the AACC mixograph method 54-40A. The peak time (min) was determined by the mixing time at maximum electric current, and the breakdown (A) represented the difference in the electric current between peak time and 2 min after peak time. The physical properties of the dough were measured by a Rheomer (model RE33005) (Yamaden Inc., Tokyo) according to the method of Yamauchi et al. (2001). Flour samples with a 2% NaCl solution were mixed up to peak, and three 10 g pieces of dough were formed into 7 cm×2.5 cm pieces after sheeting through 0.098-inch rolls twice. They were extended at a speed of 5 mm/s with a plunger (P-21) after bench time (30°C, 70% RH) for 60 min. The breaking force showed the force (N) to the breaking point of the dough, and the breaking deformation represented the deformation of the sample (mm) at the breaking point of the dough.

Baking test  
A baking test was performed by the no-time method (Takata et al., 2000): a 200 g flour sample was used for this test. The dough was divided into three 100 g and two 20 g pieces. Three loaves were baked, and CO₂ production from 20 g of dough was checked by Fermograph II (ATTO Co., Tokyo). An analysis of variance was carried out for the quality data, and significant characteristics of NILs at the 0.05 level were compared by the Tukey-Kramer test (p<0.05).

### Table 1. HMWG subunits composition of recurrent parent and donor parents.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>HMWG subunits</th>
<th>Glu-A1</th>
<th>Glu-B1</th>
<th>Glu-D1</th>
<th>Recurrent parent</th>
<th>Donor parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harunoakebono</td>
<td>2*</td>
<td>7+9</td>
<td>5+10</td>
<td></td>
<td>17+18</td>
<td></td>
</tr>
<tr>
<td>Haruyutaka</td>
<td>1</td>
<td>17+18</td>
<td>2+12</td>
<td></td>
<td>2*</td>
<td></td>
</tr>
<tr>
<td>Norin 61</td>
<td>2*</td>
<td>7+7+8</td>
<td>2+12</td>
<td></td>
<td>10+2*</td>
<td></td>
</tr>
<tr>
<td>Chihokukomugi</td>
<td>1</td>
<td></td>
<td>2+12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Underline indicates introduced HMWG subunits to Harunoakebono.

Table 2. HMWG subunits compositions, flour yield (FY), flour protein (FP) properties and SDS-sedimentation volume of Harunoakebono and near-isogenic lines (NILs) of Glu-1 alleles.

<table>
<thead>
<tr>
<th>NILs</th>
<th>HMWG subunits</th>
<th>Glu-A1</th>
<th>Glu-B1</th>
<th>Glu-D1</th>
<th>FY (%)</th>
<th>FP (%)</th>
<th>SDSS (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harunoakebono</td>
<td>2*</td>
<td>7+9</td>
<td>5+10</td>
<td></td>
<td>70.3 a</td>
<td>13.3 a</td>
<td>50.3 a</td>
</tr>
<tr>
<td>NIL 20/5+10</td>
<td>2*</td>
<td>20</td>
<td></td>
<td>5+10</td>
<td>70.7 a</td>
<td>13.0 a</td>
<td>34.8 bcd</td>
</tr>
<tr>
<td>NIL 17+18/5+10</td>
<td>2*</td>
<td>17+18</td>
<td>5+10</td>
<td></td>
<td>70.0 a</td>
<td>13.4 a</td>
<td>50.7 a</td>
</tr>
<tr>
<td>NIL 7+9/2.2+12</td>
<td>2*</td>
<td>7+7+9</td>
<td>2+12</td>
<td></td>
<td>70.0 a</td>
<td>13.4 a</td>
<td>29.3 cd</td>
</tr>
<tr>
<td>NIL 20/2.2+12</td>
<td>2*</td>
<td>20</td>
<td></td>
<td>2+12</td>
<td>70.2 a</td>
<td>13.0 a</td>
<td>10.8 e</td>
</tr>
<tr>
<td>NIL 17+18/2+12</td>
<td>2*</td>
<td>17+18</td>
<td>2+12</td>
<td></td>
<td>70.2 a</td>
<td>13.3 a</td>
<td>39.3 bc</td>
</tr>
<tr>
<td>NIL 7+9/2+12</td>
<td>2*</td>
<td>7+9</td>
<td>2+12</td>
<td></td>
<td>70.3 a</td>
<td>13.2 a</td>
<td>35.0 bcd</td>
</tr>
<tr>
<td>NIL 20/2+12</td>
<td>2*</td>
<td>20</td>
<td></td>
<td>2+12</td>
<td>69.5 a</td>
<td>13.3 a</td>
<td>29.0 cd</td>
</tr>
</tbody>
</table>

Underline shows substituted HMWG subunits.

Values shown by the same letter are not significantly different (p=0.05) by Tukey-Kramer test.

### Results

Flour properties  
HMWG subunits composition, flour yield, flour protein content, and SDS-sedimentation volume of NILs are presented in Table 2. Harunoakebono and NILs had the same electrophoretic pattern, except for the substituted HMWG subunits. The electrophoretic pattern of 10% gel is shown in Fig. 1. All lines had hard grains, and the flour yield ranged from 69.5 to 70.6%, which was not significantly different from that of Harunoakebono. The flour protein content ranged from 13.0 to 13.4% (13.5% moisture base), and no significant differences were found among NILs. It was suggested that the flour protein content did not affect the differences in physical properties of the dough among the NILs.

The SDS-sedimentation volume correlated highly with the insoluble protein content, loaf volume, and dough strength (Axford et al., 1979, Blackman & Gill 1980, Moonen et al., 1982). The SDS-sedimentation volume, dough strength property, and breaking force had a high correlation coefficient (r=0.956***) (Fig. 2). According to the SDS-sedimentation test, the NILs of the Glu-1 alleles were divided into three groups. The SDS-sedimentation volume of Harunoakebono (7+9/5+10) and NIL 17+18/5+10 was significantly higher than that of the other NILs. NIL 20/2.2+12 had the lowest volume and was significantly different with a sedimentation volume nearly one-fifth that of NIL 17+18/5+10, suggesting poor insoluble protein content. The sedimentation volume of the HMWG subunit 20 was less...
than subunits 7+9 and 17+18 at Glu-D1, but the effect differed in the subunits at Glu-D1. The sedimentation volume of NIL 20/5+10 was significantly different from those of NIL 7+9/5+10 and NIL 17+18/5+10. NIL 20/2.2+12 also had a significantly lower sedimentation volume than NIL 7+9/2.2+12 and NIL 17+18/2.2+12, while NIL 20/2+12 did not differ from NIL 7+9/2+12.

Physical properties of the dough. The mixing properties, physical properties of dough, and the baking test are presented in Table 3. The peak time of dough mixing of Harunoakebono (7+9/5+10) and NIL 17+18/5+10 was significantly longer than that of the other NILs as a result of the SDS-sedimentation volume. The long peak time indicates a strong dough property, which is an important characteristic for bread making. There were no significant differences among NILs 20/5+10, 17+18/2.2+12, 7+9/2+12, and 20/2+12, although NIL 7+9/2.2+12 had a significantly shorter peak time than the other three. HMWG 17+18 had a nearly equal peak time of subunits 7+9 with a background of subunits 5+10. However, subunits 17+18 had a longer peak time than subunits 7+9 with a background of subunits 2.2+12. NIL 20/2.2+12 showed a significantly shorter peak time than the other NILs: 1.4 min, considerably less than that of NIL 17+18/5+10 (3.4 min). The HMWG subunit 20 decreased the peak time much more significantly than subunits 7+9 with subunits 5+10 and 2.2+12 at Glu-D1. However, there was no significant difference between subunits 20 and 7+9 with subunits 2+12 at Glu-D1, nor was there any significant difference in the SDS-sedimentation volume.

The breakdown during dough mixing represents the mixing tolerance after dough development. The breakdown of NIL 17+18/5+10 was not significantly different from that of NIL 7+9/5+10 or NIL 20/5+10 but was significantly less than the lines with subunits 2.2+12 and 2+12 at Glu-D1. There were no significant differences in breakdown among NILs, except for NIL 17+18/5+10 and NIL 20/2.2+12. The breakdown of the latter, as expected, was significantly large, which meant that the dough had poor tolerance to mixing. The combination of the HMWG subunit 20 and subunits 2.2+12 had an extremely negative effect on the mixing properties.

The breaking force corresponds to the resistance of the Extensograph, which is responsible for loaf volume, and this force had a high correlation coefficient (r=0.912***). The specific loaf volume (SLV) and breaking force were not significantly different (p=0.05) by Tukey-Kramer test.
In the same way, the differences between subunits 5+10 and subunits 2+12 at Glu-D1 were 0.62 N (subunits 7+9) and 0.26 N (subunit 20). NIL 7+9/2+12 and NIL 7+9/2.2+12 had greater negative effects on the dough strength than Harunoakebono (subunits 7+9/5+10). NIL 20/2.2+12 had a large negative effect on the physical property of the dough, and NIL 20/5+10 had poor dough strength. However, the breaking force of NIL 20/2+12 was not significantly different from that of NIL 7+9/2+12. HMWG subunit 20 had different effects on the dough strength when coupled to subunits at Glu-D1.

The breaking deformation ranged from 96.5 to 141.2 mm, and there were no significant differences among NILs, except for Harunoakebono (7+9/5+10). The breaking deformation of Harunoakebono was significantly shorter than that of NILs 20/5+10, 7+9/2.2+12, 7+9/2+12, and 20/2+12. The latter had much poorer flour properties, however, the breaking deformation was no different from that of the others. As the dough strength was weaker, it stretched longer; in fact, the dough actually broke up during extension because of extremely weak dough strength.

**Baking test**

The mixing time of the bread dough was similar to the peak time (Table 3). The loaves from NILs are presented in Fig. 4. As CO₂ production from the bread dough was not significantly different among NILs (data not shown), it did not affect the loaf volume. The specific loaf volume of Harunoakebono and NIL 17+18/5+10 was significantly higher than that of the other NILs. The former showed good baking performance, and the bread dough was more handleable than the latter. NIL 20/2.2+12 had a significant negative effect and produced very poor loaves because of weak and sticky dough.

**Discussion**

The SDS-sedimentation volume correlated with the amount of total HMWG subunits and individual HMWG subunits. Some subunits were positively correlated, and the others were negatively correlated with the sedimentation volume (Seilmeier et al., 1991). Carrillo et al. (1990) reported that HMWG subunits had additives and epistatic effects on the SDS-sedimentation volume. HMWG subunits composition influenced the functional properties by modifying the glutenin polymer characteristics (Popineau et al., 1994). The change in glutenin polymer characteristics in combination with HMWG subunits was believed to be responsible for the differences in the SDS-sedimentation volume among NILs.

The results of our study indicated that the functional properties of NILs substituted for single HMWG subunits were similar to those observed in a previous study (Takata et al., 2000). HMWG subunits 17+18 were not significantly different from subunits 7+9 or 7+8 with a background of subunits 5+10 on bread-making quality, as found by Wesley et al. (1999). Subunits 17+18 had higher values of functional properties than subunits 7+9 with a background of subunits 2.2+12. Both HMWG subunits 17+18 and subunits 5+10 tended to have an equal effect on the functional properties in the null subunit lines (Lawrence et al., 1988). Subunits 17+18 might show a large positive effect in neg-
ative background such as null or subunits 2.2+12. The functional properties of NIL 20/5+10 and NIL 17+18/2.2+12 were not significantly different. It seemed that both subunit 20 and subunits 2.2+12 had a similar negative effect on the functional properties in these combinations. HMWG subunits 2.2+12 suppressed the amount of homoeologous subunits 1 at Glu-A1 and subunits 7+8 at Glu-B1 (Kolster et al., 1991). Insoluble protein of subunit 20 was lower than that of subunits 17+18, although there was no difference between these subunits in the total polymeric protein (Gupta et al., 1994). The structural properties of subunit 20 and subunits 2.2+12 have not been identified. Those subunits might be different in their structural domain such as in the number of cysteine residues of subunit 2 and subunit 5 (Shewry et al., 1992). Subunit 20 had different effects on the functional properties in three subunits at the Glu-D1 allele. Subunit 20 with a background of subunits 2+12 had a smaller negative effect than subunits 5+10 and subunits 2.2+12. This result differed from that of Gupta et al. (1994), which suggested an interaction between the HMWG subunits and low-molecular-weight glutenin subunits.

NIL 20/2.2+12 had extremely poor functional properties, as expected. However, 35% of 131 Japanese cultivars shared subunits 2.2+12, and there are no cultivars with both subunit 20 and subunits 2.2+12 (Nakamura et al., 1999). Both subunit 20 and subunits 2+12 have been observed in the cultivars Hokusin and Chihokukomugi, which are good for making noodles. The functional properties of NIL 20/2+12 were slightly inferior to those of NIL 7+9/2+12, though the difference was insignificant. That combination might relate to the quality of the noodles used for Udon. Protein quality such as HMWG subunits seemed to be associated with Udon although the starch property and protein quantity were recognized to be important for these noodles. We concluded that the combination of subunit 20 and subunits 2.2+12 produced a dough strength that was too poor to use for the Udon noodle. The quality ranking of NILs for bread-making in this study is described as follows: 7+9/5+10, 17+18/5+10>17+18/2.2+12, 7+9/2+12, 20/5+10≈20/2+12, 7+9/2+12>20/2.2+12. The first group was suitable for bread making, and 7+9/2.2+12, 20/2+12, or 7+9/2+12 of the second group was found to be suitable for making Udon noodles among Japanese cultivars. The last combination can be adapted for soft wheat with low protein content and weak dough properties. It is necessary to replace subunits 2+12 or 2.2+12 with subunits 5+10 at Glu-D1 to improve the bread-making quality of Japanese wheat, and to substitute subunit 20 by the other subunits with strong dough property at Glu-B1.

This study has researched the effects between Glu-B1 and Glu-D1 in bread-making quality. It has also been reported that there has been an interaction between the Glu-I and Glu-3 alleles (Khelifi & Branlard 1992, Gupta et al., 1994) and that some subunits at the Glu-3 locus had a strong effect on the functional properties (Wesley et al., 1999, Takata et al., 2001). We would like to go on to research relationships between Glu-I and Glu-3. Thorough understanding of the glutenin components and their relationship to quality will lead to improved wheat quality.

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