Identification of Specific Low-Molecular-Weight-Glutenin Subunits Related to Gluten Quality in Bread Wheats

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Abstract
We studied single seed descent lines derived from the Kitakeiharu 717 (KKS 717) / Grandin cross. The presence of a B subunit of KKS 717-specific low-molecular-weight-glutenin subunit (LMW-GS) significantly enhanced the gluten strength. Although the C subunit showing a difference in mobility between Grandin and KKS 717 did not affect the gluten strength, the presence of this KKS 717-specific C subunit significantly decreased the gluten index in the absence of the KKS 717-specific B subunit. Using a LMW-GS locus-specific PCR primer set, we demonstrated that the B subunit of KKS 717-specific LMW-GS was encoded by the Glu-A3 locus. The use of this PCR primer set could be suitable for breeding programs aimed at improving the gluten quality.

Discipline: Plant breeding
Additional key words: gluten index, PCR marker

Introduction

Wheat seed storage proteins are composed of 2 major fractions, gliadin and glutenin. Glutenin consists of high-molecular-weight (HMW) and low-molecular-weight (LMW) subunits. The HMW-glutenin subunits (HMW-GSs) are encoded by Glu-A1, Glu-B1, and Glu-D1 on the long arm of chromosomes 1A, 1B and 1D, respectively15. The LMW-glutenin subunits (LMW-GSs) are encoded by Glu-A3, Glu-B3, and Glu-D3 on the short arm of these chromosomes8. Glutenin subunits were also classified into A (HMW-GSs), B and C (LMW-GSs) subunits based on their mobility in SDS-PAGE analysis8. These glutenin subunits are polymerized by intermolecular disulfide bonds, which play a major role in the rheological properties of wheat flour doughs. It has been shown that allelic variations of HMW-GSs and LMW-GSs affect dough properties in various wheat cultivars6,7,10,14,16,17.

The role of individual LMW-GSs is, however, much less well characterized than that of HMW-GSs, because large numbers of the subunits display similar mobilities in SDS-PAGE analysis. Cloned sequences of the LMW-GS genes revealed that all of the predicted LMW-GS amino acid sequences contained 8 cysteine residues3,4, which are considered to be involved in the formation of intra- and inter-molecular disulfide bonds. In our previous study, we analyzed the composition of the LMW-GS genes comprehensively in a soft wheat cultivar Norin 61 and classified these genes into 12 groups based on their deduced amino acid sequence similarity9. In this study, we analyzed the relationships between the LMW-GS composition and gluten strength (gluten index) using single-seed descent lines derived from the Kitakeiharu 717 (KKS 717) / Grandin cross. Using a LMW-GS gene-specific PCR primer set, we also identified a specific LMW-GS locus and the corresponding gene related to gluten quality.
Materials and methods

1. Plant materials

Ninety five single-seed descent (SSD) F_3 lines which were developed from a cross between KKS 717 and Grandin were used in this study. These lines were grown in non-replicated widely spaced rows (1 m long with 60 cm spacing) at Kitami Agricultural Experiment Station in Hokkaido (northern Japan). Grandin is a Dark Northern Spring wheat cultivar with good breadmaking properties. KKS 717 was developed from crosses of Laura / Kitamiharu 48 // Kitamiharu 56 as a spring bread wheat line with good breadmaking properties at Kitami Agricultural Experiment Station. Data from these parental lines were expressed as means of 2 duplications. As for the HMW-GS composition, Grandin has 2 (Glu-A1), 7 + 9 (Glu-B1) and 5 + 10 (Glu-D1) subunits, whereas KKS 717 has 1 (Glu-A1), 17 + 18 (Glu-B1) and 5 + 10 (Glu-D1) subunits. Therefore, the SSD lines share the same Glu-D1 subunits (5 + 10), which exert beneficial effects on the gluten quality.

2. Determination of flour protein, wet gluten, dry gluten contents and gluten index

The wheat samples were milled using a Brabender Quadrumat Junior test mill and conditioned to a moisture content of 14% prior to milling. Flours used in this study have an extraction rate of 60% of total wheat ground. Flour protein content (%) was determined by near-infrared spectroscopy (Infra Analyzer 450, Bran+Luebbe Co., Ltd., Germany), according to the AACC Method^2. Wet gluten content (%), dry gluten content (%) and gluten index (%) were determined using the Glutomatic system (Perten Instruments Co., Ltd., Sweden), according to the AACC Method^1. The significance of the mean difference for these data was determined by variance analysis.

3. Electrophoresis of glutenin fractions

Preparation and electrophoresis of the glutenin fractions were carried out according to the method of Nagamine et al.^14. SDS-PAGE was carried out using a 15% acrylamide separation gel for 4 h at 30 mA.

4. DNA sample preparation and PCR analysis

Total DNA was prepared from embryos dissected from mature seeds by the potassium acetate method described by Dellaporta et al.^5, followed by phenol / chloroform extraction. PCR reactions were performed in a total volume of 25 µL containing 1.5 mM MgCl_2, 0.1 mM of each dNTP, 5 pmol of Glu13 (5‘-TTGGGGCTGT-GTTGTGATA-3’) and Glu22 (5‘-CGTCTTTGCCCTCCTCGCTC-3’) primer set^9 and 0.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems Co., Ltd. USA), × 1 PCR Gold buffer and about 50 ng of total DNA. Reactions were performed according to the following protocol: denaturation at 94°C for 3 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 90 s; and a final extension at 72°C for 5 min. PCR products (5 µL) were electrophoretically analyzed on 1% agarose gels in 1 × TAE buffer.

Fig. 1. SDS-PAGE pattern of glutenin fractions from KKS 717, Grandin and their progenies (SSD lines)

KKS 717-specific B subunits are denoted by an arrow. C subunits showing higher mobility in KKS 717 than in Grandin are denoted by an arrowhead.
**Results**

1. **SDS-PAGE analysis of glutenin proteins**
   
   We studied the LMW-GS composition of the SSD line derived from a cross between the KKS 717 wheat line and Grandin wheat cultivar by SDS-PAGE analysis. We detected one major B subunit of LMW-GS, which is unique to KKS 717 (indicated by an arrow in Fig. 1). KKS 717 also showed one major C subunit of LMW-GS with a higher mobility than that of Grandin (indicated by an arrowhead in Fig. 1). We detected the same variations in the LMW-GSs among the SSD lines. These LMW-GSs were transmitted to the SSD lines independently.

2. **Quality evaluation**

   We determined the protein, wet gluten, dry gluten contents and gluten index using the parents and their SSD lines, and analyzed the statistical correlation with the composition of HMW-GSs and LMW-GSs. In a comparison between the parents, no clear differences were found in these values (Table 1). Using the SSD lines, the differences in the HMW-GS alleles of *Glu-A1* and *Glu-B1* did not exert any significant effect on either the protein content, wet gluten content, dry gluten content or gluten index (Table 2). The presence of the KKS 717-specific B subunit of LMW-GSs significantly increased the gluten index, but did not affect the protein content, wet gluten content and dry gluten content (Table 3). The KKS 717-specific C subunit did not affect either the protein content, wet gluten content, dry gluten content or gluten index (Table 3). However, in the absence of the KKS 717-specific B subunit, the KKS 717-specific C subunit

<table>
<thead>
<tr>
<th>Locus (subunit)</th>
<th>Sample No.</th>
<th>Mean (%) ± s.d.</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein content</td>
<td>Wet gluten content</td>
<td>Dry gluten content</td>
<td>Gluten index</td>
</tr>
<tr>
<td>Glu-A1</td>
<td></td>
<td>15.8 ± 0.9</td>
<td>45.7 ± 3.0</td>
<td>17.0 ± 1.2</td>
<td>84.6 ± 7.2</td>
</tr>
<tr>
<td>KKS 717 (1)</td>
<td>38a</td>
<td>15.7 ± 0.7</td>
<td>44.8 ± 2.9</td>
<td>16.7 ± 1.2</td>
<td>85.9 ± 8.9</td>
</tr>
<tr>
<td>Grandin (2)</td>
<td>44</td>
<td>15.8 ± 0.8</td>
<td>45.5 ± 3.0</td>
<td>16.9 ± 1.2</td>
<td>84.4 ± 7.7</td>
</tr>
<tr>
<td>Glu-B1</td>
<td></td>
<td>15.6 ± 0.8</td>
<td>44.7 ± 2.8</td>
<td>16.7 ± 1.2</td>
<td>86.8 ± 8.6</td>
</tr>
<tr>
<td>KKS 717 (7+9)</td>
<td>51b</td>
<td>15.8 ± 0.8</td>
<td>45.5 ± 3.0</td>
<td>16.9 ± 1.2</td>
<td>84.4 ± 7.7</td>
</tr>
<tr>
<td>Grandin (17+18)</td>
<td>31</td>
<td>15.6 ± 0.8</td>
<td>44.7 ± 2.8</td>
<td>16.7 ± 1.2</td>
<td>86.8 ± 8.6</td>
</tr>
</tbody>
</table>

a, b): 37 and 50 samples were analyzed for the dry gluten content, respectively.

<table>
<thead>
<tr>
<th>LMW-GS</th>
<th>Sample No.</th>
<th>Mean (%) ± s.d.</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein content</td>
<td>Wet gluten content</td>
<td>Dry gluten content</td>
<td>Gluten index</td>
</tr>
<tr>
<td>B subunit</td>
<td></td>
<td>15.8 ± 0.9</td>
<td>45.1 ± 3.2</td>
<td>16.9 ± 1.3</td>
<td>87.1 ± 6.9**</td>
</tr>
<tr>
<td>KKS 717</td>
<td>45</td>
<td>15.8 ± 0.8</td>
<td>45.7 ± 2.9</td>
<td>16.9 ± 1.2</td>
<td>83.0 ± 8.4</td>
</tr>
<tr>
<td>Grandin</td>
<td>50a</td>
<td>15.8 ± 0.9</td>
<td>45.4 ± 3.4</td>
<td>16.8 ± 1.4</td>
<td>83.9 ± 8.5</td>
</tr>
<tr>
<td>C subunit</td>
<td></td>
<td>15.7 ± 0.8</td>
<td>45.4 ± 2.8</td>
<td>16.9 ± 1.1</td>
<td>85.7 ± 7.5</td>
</tr>
<tr>
<td>KKS 717</td>
<td>40</td>
<td>15.8 ± 0.9</td>
<td>45.4 ± 3.4</td>
<td>16.8 ± 1.4</td>
<td>83.9 ± 8.5</td>
</tr>
<tr>
<td>Grandin</td>
<td>55b</td>
<td>15.7 ± 0.8</td>
<td>45.4 ± 2.8</td>
<td>16.9 ± 1.1</td>
<td>85.7 ± 7.5</td>
</tr>
</tbody>
</table>

a, b): 49 and 54 samples were analyzed for the dry gluten content, respectively.

** Significant effect at P < 0.01.
significantly decreased the gluten index (Table 4).

3. PCR analysis of LMW-GS genes and identification of KKS 717 LMW-GS locus

To identify the gene encoding the KKS 717-specific LMW-GSs, we performed a PCR analysis with 10 LMW-GS locus-specific primer sets, which showed size variations among the wheat cultivars\(^9\) (Ikeda et al. unpublished data). Using one of the Glu-A3-specific primer set, size variation of the PCR products was detected between KKS 717 and Grandin and the lower mobility PCR product on agarose gel corresponded to the presence of the B subunit of KKS 717-specific LMW-GS among all the SSD lines (Fig. 2). These results suggested that the KKS 717-specific B subunit was encoded by the Glu-A3 locus.

Discussion

The effects of the allelic variation of HMW-GSs (Glu-A1 and Glu-B1) on the quality values were not significant in this study, partly because all the materials used contained a 5 + 10 subunit pair (Glu-D1), which was most effective in improving the gluten quality\(^8\). Although the differences in the gluten index values were not conspicuous, the presence of the B subunit of KKS 717-specific LMW-GS significantly increased the gluten strength (Table 3). The corresponding Glu-A3 allele of Grandin was assigned to the ‘e’ allele based on SDS-PAGE analysis, which was a null allele\(^8\). Payne et al.\(^17\) also reported that the lines with the ‘e’ allele at the Glu-A3 locus displayed a low breadmaking quality using Chinese Spring and a 1A chromosome substitution line. Their results which suggested that relative amounts of glutenin subunits may be involved in the differences in gluten quality are in agreement with our findings. The presence of Glu-A3 LMW-GS in KKS 717, which may be due to the higher glutenin / gliadin ratio, contributed to the increase of the gluten strength.

The Glu-A3 locus-specific PCR primer set detected the KKS 717-specific LMW-GS genes, which corresponded to the LMW-GS groups 11 and 12 based on the predicted amino acid sequence\(^9\). The amino acid sequences of these groups were characterized by the absence of cysteine residues in the N-terminal domain and the presence of all the 8 cysteine residues in the C-terminal conserved domain\(^9\). The presence of these groups of LMW-GSs may exert a different effect on gluten network formation and structure than other groups of

![Fig. 2. PCR analysis of Glu-A3-specific LMW-GS gene](image)

The specific PCR products are indicated by an arrow.

M: Molecular weight marker (φx174/HaeIII digest).

<table>
<thead>
<tr>
<th>LMW-GS</th>
<th>Sample No.</th>
<th>Protein content</th>
<th>Wet gluten content</th>
<th>Dry gluten content</th>
<th>Gluten index</th>
</tr>
</thead>
<tbody>
<tr>
<td>B subunit: KKS 717</td>
<td>C subunit: KKS 717</td>
<td>23</td>
<td>15.8 ± 0.8</td>
<td>44.8 ± 3.3</td>
<td>16.8 ± 1.3</td>
</tr>
<tr>
<td>B subunit: Grandin</td>
<td>C subunit: KKS 717</td>
<td>22</td>
<td>15.9 ± 0.9</td>
<td>45.4 ± 3.1</td>
<td>17.0 ± 1.3</td>
</tr>
<tr>
<td>B subunit: Grandin</td>
<td>C subunit: KKS 717</td>
<td>17</td>
<td>15.8 ± 1.1</td>
<td>46.3 ± 3.5</td>
<td>17.0 ± 1.5</td>
</tr>
<tr>
<td>B subunit: Grandin</td>
<td>C subunit: KKS 717</td>
<td>33(^a)</td>
<td>15.7 ± 0.7</td>
<td>45.4 ± 2.6</td>
<td>16.8 ± 1.0</td>
</tr>
</tbody>
</table>

\(^a\): 32 samples were analyzed for the dry gluten content.
** Significant effect at \(P < 0.01\).
LMW-GSs. Further analysis should be carried out to determine the role of these LMW-GS groups. The PCR products amplified with the Grandin-specific Glu-A3 LMW-GS gene corresponded to a pseudogene, since this locus of Grandin did not express any LMW-GSs.

The effect of the C subunit on the gluten strength was significant only in the combination of the Grandin decrease of the gluten strength (Table 4). The C subunit of KKS 717 exerted a distinct effect on the gluten strength. It could be a possible that an unidentified locus of Grandin did not express any LMW-GSs.

The effect of the C subunit on the gluten strength was significant only in the combination of the Grandin Glu-A3 (null) locus, suggesting that the B subunit of KKS 717 Glu-A3 exerted a more pronounced effect on the gluten strength than the C subunit. However, in combination with the B subunit (null) of Grandin Glu-A3, the C subunit of KKS 717 exerted a distinct effect on the decrease of the gluten strength (Table 4). Since the C subunit of KKS 717 and Grandin displayed an almost equal amount of bands in SDS-PAGE analysis (Fig. 1), structural differences in the C subunit between them might be involved in the difference in gluten strength. In this study, we could not identify the locus of the C subunit. It could be a γ-gliadin type subunit, because previous studies showed that a major C subunit of Norin 61 was encoded by Glu-D3 containing a γ-gliadin-like N-terminal amino acid sequence\(^9\). Other studies\(^{11,12}\) revealed that most of the LMW-GSs in the C subunits shared a similarity of amino acid sequences to those of α- and γ-gliadins that carry a single additional cysteine residue involved in the formation of an intermolecular disulfide bond. Therefore, this LMW-GS could be encoded by the Gli-D1 locus tightly linked to Glu-D3 locus. It is also possible that an unidentified Glu-D3 LMW-GS affects the gluten strength. Further studies should be carried out to identify the LMW-GSs involved in the gluten strength.

Based on this analysis, we identified 2 LMW-GSs associated with the gluten strength in the presence of the HMW-GS 5 + 10 pair. The use of PCR analysis to identify the KKS 717-specific B subunit may enable to accelerate breeding programs aimed at improving the gluten strength.

References


