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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, respectively[15]. The LMW-glutenin subunits (LMW-GSs) are encoded by *Glu-A3*, *Glu-B3*, and *Glu-D3* on the short arm of these chromosomes[8]. Glutenin subunits were also classified into A (HMW-GSs), B and C (LMW-GSs) subunits based on their mobility in SDS-PAGE analysis[8]. 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 cultivars[6,7,10,14,16,17]. 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 residues[3,4], 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 similarity[9]. 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.

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Materials and methods

1. Plant materials

Ninety five single-seed descent (SSD) F_5 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-TGTTGCTGATA-3') and Glu22 (5'-CGTCTTTGC-CCTCCTCGCTC-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.

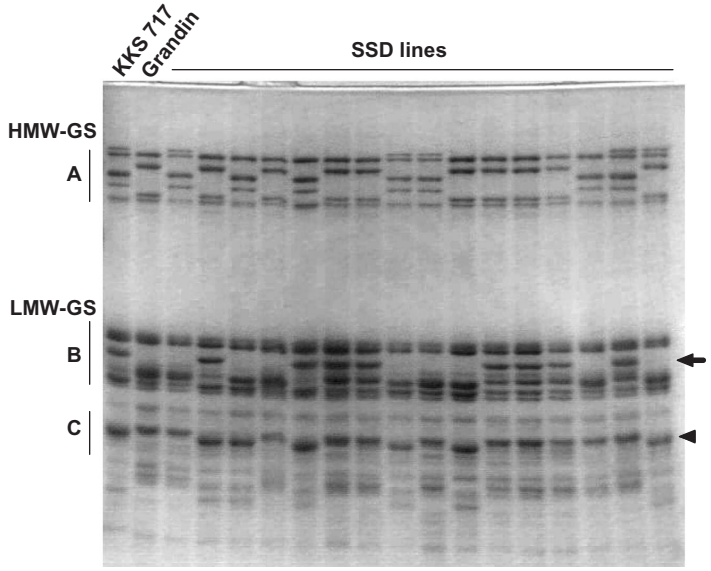

Fig. 1. SDS-PAGE pattern of glutenin fractions from KKS 717, Grandin and their progenies (SSD lines)
KKS 717-specific B subunits are denoted by an arrow. C subunits showing higher mobility in KKS 717 than in Grandin are denoted by an arrowhead.

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 1. Comparison of quality values (%) between parents[a)]

Parents	Protein content	Wet gluten content	Dry gluten content	Gluten index
KKS 717	15.7	45.8	16.1	83.1
Grandin	16.3	47.6	17.3	83.3

a): These values are the means of 2 measurements.

Table 2. Effect of HMW-GS subunit on quality values

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

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

Table 3. Effect of KKS 717-specific B and C subunits on quality values

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

a, b): 49 and 54 samples were analyzed for the dry gluten content, respectively.
** Significant effect at $P < 0.01$.

Table 4. Effect of a specific C subunit in different B subunit combinations

LMW-GS		Sample No.	Mean (%) ± s.d.			
			Protein content	Wet gluten content	Dry gluten content	Gluten index
B subunit:	KKS 717					
C subunit:	KKS 717	23	15.8 ± 0.8	44.8 ± 3.3	16.8 ± 1.3	87.6 ± 7.4
	Grandin	22	15.9 ± 0.9	45.4 ± 3.1	17.0 ± 1.3	86.7 ± 6.4
B subunit:	Grandin					
C subunit:	KKS 717	17	15.8 ± 1.1	46.3 ± 3.5	17.0 ± 1.5	79.0 ± 7.4**
	Grandin	33[a)]	15.7 ± 0.7	45.4 ± 2.6	16.8 ± 1.0	85.1 ± 8.1

a): 32 samples were analyzed for the dry gluten content.
** Significant effect at $P < 0.01$.

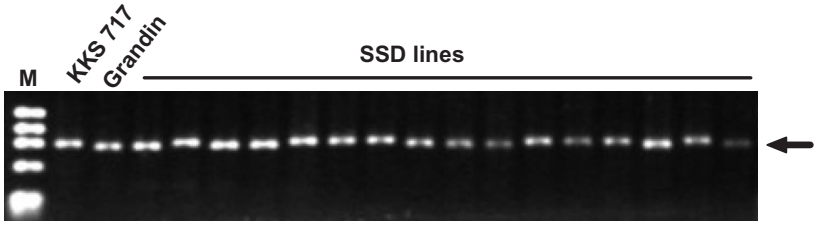

Fig. 2. PCR analysis of *Glu-A3*-specific LMW-GS gene
The specific PCR products are indicated by an arrow.
M: Molecular weight marker (ϕx174/*Hae*III digest).

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 qualiy[16]. 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

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[8].

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[13]. 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

1. American Association of Cereal Chemists (2000) Approved methods of the AACC, 10th ed. Method 38–12A Wet gluten, dry gluten, water-binding capacity, and gluten index. The American Association of Cereal Chemists, Inc., St. Paul, MN, USA.
2. American Association of Cereal Chemists (2000) Approved methods of the AACC, 10th ed. Method 39–11 Near-infrared reflectance method for protein determination in wheat flour. The American Association of Cereal Chemists, Inc., St. Paul, MN, USA.
3. Cloutier, S. et al. (2001) Cloning and expression of a LMW-i glutenin gene. *J. Cereal Sci.*, **33**, 143–154.
4. D'Ovidio, R. et al. (1999) Sequence similarity between allelic *Glu-B3* genes related to quality properties of durum wheat. *Theor. Appl. Genet.,* **98**, 455–461.
5. Dellaporta, S. L., Wood, J. & Hicks, J. B. (1983) A plant DNA minipreparation: Version II. *Plant Mol. Biol. Rep.,* **1**, 19–21.
6. Gupta, R. B., Bekes, F. & Wrigley, C. W. (1991) Prediction of physical dough properties from glutenin subunit composition in bread wheats. *Cereal Chem.,* **68**, 328–333.
7. Gupta, R. B. et al. (1994) Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3* and *Gli-1*, of common wheat. I. Its additive and interaction effects on dough properties. *J. Cereal Sci.,* **19**, 9–17.
8. Gupta, R. B. & Shepherd, K. W. (1990) Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutelin. 1. Variation and genetic control of the subunits in hexaploid wheats. *Theor. Appl. Genet.,* **80**, 65–74.
9. Ikeda, T. M. et al. (2002) Characterization of new low-molecular-weight glutenin subunit genes in wheat. *Theor. Appl. Genet.,* **104**, 680–687.
10. Khelifi, D. & Branlard, G. (1992) The effects of HMW and LMW subunits of glutenin and of gliadins on the technological quality of progeny from four crosses between poor breadmaking quality and strong wheat cultivars. *J. Cereal Sci.,* **16**, 195–209.
11. Lew, E. J.-L., Kuzmicky, D. D. & Kasarda, D. D. (1992) Characterization of low molecular weight glutenin subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-terminal amino acid sequencing. *Cereal Chem.,* **69**, 508–515.
12. Masci, S. et al. (2002) Characterisation and chromosomal localisation of C-type low-molecular-weight glutenin subunits in the bread wheat cultivar Chinese Spring. *Theor. Appl. Genet.,* **104**, 422–428.
13. Nagamine, T. et al. (2000) Effects of HMW and LMW glutenin subunit genotypes on rheological properties in Japanese wheat. *In* 7th International Workshop Gluten 2000. eds. Shewry, P. R. & Tatham, A. S., The Royal Society of Chemistry, The University of Bristol, UK, 404–407.
14. Nagamine, T. et al. (2000) Allelic variations of glutenin subunit loci *Glu-1* and *Glu-3* in southern Japanese wheat and their effects on dough and gluten properties. *Cereal Sci.,* **32**, 123–135.
15. Payne, P. I., Law, C. N. & Mudd, E. E. (1980) Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. *Theor. Appl. Genet.,* **58**, 113–120.
16. Payne, P. I. et al. (1987) The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J. Sci. Food Agric.,* **40**, 51–65.
17. Payne, P. I. et al. (1987) Allelic variation of glutenin subunits and gliadins and its effect on breadmaking quality in wheat: Analysis of F5 progeny from Chinese Spring × Chinese Spring (Hope 1A). *J. Cereal Sci.,* **6**, 103–118.