Improvement of dough strength for bread-making quality in Japanese common wheat

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ABSTRACT

Many cultivars in Europe and America showing good bread-making characters contain an allele of the high-molecular-weight glutenin subunit, *Glu-D1d*, located on chromosome 1D. We introduced this allele into four Japanese leading cultivars with low bread-making quality by the recurrent backcrossing method and produced near isogenic lines. The dough of these lines with *Glu-D1d* was stronger than the recipient cultivars. However, the strength did not reach the level of the donor cultivar, 'Haruhikari', with a good bread-making quality. To reveal the other factors of 'Haruhikari' on dough strength, we analyzed the effect of low-molecular-weight glutenin subunit (LMW-GS) using locus-specific primers of LMW-GS genes and gliadin bands tightly linked to LMW-GS genes. Segregation analysis of the F_2 between 'Haruhikari' and 'Asakaze-komugi' with a poor bread-making quality revealed that an amplified LMW-GS gene involved in the multigenes on *Glu-B3* of 'Haruhikari' had the significant effect on dough strength. The gene of 'Haruhikari', comparing to 'Asakaze-komugi', had a seven amino-acid deletion in the repetitive domain and three amino-acid substitutions, changing the hydrophilicity. The presence of the gene and the other genes on *Glu-B3* tightly linked with it must affect the dough strength.

INTRODUCTION

Wheat flour contains two major proteins, glutenin and gliadin. Glutenins are further classified into high-molecular-weight glutenin subunit (HMW-GS) and low-molecular-weight glutenin subunit (LMW-GS). HMW- and LMW-GSs are held together by disulphide linkages, whereas gliadins are monomeric proteins. The elasticity of wheat dough depends mainly on these proteins, so they are important determinants of bread-making quality (Payne *et al*., 1979, 1981).

Comparing many cultivars in Europe and America, Payne *et al*. (1987a) observed that most cultivars showing good bread-making characteristics contained an allele of the HMW-GS, *Glu-D1d*, on chromosome 1D. Payne *et al*. (1987b) also suggested that *Gli-1*/*Glu-3* alleles are also related to dough quality and that LMW-GSs may be the significant contributor, rather than gliadins, because the texture and volumes of the loaves baked from grains from the four storage-protein genotypes reflected exactly the SDS sedimentation values for aggregates of glutenin.

In this study, the *Glu-D1d* allele was introduced into four leading Japanese cultivars that exhibit low bread-making quality, and an analysis was made of how much the introduced gene improved the quality and quantity of the proteins. We also show evidence that LMW-GSs significantly affect dough strength, and suggest the possibility that alleles of gliadin (*Gli-1* loci) tightly linked to the LMW-GSs (the *Glu-3* locus) can serve as genetic markers for selection of particular *Glu-3* alleles.

MATERIALS AND METHODS

Common wheat cultivar 'Haruhikari' carries the *Glu-D1d* gene for HMW-GS. The bread-making quality of 'Haruhikari' is exceptional among Japanese cultivars because it was bred by crosses with good-quality cultivars from United States. The *Glu-D1d* allele was introduced by recurrent backcrossing into four cultivars grown in different regions of Japan; 'Asakaze-komugi' and 'Norin 61' (cultivars in the western Kanto district), 'Ushio-komugi' and 'Shirasagi-komugi' (cultivars in the coastal area of Setonaikai Sea). These recipient cultivars originally carried the *Glu-D1f* allele. After backcrossing 10 times, plants heterozygous for *Glu-D1d* were self-pollinated, and homozygotes for *Glu-D1d* were selected for near-isogenic lines (NILs) in the next generation. To identify the chromosome locations of LMW-GS genes concerned, monosomics of 'Chinese Spring' for chromosomes 1A, 1B and 1D (Sears 1954) were crossed with 'Norin 26' carrying the same ω-gliadin as 'Asakaze-komugi'. The monosomic F_1 plants were selected by observing the chromosomes. Self-pollinated progeny were used in the analysis. These cultivars and monosomic lines were maintained at Tottori University as part of the National Bioresource Project-Wheat.

The compositions of HMW-GSs and gliadins were determined by SDS-PAGE and A-PAGE, respectively, according to the procedure of Tanaka *et al*. (2003). LMW-GS alleles were analysed by PCR (polymerase chain reaction) with locus-specific primers developed by Van Campenhout *et al*. (1995) for LMW-GS genes on each of the group 1 chromosomes of hexaploid wheat. The DNA sequences of the amplified LMW-GS genes were determined by the procedure of Tanaka *et al*. (2005).

To identify the effect of HMW-GS from *Glu-D1d* allele and LMW-GSs on dough strength, NILs' seeds and bulked F_3 seeds from crosses between 'Haruhikari' and 'Asakazekomugi' were harvested. The protein content was measured by the method of Zaman and Verwilghen (1979). The SDS sedimentation value,

which is highly correlated with dough strength, was measured on a small scale according to the method of Takata *et al*. (1999).

RESULTS

The SDS sedimentation values of the NILs for *Glu-D1d* were much higher than those of the corresponding recurrent parents, but did not reach the level of 'Haruhikari' (Table 1). All four recipient cultivars and the NILs with the *Glu-D1d* gene showed similar amounts of proteins.

Table 1. SDS sedimentation value (SDS-SV) and protein content (PC) in the NILs with the *Glu-D1d* gene

Cultivar or line	SDS-SV (ml)	PC (%)
Haruhikari	9.6 f	13.3 d
Asakaze-komugi	4.5 a	8.9 ab
Asakaze-komugi with *Glu-D1d*	6.4 cd	9.7 c
Ushio-komugi	4.4 a	9.4 bc
Ushio-komugi with *Glu-D1d*	6.8 d	9.0 ab
Shirasagi-komugi	4.1 a	8.8 a
Shirasagi-komugi with *Glu-D1d*	6.1 c	9.2 abc
Norin 61	5.4 b	9.3 abc
Norin 61 with *Glu-D1d*	7.3 e	9.7 c

The same alphabets after values indicate no significant difference at the 5% level by the new multiple range test (Duncan, 1955).

With the *Glu-B3* specific primer set, the amplified fragment from 'Haruhikari' was shorter than that from 'Asakaze-komugi' and 'Chinese Spring' (Figure 1).

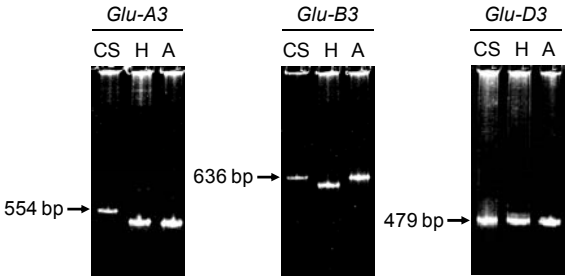

Figure 1. Polymorphisms of PCR amplicons in *Glu-3* locus on chromosome 1B. CS: 'Chinese Spring', H: 'Haruhikari', A: 'Asakazekomugi'. Chromosome specific primers of *Glu-3* loci were used (Van Campenhout *et al*., 1995).

Therefore, this primer set was used for identifying the types of LMW-GS genes in the F_2 segregating population between 'Haruhikari' and 'Asakaze-komugi'.

To identify polymorphisms between 'Haruhikari' and 'Asakaze-komugi' on *Glu-A3* and *Glu-D3* loci that were not detected by the PCR method, we analysed polymorphic bands of ω-gliadin tightly linked to the LMW-GS locus, *Glu-3* (data not shown). Three bands, 3, 4 and 5 showed polymorphisms between 'Haruhikari' and 'Asakaze-komugi'. Bands 3, 4 and 5 could were associated, and are hereinafter referred to as bands 3-4-5. To identify the chromosomal location of bands 3-4-5, we carried out monosomic analysis by A-PAGE. All of the 20 self-pollinated plants of monosomic F_1 hybrids between monosomic 1A of 'Chinese Spring' and 'Norin 26' carried bands 3-4-5. This result showed that the monosomic F_2 ratio was significantly different from a 3:1 ratio, and indicated that the gene(s) was on chromosome 1A. Therefore, bands 3-4-5 of ω-gliadin could be used to identify the type of LMW-GS gene on chromosome 1A in the F_2 segregating population between 'Haruhikari' and 'Asakaze-komugi'.

The protein content is highly correlated with the SDS sedimentation value (Moonen *et al*., 1982). Therefore, to evaluate the qualitative effect of LMW-GSs on dough strength, SDS sedimentation values were substituted for the protein contents in this study. The genotype of the corresponding F_2's indicated that the allele of LMW-GS on chromosome 1B in 'Haruhikari', significantly affected the SDS sedimentation value. However, the LMW-GS allele on chromosome 1A had no effect on the value (Table 2).

Table 2. Evaluation of dough strength for the types of LMW-GS genes and gliadin bands in an F_2 population of 'Haruhikari' × 'Asakaze-komugi'

Chromosomal location	LMW-GS alleles	Gliadin bands	Number of plants	SDS sedimentation value (ml)
1A		Presence of band 3-4-5	52	4.40 a
		Absence of band 3-4-5	15	4.58 a
1B	HH		17	4.64 a
	HA		41	4.38 a
	AA		9	3.89 b

HH: homozygous type of LMW-GS genes from 'Haruhikari'; HA: heterozygous type of LMW-GS genes from 'Haruhikari' and 'Asakaze-komugi'; AA: homozygous type of LMW-GS genes from 'Asakaze-komugi'.

The same alphabets after values indicate no significant difference at the 5% level by the new multiple range test (Duncan, 1955).

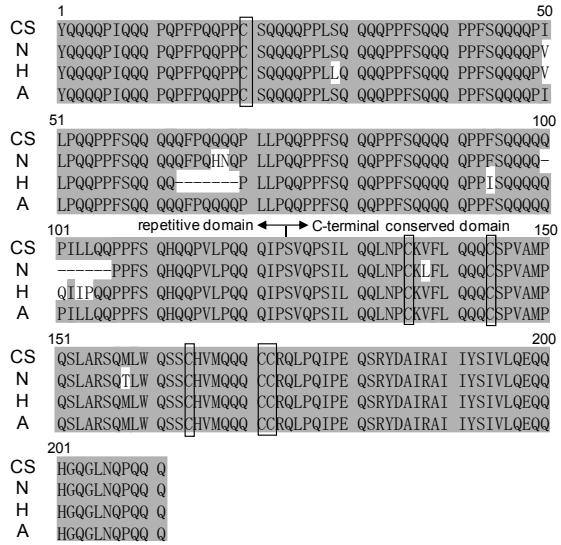

Figure 2. Comparison of the deduced amino-acid sequences of the LMW-GS genes amplified by PCR. Identical residues are shaded. Cysteines are boxed. CS: 'Chinese Spring', N: 'Norin 61', H: 'Haruhikari' and A: 'Asakaze-komugi'. The new sequences of

'Haruhikari' and 'Asakaze-komugi' have been deposited in DDBJ under Accession Nos. AB178884 and AB178885, respectively.

Figure 2 shows the deduced amino-acid sequences of the *Glu-B3* genes from 'Chinese Spring', 'Norin 61', 'Haruhikari' and 'Asakaze-komugi'. All of them contained six cysteine residues, which are conserved among all of the published LMW-GS sequences. The relative positions of the cysteines were also conserved. However, between 'Haruhikari' and 'Asakaze-komugi', we found six amino-acid substitutions (S29L, I50V, F94I, P101Q, L103I and L104P) and one deletion of 7 aminoacids (from position 63 to position 69 in the repetitive domain) in the amplified *Glu-B3* genes.

DISCUSSION

The present study using NILs clearly indicated that an allele of glutenin, *Glu-D1d*, is a major determinant of SDS sedimentation value. However, it also suggested that the other factors must take part in this character, one of which is the quantity of protein. If the level of protein content in the NILs were to equal that of 'Haruhikari', the SDS sedimentation value might be comparable with that of 'Haruhikari'. However, these values did not reach that level, even if they were corrected for protein content.

To increase this level, we focused on other seed storage proteins, such as LMW-GS that must be associated with the SDS sedimentation value as suggested by Payne *et al*. (1987b) and Pogna *et al*. (1988) in relation to pasta- and bread-making quality. We found a length polymorphism in the amplified LMW-GS genes on chromosome 1B (*Glu-B3*) between 'Haruhikari' and 'Asakaze-komugi' by PCR. The SDS sedimentation value associated with protein quality determines the extent of protein gel formation in solutions of SDS and lactic acid, which is a function of glutenin, not gliadin (Moonen *et al*., 1982). Therefore, this value would implicate the function of glutenin. Consequently, the presence of the allele of *Glu-B3* in 'Haruhikari' must have affected the dough strength.

This allele of 'Haruhikari' had many deletions and substitutions compared to that of 'Asakaze-komugi'. Deletions in the repetitive domain have often been found in LMW-GS sequences (Van Campenhout *et al*., 1995). The repetitive domain has a beta spiral structure, which consists of consecutive beta-pleated sheets, and elasticizes the LMW-GS like a spring. Graßberger *et al*. (2003) reported that the addition of reoxidized HMW-GSs caused an increase in dough extensibility and resistance, whereas with reoxidized LMW-GSs, only the dough resistance was increased. This means that a higher content of the short-spring, i.e., LMW-GS makes dough stronger than a lower content. Therefore, the deletion in this domain may affect the elastic behaviour of the LMW-GS. Three of the 6 amino-acid substitutions, positioned at 29, 101 and 104, change the hydrophilicity. Furthermore, proline, positioned at 101, gives the LMW-GS extensibility by making the amino-acid chain form a zigzag shape, whereas glutamine is linked by hydrogen bonds between the chains that form a zigzag shape, and strengthens the gluten network. Therefore, amino-acid substitutions, especially proline to glutamine, may affect the dough strength of wheat flour. These deletions and substitutions may be correlated with the positive effect on bread-making quality. However, we have to consider the other loci of 'Haruhikari' in the gene complex of LMW-GSs on chromosome 1B, and the other gene-complex of LMW-GSs on chromosome 1D. In this study, although the gene complex of LMW-GSs on chromosome 1A also consists of many loci, we could not identify a difference in SDS sedimentation values between 'Haruhikari' and 'Asakaze-komugi'. Therefore, differences in the gene complexes between 'Haruhikari' and 'Asakaze-komugi' on chromosome 1B might have a significant effect on dough strength.

REFERENCES

Duncan, D. B. (1955) Biometrics 11, 1–42.

Graßberger, A., P. Schieberle and P. Koehler (2003) Eur. Food Res. Technol. 216: 204–211.

Moonen, J.H.E., A. Scheepstra and A. Graveland (1982) Euphytica 31: 677–690.

Payne, P. I., K. G. Corfield, and J. A. Blackman (1979) Theor. Appl. Genet. 55, 153–159.

Payne, P.I., K.G. Corfield, L.M. Holt and J.A. Blackman (1981) J. Sci. Food Agric. 32: 51–60.

Payne, P. I., M. A. Nightingale, A. F. Krattiger, and L. M. Holt (1987a) J. Sci. Food Agric. 40, 51–65.

Payne, P.I., J.A. Seekings, A.J. Worland, M.G. Jarvis and L.M. Holt (1987b) J. Cereal Sci. 6: 103–118.

Pogna, N., D. Lafiandra, P. Feillet, and J. C. Autran (1988) J. Cereal Sci. 7, 211–214.

Sears, E.R. (1954) Mo. Agr. Exp. Stn. Res. Bull. 572: 1–59.

Takata, K., H. Yamauchi, N. Iriki, and T. Kuwabara (1999) Breeding Sci. 49, 221–223.

Tanaka, H., M. Tomita, H. Tsujimoto and Y. Yasumuro (2003) Euphytica 132: 167–174.

Tanaka, H., R. Shimizu, H. Tsujimoto (2005) Euphytica 141: 157-162.

Van Campenhout, S., J. Vander Stappen, L. Sagi and G. Volckaert (1995) Theor. Appl. Genet. 91: 313–319.

Zaman, Z., and R. L. Verwilghen (1979) Anal. Biochem. 100, 64–69.