Journal of Cereal Science 86 (2019) 15–21

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

# Contribution of α-gliadin alleles to the extensibility of flour dough in Japanese wheat cultivars

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ARTICLE INFO

*Keywords:*
*Triticum aestivum*
α-Gliadin
Flour dough extensibility
DNA marker

ABSTRACT

Alpha-gliadins are one of the major seed storage proteins in wheat and considered involved in wheat dough extensibility. We investigated the relationship between α-gliadin allele types and the extensibility of the resulting flour dough. In a preliminary survey, we identified 26 unique α-gliadin genes expressed in *Triticum aestivum* L. 'Chinese Spring', an experimental standard cultivar. These genes are encoded in three *Gli-2* homoeoloci, and consist of seven *Gli-A2*, twelve *Gli-B2*, and seven *Gli-D2* genes. Using specific primers for each gene, we then found that all three homoeoloci are associated with flour dough extensibility, as measured on an AlveoConsistograph. Importantly, the relationship between *Gli-A2* genes and flour dough extensibility is robust, as surveyed in available cultivars from different seasons. These results suggest that α-gliadin genes in chromosome 6A contribute to dough extensibility and that genotyping such genes may facilitate breeding programs to improve flour dough quality.

## 1. Introduction

Wheat flour can be processed into bread, pasta, noodles, and other foods due to its unique viscoelastic properties, which mostly depend on the gluten network formed when wheat flour is mixed with water. This gluten network is mainly composed of seed storage proteins, namely glutenins and gliadins. Glutenins are classified as high-molecular weight glutenin subunits (HMW-GSs) and low-molecular weight glutenin subunits (LMW-GSs), according to their mobility on sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE; Payne and Corfield, 1979), that polymerize via intermolecular disulfide bonds and that are thought to determine dough elasticity. On the other hand, gliadins are classified into α- (same as α/β), γ-, and ω-gliadins, also depending on electrophoretic mobility (Jackson et al., 1983), and contain intramolecular disulfide bonds that are thought to affect dough extensibility (Müller and Wieser, 1995). Part of gliadins are suggested to participate in the gluten network through intermolecular disulfide bonds during the process such as dough mixing (Johansson et al., 2013).

Wheat seed storage proteins are encoded by multigene families with highly variable alleles. As HMW-GSs are in relatively low copy numbers, allelic variations have been investigated in relation to flour dough properties. For instance, Greene et al. (1988) reported that wheat varieties with the *Glu-D1d* allele, consisting of genes 1Dx5 − 1Dy10, produce stronger dough than that with the *Glu-D1a* allele, which consists of genes 1Dx2 − 1Dy12. Accordingly, PCR primers were designed to discriminate between these alleles (Ahmad, 2000). Furthermore, a large number of researches on associations between HMW-GS alleles and breadmaking quality were investigated and a *Glu-1* quality score assigned to individual HMW-GS alleles was reported (e.g. Payne et al., 1987). The relationship between LMW-GSs and flour dough quality has also been investigated, although there are 30–40 copies of these genes in bread wheat cultivars (Cassidy et al., 1998). For example, Gupta et al. (1989) found that banding patterns of LMW-GSs on SDS-PAGE were associated with dough elasticity, measured as $R_{max}$ on an Extensograph. Thus, DNA markers for LMW-GSs were also developed (e.g. Wang et al., 2009) to facilitate breeding programs aiming to improve flour dough quality.

On the other hand, relatively little is known on the relationship between gliadin alleles and flour dough properties, even though gliadin accounts for approximately 50% of the proteins stored in wheat seeds. Studies on the association between gliadin alleles and wheat dough qualities have also been performed. For example, it was found that alleles at *Gli-A2*, which were assigned using acid polyacrylamide gel electrophoresis (A-PAGE; Metakovsky, 1991), correlated strongly with $R_{max}$ and alleles at *Gli-A1*, *Gli-B2* and *Gli-D2* were associated with

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https://doi.org/10.1016/j.jcs.2018.12.017
Received 13 October 2018; Received in revised form 27 December 2018; Accepted 27 December 2018
Available online 28 December 2018
0733-5210/ 

extensibility on an Extensigraph in Australian wheats (Branlard and Metakovsky, 2006). However, their genotyping of gliadins by A-PAGE needs a little cumbersome procedures because gliadin genes are in extremely high copy numbers, surveying such effects is complicated. Even in comparison to other gliadins, α-gliadin genes at *Gli-2* loci in the short arm of the homoeologous group 6 chromosome (Payne, 1987) are in especially high copy numbers in the genome, ranging from 60 in Chinese Spring wheat to 150 in Cheyenne wheat (Okita et al., 1985). In comparison, copy numbers of HMW-GS genes are only between three and five. In addition, half of α-gliadin genes are pseudogenes with premature stop codons (Noma et al., 2016), further complicating the relationship between α-gliadin alleles and flour dough quality.

Previously, we identified 90 unique α-gliadin genes in the genome of Chinese Spring wheat, which we classified into 11 groups based on phylogenetic analysis (Noma et al., 2016). Notably, the expression of these genes in each group differed in response to environmental conditions. However, we were unable to infer the relationships between α-gliadins and dough quality because the primers we used in quantitative real time (qRT)—PCR amplified not only intact genes that are translated and stored as proteins in the endosperm, but also pseudogenes that may or may not be transcribed or translated. Therefore, in the present study, we surveyed the α-gliadin genes actually expressed in immature seeds of Chinese Spring wheat. Gene-specific primers were designed and used to genotype 22 Japanese wheat cultivars, and these genotypes were then analyzed in relation to dough properties measured on an Alveo-Consistograph.

## 2. Materials and methods

### 2.1. Plant materials

*Triticum aestivum* L. 'Chinese Spring' and derivative nullisomic–tetrasomic lines for chromosome group 6 were obtained from the National Bioresource Project-Wheat, Japan. Plants were grown in a field at Kihara Institute for Biological Research, Yokohama, Japan, during the growing season 2011–2012. Seeds were obtained 12 days post-anthesis, and immediately stored at −80 °C.

Grains from Japanese wheat cultivars were provided by Nisshin Flour Milling Inc., Tokyo, Japan. Samples were milled to 60% extraction using a Bühler experimental mill (Bühler, Uzwil, Switzerland) according to AACC Method 26−21 (AACC, 2000). Moisture, ash, and protein contents of the flour samples were determined according to AACC Method 44−19, 08−01, and 46−12, respectively (AACC, 2000).

### 2.2. Extraction of genomic DNA and total RNA

Genomic DNA was extracted from a fresh leaf or a single ground seed in a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan), using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Total RNA was isolated from immature seeds of Chinese Spring wheat using the NucleoSpin RNA Plant (Takara Bio, Shiga, Japan) following the manufacturer's protocol, and reverse-transcribed using an oligo dT20 primer and a ThermoScript RT-PCR system (Invitrogen, Carlsbad, CA, USA).

### 2.3. Cloning and sequencing of α-gliadin genes

New group-specific primers (AS1-AS11) for cloning α-gliadin genes were designed based on our previous study (Noma et al., 2016), and are listed in Supplementary Table 1. Primers AS2 and AS7 were designed to amplify *Gli-A2* genes, primers AS3, AS4, AS5, and AS6 to amplify *Gli-B2* genes, and primers AS1, AS8, AS9, AS10, and AS11 to amplify *Gli-D2* genes. Targets were amplified by reverse transcription (RT)—PCR using high-fidelity KOD-plus DNA polymerase (Toyobo, Osaka, Japan), as previously described (Noma et al., 2016). After adding a single deoxyadenosine at the 3′-end using 10 × A-attachment Mix (Toyobo), PCR products were purified, ligated to pGEM-T Easy Vector (Promega, Madison, WI, USA), and transformed into DH5α. After extracting the plasmid DNA, inserts were sequenced by Genome Lab Dye Terminator Cycle Sequencing on a CEQ8000 Genetic Analysis System (Beckman Coulter, Brea, CA, USA), using the Quick Start Kit (Beckman Coulter).

A sequence found in more than one clone and identical to a sequence in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/nucleotide/) was considered a unique α-gliadin gene expressed in immature Chinese Spring seeds. Such sequences were deposited in DNA Data Base of Japan (DDBJ) with accession numbers LC335724 through LC335749.

### 2.4. Design of gene-specific markers and genotyping of Japanese wheat cultivars

Sequences of unique α-gliadin genes identified as described above were aligned in ClustalW (http://www.clustalw.ddbj.nig.ac.jp/) to design gene-specific primers that discriminate nucleotide polymorphisms. Sequences of the primers are listed in Supplementary Table 2. Specificity was examined by PCR, using the genomic DNA from nullisomic–tetrasomic lines, and specific primers were then used to genotype 22 Japanese wheat cultivars. The PCR was performed in 10-μL reactions containing 0.5 U AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 1 × PCR Buffer II, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 0.3 μM of each primer, and 20 ng genomic DNA. Targets were amplified by denaturation at 95 °C for 10 min, followed by 35 cycles at 95 °C for 15 s, annealing for 30 s at the temperatures listed in Supplementary Tables 2 and 72 °C for 30 s, and final extension at 72 °C for 5 min. Amplicons were resolved on 2% agarose gels, and visualized under ultraviolet light with ethidium bromide.

### 2.5. Dough rheology

AlveoConsistograph characteristics of flour samples were determined according to AACC Method 54−30 (AACC, 2000), using the water absorption values measured according to AACC Method 54−50 (AACC, 2000) to obtain dough with a maximum consistency of 2200 mb (Dubois et al., 2008). Data were collected in Alveolink NG software (Chopin Technologies, Villeneuve La Garenne, France), including maximum overpressure (T) and average abscissa at rupture (Ex), which correspond to resistance to extension and dough extensibility, respectively (Dubois et al., 2008).

### 2.6. Statistical analysis

Data were analyzed by Tukey-Kramer multiple tests in JMP 11.2.0 (SAS Institute, Cary, NC, USA).

## 3. Results

### 3.1. Development of specific markers for α-gliadin genes

In total, 139 α-gliadin sequences expressed in immature Chinese Spring seeds were isolated by RT-PCR. Twenty-six unique sequences were obtained after selecting the genes that exactly matched nucleotide sequences deposited in the NCBI database, and designated α1 to α26 (Table 1). Of these, seven were located in *Gli-A2*, with three genes amplified by primer AS2 and four amplified by AS7. Twelve *Gli-B2* genes were also isolated: five were amplified by AS3, three were amplified by AS4, one was amplified by AS5, and three were amplified by AS6. Finally, seven *Gli-D2* sequences were obtained, including five amplified by AS8 and one each amplified by AS1 and AS11. Twenty-four of the 26 unique genes were intact, while two (α14* and α15*) were pseudogenes with premature stop codons. Genes α2 and α3, as well as α24 and α25, differed only in the number of glutamine codons

**Table 1**
Unique α-gliadin genes expressed in immature seeds of Chinese Spring wheat.

| Chromosome | Primer Name | Gene Name | Accession No. (gene name) exactly matching cDNA | Reference |
|---|---|---|---|---|
| 6A | AS2 | α1 | AP012286 (BAC3a-gli-1) | Kawaura et al. (2012) |
| | | α2 | AP012286 (BAC3a-gli-2) | Kawaura et al. (2012) |
| | | α3 | AB982235 (clone2) | Noma et al. (2016) |
| | AS7 | α4 | AB982245 (clone12) | Noma et al. (2016) |
| | | α5 | AP012289 (BAC11a-gli-2) | Kawaura et al. (2012) |
| | | α6 | AB982242 (clone9) | Noma et al. (2016) |
| | | α7 | AB982278 (clone45) | Noma et al. (2016) |
| 6B | AS3 | α8 | AP012287 (BAC5a-gli-1) | Kawaura et al. (2012) |
| | | α9 | AP012287 (BAC5a-gli-3) | Kawaura et al. (2012) |
| | | α10 | AP012287 (BAC5a-gli-4) | Kawaura et al. (2012) |
| | | α11 | AP012287 (BAC5a-gli-5) | Kawaura et al. (2012) |
| | | α12 | AP012287 (BAC5a-gli-7) | Kawaura et al. (2012) |
| | AS4 | α13 | AP012288 (BAC6a-gli-1) | Kawaura et al. (2012) |
| | | α14* | AB982290 (clone57*) | Noma et al. (2016) |
| | | α15* | AB982244 (clone11*) | Noma et al. (2016) |
| | AS5 | α16 | AB982273 (clone40) | Noma et al. (2016) |
| | AS6 | α17 | KX173902 | Dubois et al. (2016) |
| | | α18 | KX174126 | Dubois et al. (2016) |
| | | α19 | AB982286 (clone53) | Noma et al. (2016) |
| 6D | AS1 | α20 | AP012290 (BAC2a-gli-1) | Kawaura et al. (2012) |
| | AS8 | α21 | JX141486 | Unpublished |
| | | α22 | LT627606 | Unpublished |
| | | α23 | AB982248 (clone15) | Noma et al. (2016) |
| | | α24 | JX828238 | Unpublished |
| | | α25 | LT627597 | Unpublished |
| | AS11 | α26 | AB982256 (clone23) | Noma et al. (2016) |

in the polyglutamine domain.

Specific primers discriminating nucleotide polymorphisms were then designed for each unique α-gliadin gene (Supplementary Table 2), although primers for α2 and α24 would also detect α3 and α25, respectively. Primer specificity and optimum annealing temperature were evaluated using plasmid DNA clones. The PCR against the genome of nullisomic-tetrasomic lines for chromosome group 6, allowed mapping seven genes (α1–α7) to chromosome 6A, twelve genes (α8–α19) to chromosome 6B, and seven genes (α20–α26) to chromosome 6D (Fig. 1A), in agreement with previous results (Noma et al., 2016).

### 3.2. Genotyping of α-gliadin genes in Japanese wheat cultivars

Gene-specific primers were used to survey α-gliadin types in 22 wheat cultivars commercially cultivated in Japan in recent years, and popularly used for various products such as breads and noodles. We found two, six, and three *Gli-A2*, *Gli-B2*, and *Gli-D2* allele types, respectively. *Gli-A2* types, designated a-1 and a-2 (Figs. 1B and 2A), contained all seven or none of the *Gli-A2* α-gliadin genes, respectively. Seven cultivars were genotyped a-1 and 15 were genotyped a-2 (Fig. 2A).

The *Gli-B2* types were designated b-1, b-2, b-3, b-4, b-5, and b-6 (Figs. 1B and 2B). Type b-1, which was found in ten cultivars, contained all twelve *Gli-B2* α-gliadin genes, while b-2, also containing all *Gli-B2* α-gliadin genes except α16, was found in a single cultivar (Kitanokaori). Six cultivars were genotyped b-3 with all *Gli-B2* α-gliadin genes except α17. Type b-4, found in one cultivar (Norin 61), had neither α16 nor α17, while b-6, found in one cultivar (Horoshirikomugi), contained only α16 and α17 (Fig. 2B, Table 2). Type b-5, found in three cultivars, contained only α13, α16, α18, and α19.

The *Gli-D2* types were designated d-1, d-2, and d-3 (Figs. 1B and 2C). The first, found in nine cultivars, contained all seven *Gli-D2* α-gliadin genes, while the second, found in three cultivars, contained all *Gli-D2* α-gliadin genes but a shorter than typical α24 that was therefore designated α24' (Figs. 1B and 2C). This difference was only in the number of triplets in a repeat domain (data not shown). Finally, d-3, found in ten cultivars, contained all *Gli-D2* α-gliadin genes except α25 (Fig. 2C). Notably, α20, α21, α22, α23, and α26 were present in all cultivars (Fig. 1B). These α-gliadin types were not linked to HMW-GS (*Glu-A1, Glu-B1, and Glu-D1*) alleles (Table 2).

### 3.3. Relationship between α-gliadin types and flour dough quality

The AlveoConsistograph water absorption, Ex (average abscissa at rupture), and T (maximum overpressure) values differed among the 22 Japanese cultivars genotyped (Table 2). Strikingly, flour dough from type a-1 was significantly more extensible, as indicated by higher Ex value, than flour dough from type a-2 cultivars (Fig. 3A), although T values were similar (Fig. 3D). These results suggested that seven α-gliadins in *Gli-A2* enhance dough extensibility. Similarly, type b-5 produced significantly more extensible flour dough than types b-1 and b-3 (Fig. 3B). However, flour from type b-1 had significantly higher T values than flour from type b-5 cultivars (Fig. 3E). These results suggested that α8, α9, α10, α11, α12, α14*, and α15*, which are present in type b-1 and b-3 but absent from type b-5 (Fig. 2B), reduce dough extensibility. Types b-2, b-4, and b-6 were excluded from this analysis because they were found in only one cultivar each. Finally, flour from α-gliadin type d-1 was significantly more extensible than flour from type d-3 (Fig. 3C), although T values were comparable (Fig. 3F), implying that α25, which is present in type d-1 but not in type d-3 (Fig. 2C), enhances dough extensibility.

### 3.4. Robustness of the relationship between Gli-2 allele types and dough extensibility

As specific *Gli-A2* and *Gli-D2* types seemed to be associated with the extensibility of wheat flour harvested in 2013, the robustness of this relationship was investigated in flour samples from another year. We found that type a-1 also produced significantly more extensible flour than type a-2 in 2012 (Fig. 4A), based on samples of cultivars Chikugoizumi, Haruyokoi, Kitahonami, Kitanokaori, Norin 61, Sanukinoyume 2009, Satonosora, and Shiroganekomugi that were available for analysis. On the other hand, flour from type d-1 harvested in 2012 was of similar extensibility to that of typed-3 harvested in the same year (Fig. 4C). These results suggested that the effect of *Gli-A2* on dough extensibility is robust and less sensitive to environmental factors than

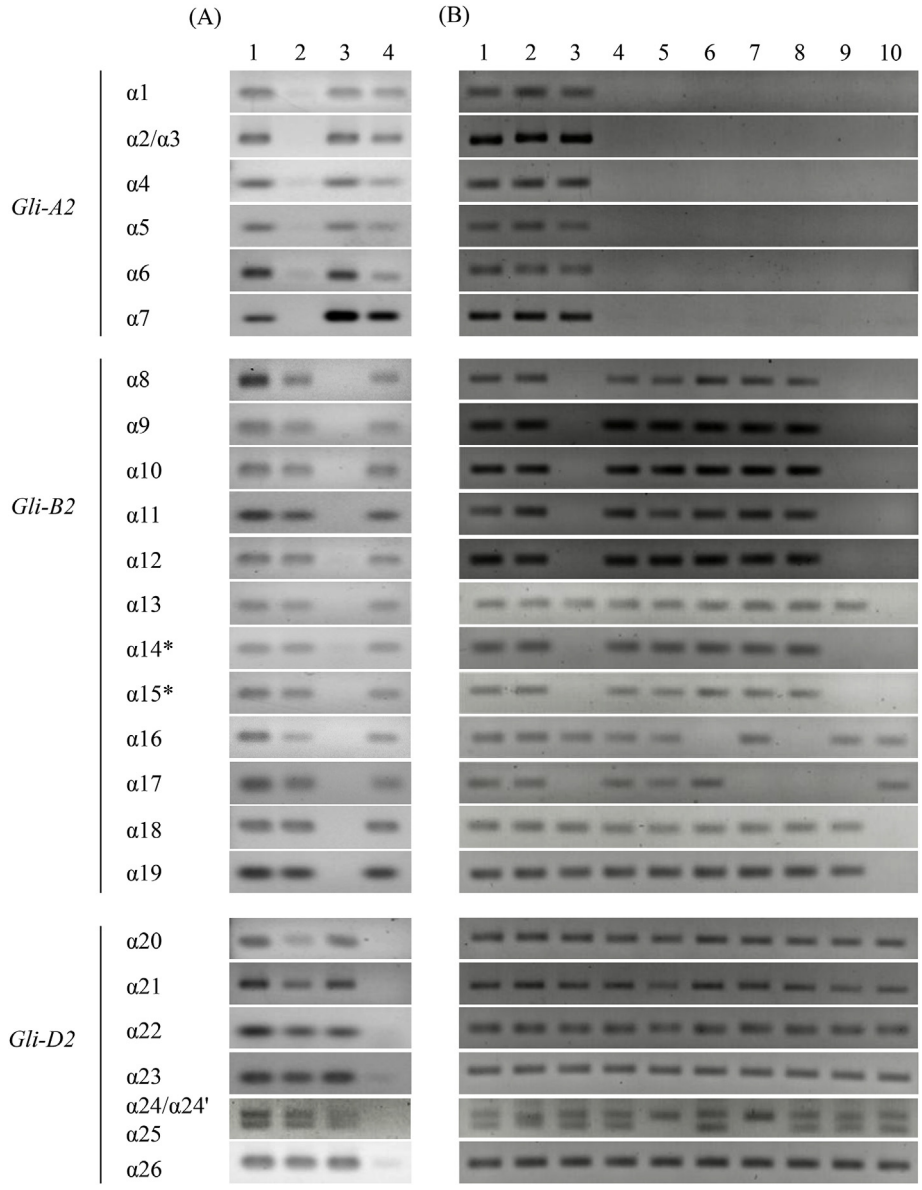


**Fig. 1.** Agarose gel electrophoresis of 26 α-gliadin genes amplified by PCR from (A) Chinese Spring nullisomic–tetrasomic lines for chromosome group 6, and (B) 10 representative Japanese wheat cultivars. (A) Lane 1, Chinese Spring; lane 2, Nulli6A-Tetra6D; lane 3, Nulli6B-Tetra6A; lane 4, Nulli6D-Tetra6B. (B) Lane 1, Kitahonami; lane 2, Harukirari; lane 3, Chihokukomugi; lane 4, Takunekomugi; lane 5, Kinuakari; lane 6, Kitanokaori; lane 7, Chikugoizumi; lane 8, Norin 61; lane 9, Taisetsukomugi; lane 10, Horoshirikomugi.

other α-gliadin allele types.

## 4. Discussion

Twenty-six unique α-gliadin genes were expressed in immature seeds of Chinese Spring wheat according to RT-PCR. Seven of these genes are located in chromosome 6A, twelve in 6B, and seven in 6D (Fig. 1A). Notably, more genes were identified by genomic PCR and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Indeed, we previously isolated 90 α-gliadin genes by genomic PCR, including 40 pseudogenes (Noma et al., 2016). In addition, 10, 10, and 16 spots on 2D-PAGE attributed to α-gliadins in chromosomes 6A, 6B, and 6D, respectively (Kawaura et al., 2018). Comparison of number of genes isolated in this study to that identified by genomic PCR and 2D-PAGE inferred that genes expressed in relatively low level might not be cloned by RT-PCR. On the other hand, using RNA sequencing, Wang et al. (2017) found that 25 intact α-gliadin genes were expressed in Xiaoyan 81 wheat 25 days after flowering, of which eight, ten, and seven genes were mapped to chromosomes 6A, 6B, and 6D, respectively. Therefore, we believe that all major α-gliadin genes expressed in Chinese Spring have been identified, considering copy number variations among cultivars. Of note, two of these genes, α14* and α15*, are pseudogenes with premature stop codons in the first polyglutamine domain. Strikingly, Xu and Messing (2009) reported that prolamin genes with premature stop codons are transcribed in rice, implying that some α-gliadin pseudogenes might be expressed and accumulate as proteins, although most of the other 40 α-gliadin pseudogenes (Noma et al., 2016) are not.

Based on the α-gliadin genes expressed in Chinese Spring, we genotyped 22 Japanese cultivars, and found that 2, 6, and 3 *Gli-A2*, *Gli-B2*, and *Gli-D2* allele types (Table 2) and the three homoeologous *Gli-2* loci containing these genes are associated with Ex values, a measure of dough extensibility. The number of these α-gliadin allele types was very low compared to that of alleles reported by Metakovsky (1991), which identified 24, 22, and 19 alleles for *Gli-A2*, *Gli-B2*, and *Gli-D2* loci, respectively. Additionally, Metakovsky (1991) classified *Gli-2* alleles by

(A) *Gli-A2*

| Type | No. of cultivars | α-gliadin genes | | | | | |
|---|---|---|---|---|---|---|---|
| | | α1 | α2/α3 | α4 | α5 | α6 | α7 |
| a-1 | 7 | + | + | + | + | + | + |
| a-2 | 15 | - | - | - | - | - | - |

(B) *Gli-B2*

| Type | No. of cultivars | α-gliadin genes | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | α8 | α9 | α10 | α11 | α12 | α13 | α14* | α15* | α16 | α17 | α18 | α19 |
| b-1 | 10 | + | + | + | + | + | + | + | + | + | + | + | + |
| b-2 | 1 | + | + | + | + | + | + | + | + | - | + | + | + |
| b-3 | 6 | + | + | + | + | + | + | + | + | + | - | + | + |
| b-4 | 1 | + | + | + | + | + | + | + | + | - | - | + | + |
| b-5 | 3 | - | - | - | - | - | + | - | - | + | - | + | + |
| b-6 | 1 | - | - | - | - | - | - | - | - | + | + | - | - |

(C) *Gli-D2*

| Type | No. of cultivars | α-gliadin genes | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | α20 | α21 | α22 | α23 | α24/α24' | α25 | α26 |
| d-1 | 9 | + | + | + | + | +/- | + | + |
| d-2 | 3 | + | + | + | + | -/+ | + | + |
| d-3 | 10 | + | + | + | + | +/- | - | + |

**Fig. 2.** α-Gliadin types in Japanese cultivars. (A) Allele types of *Gli-A2* based on seven α-gliadin genes. (B) Allele types of *Gli-B2* based on twelve α-gliadin genes. (C) Allele types of *Gli-D2* based on seven α-gliadin. +, gene present in genome; -, gene absent from genome.

A-PAGE analysis using 45 crosses and about 360 common wheat cultivars from several countries. Therefore, α-gliadin allele types determined in this study were not directly compared to those designated in the previous study (Metakovsky, 1991). On the other hand, Tanaka et al. (2003) reported that 14 different banding patterns in α- and β/γ-gliadins by A-PAGE analysis using 107 Japanese common wheat cultivars and landraces. Although it is not clear whether all α-gliadin genes which affect dough extensibility were fully isolated, we believe that 24 primer pairs designed in this study are available to genotype the 22 Japanese cultivars.

In particular, cultivars with *Gli-A2* allele type a-1 produce significantly more extensible flour than type a-2 cultivars (Fig. 3A), suggesting that seven *Gli-A2* α-gliadin genes (α1, α2, α3, α4, α5, α6, and α7), which are present in the former but not in the latter (Figs. 1B and 2A), enhance dough extensibility. This relationship was observed in two crop seasons (Fig. 4A). In addition, 2D-PAGE profile showed that protein spots which were derived from *Gli-A2* were absent in Norin 61, which is one of type a-2 cultivars (Supplementary Fig. 1). These results indicate that the number of *Gli-A2* α-gliadins is important for flour dough extensibility. Moreover, Ex values varied among type a-1

**Table 2**
α-Gliadin and glutenin alleles in 22 Japanese wheat cultivars, and corresponding flour quality as assessed on an AlveoConsistograph.

| Cultivar | α-Gliadin types | | | Glutenin alleles | | | Moisture (%) | Ash (%)[d] | Protein (%)[d] | AlveoConsistograph characteristics | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Gli-A2* | *Gli-B2* | *Gli-D2* | *Glu-A1*[a] | *Glu-B1*[b] | *Glu-D1*[c] | | | | Water absorption (%)[d] | Ex (mm)[e] | T (mm $H_2O$)[e] |
| Chihokukomugi | a-1 | b-5 | d-1 | a | e | a | 13.1 | 0.40 | 9.4 | 48.4 | 167 ± 14 | 46 ± 1 |
| Chikugoizumi | a-2 | b-3 | d-3 | b | u | f | 13.2 | 0.37 | 7.6 | 48.1 | 69 ± 1 | 73 ± 1 |
| ChikushiW 2 | a-2 | b-3 | d-3 | a | c | f | 13.6 | 0.49 | 9.9 | 51.8 | 71 ± 3 | 97 ± 3 |
| Harukirari | a-1 | b-1 | d-2 | b | c | d | 13.5 | 0.48 | 11.4 | 54.1 | 78 ± 1 | 74 ± 1 |
| Haruyokoi | a-1 | b-1 | d-2 | b | c | d | 13.6 | 0.49 | 12.6 | 56.8 | 105 ± 4 | 83 ± 1 |
| Haruyutaka | a-1 | b-1 | d-2 | a | i | a | 13.4 | 0.52 | 11.0 | 54.1 | 66 ± 1 | 98 ± 1 |
| Hokushin | a-1 | b-5 | d-1 | a | e | c | 12.8 | 0.43 | 8.4 | 48.5 | 141 ± 9 | 40 ± 2 |
| Horoshirikomugi | a-2 | b-6 | d-1 | a | c | a | 13.2 | 0.47 | 9.3 | 51.7 | 65 ± 2 | 91 ± 1 |
| Hukuhonoka | a-2 | b-3 | d-3 | b | u | f | 12.8 | 0.37 | 7.7 | 49.8 | 61 ± 2 | 58 ± 1 |
| Iwainodaichi | a-2 | b-3 | d-3 | c | u | a | 14.0 | 0.37 | 7.6 | 48.4 | 36 ± 2 | 64 ± 1 |
| Kinuakari | a-2 | b-1 | d-3 | b | u | f | 13.1 | 0.33 | 8.3 | 49.8 | 88 ± 2 | 65 ± 1 |
| Kitahonami | a-1 | b-1 | d-1 | a | u | a | 14.3 | 0.35 | 9.9 | 47.8 | 101 ± 5 | 67 ± 2 |
| Kitamoe | a-1 | b-1 | d-1 | a | u | c | 13.4 | 0.44 | 9.4 | 47.8 | 121 ± 14 | 51 ± 1 |
| Kitanokaori | a-2 | b-2 | d-1 | a | c | d | 14.1 | 0.53 | 13.0 | 57.4 | 50 ± 4 | 123 ± 1 |
| Minaminokaori | a-2 | b-1 | d-3 | a | u | f | 13.2 | 0.48 | 10.0 | 52.6 | 109 ± 3 | 77 ± 1 |
| Norin 61 | a-2 | b-4 | d-1 | b | u | f | 13.2 | 0.43 | 7.3 | 48.4 | 43 ± 4 | 78 ± 1 |
| Sanukinoyume 2009 | a-2 | b-1 | d-3 | c | u | a | 13.5 | 0.39 | 7.8 | 49.6 | 63 ± 2 | 68 ± 1 |
| Satonosora | a-2 | b-1 | d-3 | c | c | f | 13.6 | 0.35 | 8.2 | 47.7 | 65 ± 8 | 55 ± 0 |
| Shiroganekomugi | a-2 | b-3 | d-3 | c | u | f | 13.6 | 0.35 | 7.9 | 49.4 | 62 ± 5 | 64 ± 1 |
| Taisetsukomugi | a-2 | b-5 | d-1 | a | c | a | 13.0 | 0.41 | 8.1 | 47.3 | 110 ± 6 | 56 ± 3 |
| Takunekomugi | a-2 | b-1 | d-1 | a | d | c | 13.0 | 0.62 | 11.0 | 52.6 | 42 ± 1 | 109 ± 1 |
| Tsurupikari | a-2 | b-3 | d-3 | b | u | f | 13.5 | 0.42 | 8.0 | 51.6 | 59 ± 3 | 69 ± 2 |

[a] *Glu-A1* alleles were determined by PCR according to Takata et al. (2008).
[b] *Glu-B1* alleles were determined by PCR according to Zaitseva et al. (2017) and SDS-PAGE according to Nakamura et al. (1990).
[c] *Glu-D1* alleles were determined by SDS-PAGE according to Nakamura et al. (1990).
[d] Moisture basis 14%.
[e] Mean ± SD (n = 3).

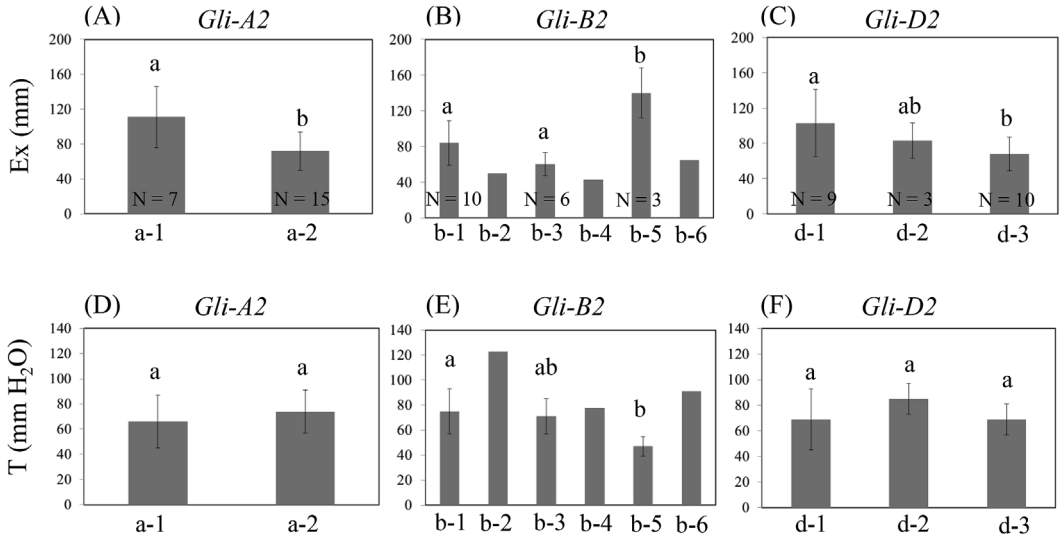


**Fig. 3.** AlveoConsistograph characteristics of flour from Japanese cultivars with allele types of *Gli-A2*, *Gli-B2*, and *Gli-D2* indicated on x-axes. (A–C) Ex value. (D–F) T value. Data are means ± SD. Different letters indicate $p < 0.05$.

cultivars (Table 2): Haruyokoi showed significantly higher Ex value than Harukirari and Haruyutaka. Although this data was shown by only a few cultivars, one possibility is that the expression level of these *Gli-A2* α-gliadins was differed among these cultivars. On the other hand, alleles of HMW-GS (*Glu-A1, Glu-B1,* and *Glu-D1*) were not associated with dough extensibility (Table 2).

Overall, allele types of *Gli-B2* were more diverse than *Gli-A2* and *Gli-D2*, and flour from type b-5 was more extensible than flour from type b-1 and b-3 (Fig. 3B). Type b-1 and b-3 share eleven genes (α8, α9, α10, α11, α12, α13, α14*, α15*, α16, α18, and α19) of which only α13, α16, α18, and α19 are present in type b-5 (Figs. 1B and 2B). On the other hand, type b-2 and b-4 share seven genes with type b-1 and b-3 (α8, α9, α10, α11, α12, α14*, and α15*), but produce less extensible flour than type b-5. Taken together, these results suggest that these seven genes containing α14* and α15* pseudogenes with premature stop codons in polyglutamine domain-1 (Table 1) are associated with lower dough extensibility. In addition, α14* and α15* do not contain the conserved cysteine residues, instead of the normal six, which form intramolecular disulfide bonds and stabilize the monomeric structure (Müller and Wieser, 1995). Hence, these α-gliadins may not function as monomeric proteins incorporated into gluten matrixes, thereby lowering dough extensibility. On the other hand, the five intact α-gliadins (α8, α9, α10, α11, and α12) in type b-1, b-2, b-3, and b-4 (Table 2, Fig. 3B) do not appear to enhance dough extensibility, in contrast to intact genes in *Gli-A2*, implying that these genes might not be expressed or are expressed at low levels.

Allele types of *Gli-D2* also appear to be associated with dough extensibility, as type d-1 produces significantly more extensible flour than type d-3 (Fig. 3C). Notably, all genes in type d-1 are also present in type d-3, except α25 (Figs. 1B and 2C), which contains an additional cysteine residue in unique domain-2. Thus, α25 might be incorporated into glutenin polymers similar to α-gliadins with a 7th cysteine residue (Kasarda, 1989), which are found in the glutenin fraction (Muccilli et al., 2005). Accordingly, α25 might moderately disrupt

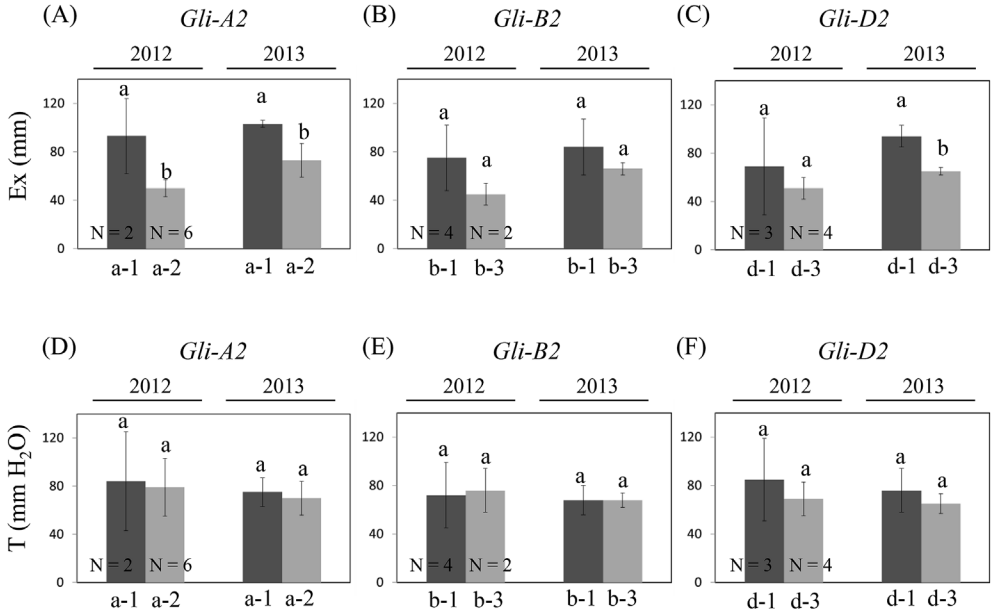


**Fig. 4.** AlveoConsistograph characteristics of flour from Japanese wheat harvested in 2012 and 2013. Ex value (A–C) and T value (D–F) of flour from cultivars with allele types with *Gli-A2* (A, D), *Gli-B2* (B, E), and *Gli-D2* (C, F). Data are means ± SD. Different letters indicate $p < 0.05$.

intermolecular disulfide bonds between glutenins, and therefore confer high extensibility to the flour dough. However, the extensibility of type d-1 flour harvested in a different year was only marginally higher than that of type d-3 flour harvested in the same year (Fig. 4C), suggesting that the effect of α25 on flour extensibility might depend on its expression in response to environmental conditions, as previously observed (Noma et al., 2016). In addition, expression of genes encoding α-gliadins, ω-gliadins, and HMW-GSs is enhanced by fertilization, while that of γ-gliadins and LMW-GS is not (Dupont et al., 2006; Altenbach and Kothari, 2007). Collectively, these observations suggest that the effect of *Gli-D2* allele types on dough extensibility is due not only to α25 but also to glutenins or to the glutenin/gliadin ratio.

In summary, we surveyed *Gli-2* allele types in Japanese wheat cultivars based on 26 α-gliadin genes expressed in Chinese Spring wheat. We found two *Gli-A2* allele types, one of which was null and robustly associated with dough extensibility. Furthermore, we developed DNA markers related to α-gliadin alleles. However, the number of cultivars surveyed in this study was limited because we used cultivars which were mainly cultivated in Japan in recent years. In addition, they had different genetic backgrounds and were cultivated in various locations. Further studies are needed to confirm the *Gli-A2* contribution to dough extensibility, for example, comparison with near isogenic lines grown in the same agronomic practices. Consequently, specific markers for α-gliadin genes in these allele types might assist breeding programs aiming to improve flour dough quality.

## Conflicts of interest

The authors declare that they have no conflict of interest.

## Acknowledgements

We thank the National BioResource Project (NBRP)-Wheat, supported by the NBRP of the MEXT, Japan, for providing Chinese Spring aneuploids. This research was partially supported by a JSPS KAKENHI grant number 15K07261.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2018.12.017.

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