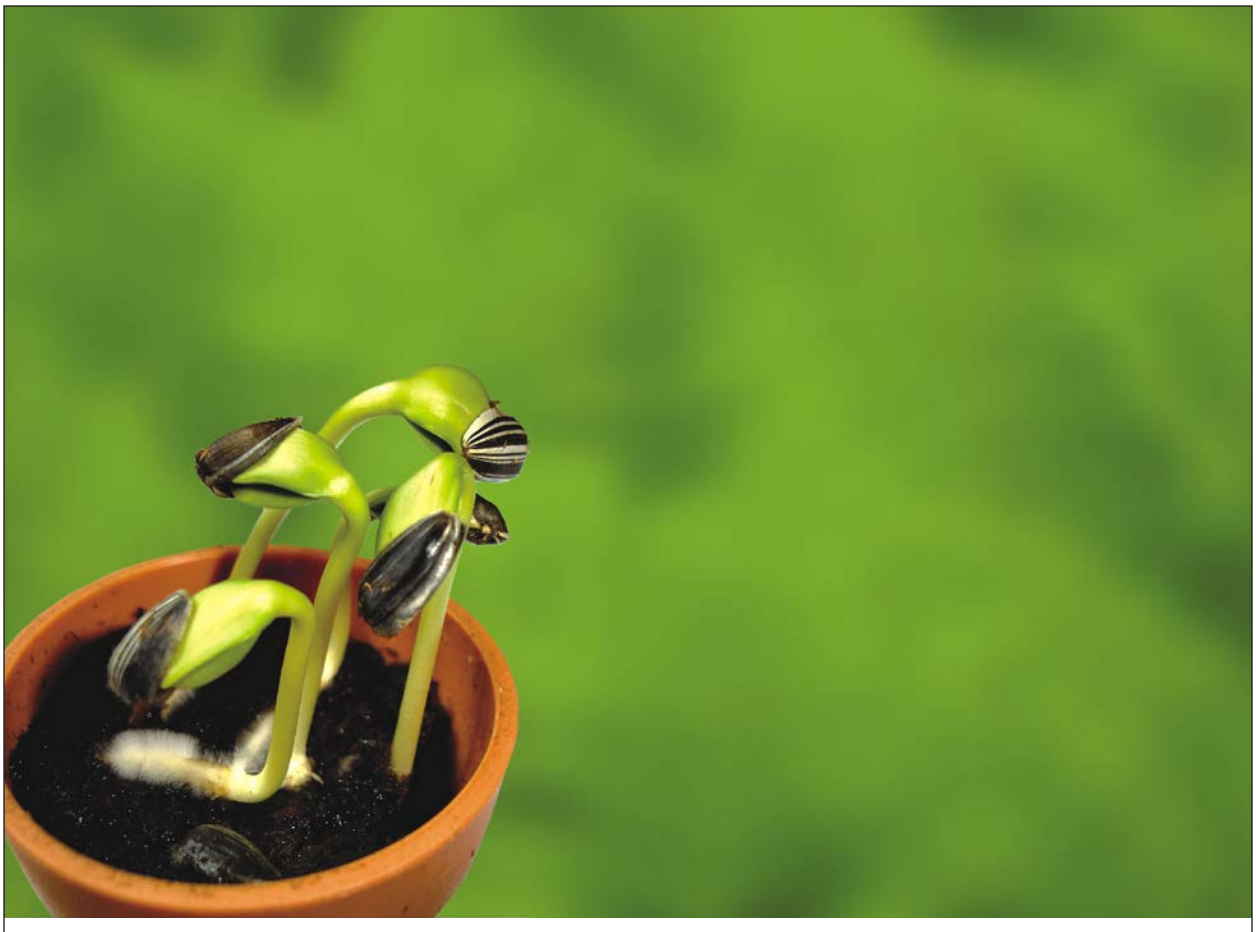

International Journal of Plant Breeding and Genetics

ISSN 1819-3595

Academic Journals Inc.

www.academicjournals.com

International Journal of Plant Breeding and Genetics 4 (4): 228-237, 2010
ISSN 1819-3595

Molecular Cloning, Sequencing and N-Terminal Amino Acids Sequences Analysing of ω-gliadin from *T. turgidum* sp. *paleocolchicum*

[1,2]X. Chen, [1,2]G.Y. Chen and [1,2,3]W. Li
[1]Triticeae Research Institute, Sichuan Agricultural University, Yaan, Sichuan 625014, China
[2]Ministry of Education Key Laboratory for Crop Genetic Resources and Improvement in Southwest China, Sichuan Agricultural University, Yaan, Sichuan 625014, China
[3]Agronomy College, Sichuan Agricultural University, Yaan, Sichuan 625014, China

Abstract: In this study, we aim to get the DNA sequences of ω-gliadin gene from *T. turgidum* sp. *paleocolchicum*. Three wheat ω-gliadin gene sequences (*ω-gli-1*, *ω-gli-2* and *ω-gli-3*) were isolated from *T. turgidum* sp. *paleocolchicum* (2n = 4x = 28, AABB) using PCR primers, designed from published ω-gliadin gene sequences. *ω-gli-3* could encode putative mature protein, while *ω-gli-1* and *ω-gli-2* were assumed to be pseudogenes due to their in-frame stop codon, which is attributed to the single base change C to T. The deduced ω-gliadin amino acid sequences were compared with all the ω-2 type ω-gliadins at GenBank. The ω-gliadin genes described here have a long repetitive domain with a repeat unit $PFPQ_{1-2}PQQ$, which is identical with that proposed for 1A, 1D ω-gliadins, C-hordein and ω-secalin genes, but different from that proposed for 1B ω-gliadins. These three ω-gliadin genes are of the ARQ-/ARE-variant type as categorized by the derived N-terminal amino-acid sequences and amino acid compositions. Based on the N-terminal protein sequences, ω-gliadins could be divided into KEL, ARQ, ARE, XRQ, or RQ and SRL types, only the ARH-/ARQ-/ARE- and SLK-type ω-gliadin were deduced from all the ω-gliadin DNA sequences at GenBank.

Key words: Store protein, glutenin, gene structure, N-terminal sequences, repetitive domain

INTRODUCTION

Wheat grain is a major source of proteins and is important food in the world. The wheat seed protein fraction is composed mainly of prolamins, which consist of gluteins and gliadins. The latter can be separated into α-, γ- and ω-gliadins based on the electrophoretic mobility in acidic polyacrylamide gel electrophoresis (A-PAGE) (Metakovsky *et al.*, 1984). Of the three main wheat gliadin families, the ω-gliadins differ from the others because they generally have no cysteine or methionine, They are therefore defined as sulfur-poor prolamins, together with the ω-secalins of rye (*Secale cereale*) and the C-hordeins of barley (*Hordeum vulgare*) (Shewry *et al.*, 1986; Tatham and Shewry, 1995).

N-terminal protein sequences have been used to classify the ω-gliadins based on the first three amino acids of the mature protein: the ω-1 type begins with KEL, the ω-2 type with

Corresponding Author: Wei Li, Triticeae Research Institute, Sichuan Agricultural University, Yaan, Sichuan 625014, China Tel: +86 835 2882620 Fax: +86 835 2882336

ARQ, ARE, XRQ, or RQ and the ω-5 type with SRL, the ω-5 type corresponds to the 1B ω-gliadins, whereas the 1A and 1D ω-gliadins included both the ω-1 and ω-2 types (Kasarda *et al*., 1983).

Gliadins are the most abundant proteins in wheat endosperm and are encoded by gene families located on the short arms of homoeologous group one and six. All the ω-gliadin and the majority of γ-gliadin genes are encoded by the *Gli-1* loci while most α- and some γ-gliadins are encoded at *Gli-2* loci (Metakovsky *et al*., 1984). In this study, we refer to the ω-gliadins encoded by the *Gli-A1*, *Gli-B1* and *Gli-D1* as the 1A, 1B and 1D ω-gliadins, respectively.

Various coding gene sequences for the α- and γ-gliadin have been characterized (Anderson *et al*., 1984; Bartels *et al*., 1986; Scheets and Hedgcoth, 1988; Anderson *et al*., 1997; Pistóna *et al*., 2006). However, there are only a few reports on ω-gliadin gene sequence. The difficulty in cloning ω-gliadin genes has been reported. ω-gliadin genes are unstable in the *E. coli* vector resulting in numerous deletions of various sized fragments (Hsia and Anderson, 2001; Hassani *et al*., 2008). The reason is not confirmed but it is assumed to be related to the character of the ω-gliadin repetitive region, which composes of a series of short DNA repeats and covers up to 96% of the coding region (Anderson *et al*., 2009). The N-terminal peptide was described based on protein sequencing, e.g., Kasarda *et al*. (1983), DuPont *et al*. (2000, 2004), Seilmeier *et al*. (2001), Matsuo *et al*. (2005) and Hassani *et al*. (2008), whereas few studies focus on the DNA sequences.

T. turgidum sp. *paleocolchicum* (2n = 4x = 28, AABB) is a valuable source of genes for wet resistance and diseases, such as stripe rust, leaf rust and dust band. To date, several studies about its agronomic characters, phylogeny and α-gliadin have been reported by Mori *et al*. (1997) and Chen *et al*. (2008). There is no literature report on the characterization of its ω-gliadin genes.

In this study, we aim to get the DNA sequences of ω-gliadin gene from *T. turgidum* sp. *Paleocolchicum* using a PCR approach. Three ω-gliadin DNA sequences, designated *ω-gli-1*, *ω-gli-2* and *ω-gli-3*, were identified and characterized. The repeat structure and N-terminal protein sequences were compared with those of other prolamins, C-hordeins and ω-secalins. The N-terminal amino acid sequences deduced from ω-gliadin DNA sequences at GenBank are further discussed.

MATERIALS AND METHODS

Plant Materials and DNA Isolation

Seeds of *T. turgidum* sp. *paleocolchicum* accession AS2275, were collected and conserved by the Triticeae Research Institute of Sichuan Agricultural University in 2007.

Seeds were germinated in Petri dishes under the dark at 25°C for 1 week, young leaves were harvested and crushed into powder with the aid of liquid nitrogen and the genomic DNA was extracted by a CTAB method (Yan *et al*., 2002).

PCR Amplification

A pair of primers (PF1 and PR1) was designed to amplify the complete ORF (open reading frame) based on known ω-gliadin gene sequences. The sequences of primers were:

Pwf1: 5'-ATGAAGACCTTCCTCATCTTTG-3',
Pwr1: 5'-TCATTGGCCACCGATGCTTGT -3'

PCR amplifications were performed in 50 μL reaction volume, which containing 1.5 U Taq plus DNA polymerase, 100 ng templet DNA, 5 μL PCR buffer (supplied with Taq plus DNA polymerase), 1.5 mM $MgCl_2$, 100 mM of each dNTP and 150 ng each primer. The reactions were conducted in a PTC-100 (Bio-Rad) using the following program: 94°C for 4 min denaturation, followed by 35 cycles of 45 sec at 94°C, 1 min at 63°C, 1.5 min at 72°C and 10 min at 72°C.

Cloning and Sequencing of PCR Products

PCR products were separated on 1.0% agarose gels. The expected fragments were purified from the gels using Quick DNA extraction kit (OMIGA). Subsequently purified products were ligated into pMD18-T vector (TaKaRa, Dalian, China) and transformed into competent cells of *E. coli* (DH-5α), the recombinant colonies were amplified using the primers PF1 and PR1 to identify the clones with an insert. The positive clones were sequenced by TaKaRa (Dalian, China).

Sequence Analyses

The obtained sequences were compared with known sequences using BLAST (http://www.ncbi.nlm.nih/gov). The nucleotide and deduced amino acid sequence analyses were conducted by using programs deposited in the NCBI network. Sequence alignment was completed by DNAMAN 5.2.2 (http://www.lynnon.com).

RESULTS

Isolation and Sequencing of ω-Gliadin Clones

Specific PCR products were ligated into pMD18-T vector and then transformed into *E. coli* DH-5α competent cells. Most product sizes were around 1200 bp, however, several products with various sizes ranging from 300 to 600 bp were also found. The clones with insert size of about 1200 bp were sequenced. Three sequences, designated *ω-gli-1*, *ω-gli-2* and *ω-gli-3*, were determined and deposited in the GenBank of NCBI under the accession numbers GU220053, GU220052 and GU220054.

Nucleotide Sequence Analysis

The presumptive ω-gliadin sequences were analyzed using BLAST (Altschul *et al*., 1997). It was found that the cloned gliadin genes had the closest homology with other ω-gliadins registered at GenBank, confirming the identity of the new sequences as the members of ω-gliadin family. Length of *ω-gli-1*, *ω-gli-2* and *ω-gli-3* was 984, 1080 and 1080 bp, respectively.

Comparison of nucleotide sequences of the three ω-gliadin genes with the other prolamin genes, C-hordein from barley and ω-secalin from rye (Table 1) revealed that *ω-gli-1*, *ω-gli-2* and *ω-gli-3* were highly homologous to the ω-gliadin genes from 1A and 1D genome (79.64-89.41%), slightly less homologous to the C-hordein (72.25-73.94%) and ω-secalin (70.49-70.61%) genes, moderately homologous to the ω-gliadin genes encode by 1B genome (40.59-52.80%) and has much less homologous to the α-gliadin and γ-gliadin genes (37.29-44.24%).

Amino Acid Sequences Analyses

The derived amino-acid sequences were aligned with all known 1A and 1D ω-gliadin amino acid sequences deposited at GenBank (Fig. 1). As it is the case for other storage

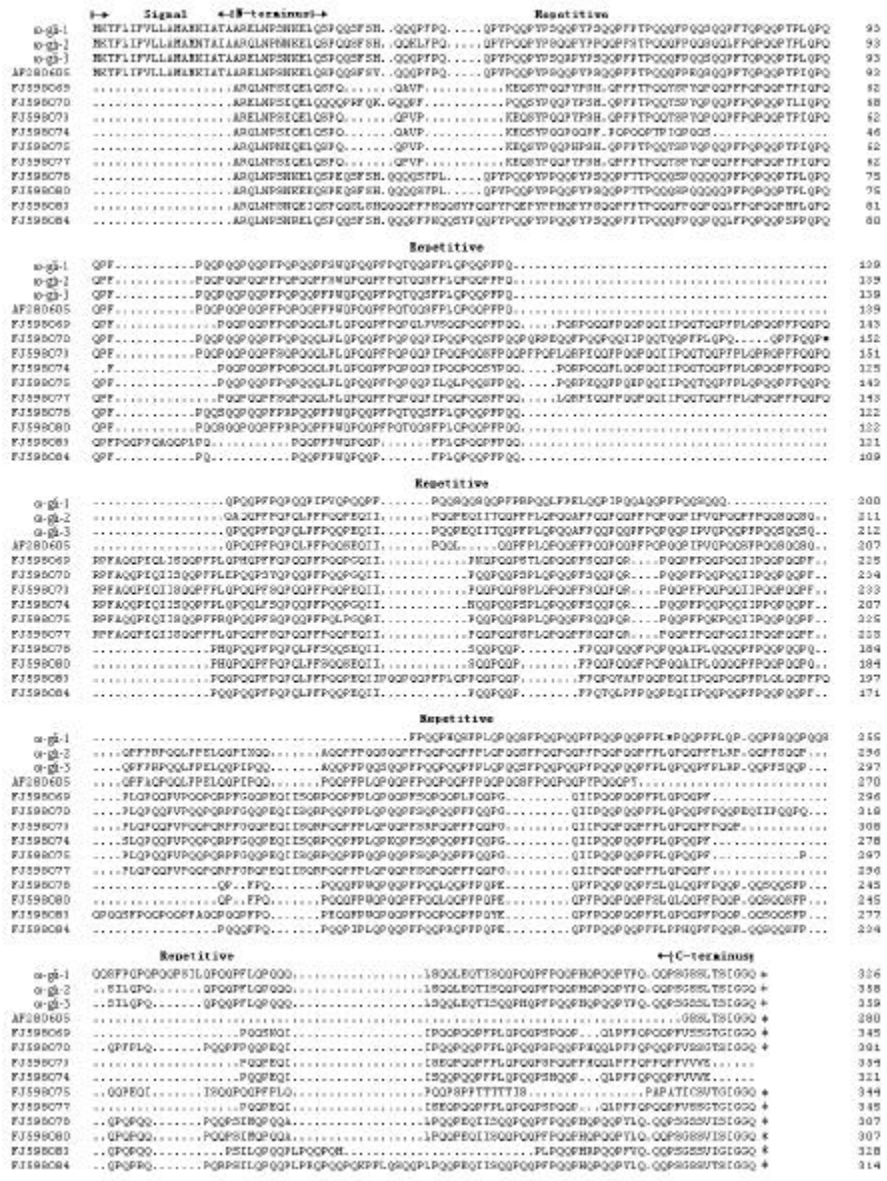

Fig. 1: Comparison of the deduced amino acids sequences with all ARE-/ARQ type ω-gliadin amino acid sequences deposited at GenBank (pseudogenes were not included). "*" represents the stop condons, "."marks the deletions

protein genes (Rafalski, 1989), the genes had no intron. They had a structure similar to previously characterized ω-gliadin genes, which consist of a 19-residue putative signal peptide, 10-11 residue non-repetitive N-terminus, a repetitive region encompassing 90-96% of the protein and a 10-11 residue C-terminus. *ω-gli-1* and *ω-gli-2* were considered as pseudogenes, due to the premature stop codon. *ω-gli-3* could encode a putative mature proteins with 359 amino acid residues.

Table 1: Comparison of nucleotide sequences of ω-gliadin genes

Genes type	Accession	Origin	Identity (%)			References
			ω-gli-1	*ω-gli-2*	*ω-gli-3*	
ω-gliadin	AF280606	*T. aestivum*	84.07	88.77	89.41	Hsia and Anderson (2001)
ω-gliadin	AY667097	*Ae. tauschii*	80.32	84.42	86.18	Unpublished
ω-gliadin	AUS18913	*Ae. tauschii*	79.64	82.75	85.18	Hassani *et al.* (2008)
ω-gliadin	AB181300	*T. aestivum* (1B)	40.59	42.17	44.70	Matsuo *et al.* (2005)
ω-gliadin	AB181301	*T. aestivum* (1B)	46.91	50.76	52.80	Matsuo *et al.* (2005)
C-hordein	M36941	*H. vulgare*	72.25	73.86	73.95	Entwistle (1988)
ω-secalin	X60294	*S. cereale*	70.49	70.43	70.61	Hull *et al.* (1991)
α-gliadin	EU401787	*T. turgidum* sp. *paleocolchicum*	44.44	41.27	41.43	Chen *et al.* (2008)
γ-gliadin	AF234644	*T. aestivum*	37.29	38.52	38.63	Anderson *et al.* (2001)

Table 2: Comparison of N-terminal sequences of *ω-gli-1*, *ω-gli-2* and *ω-gli-3* with ω-gliadins from A, B and D genome of wheat, C-hordein of barley and ω-secalin of rye

Protein	Source	N-terminal amino acid sequence	References
Wheat			
ω-gli-1	*T. turgidum* sp. *paleocolchicum*	ARELNPSNKELQSPQQSFSHQQQP	This study
ω-gli-2	*T. turgidum* sp. *paleocolchicum*	ARQLNPSNKELQSPQQSFSHQQKL	This study
ω-gli-3	*T. turgidum* sp. *paleocolchicum*	ARELNPSNKELQSPQQSFSHQQQP	This study
ω-2	*T. monococcum*	ARQLNPSDQELQSPQQLYPQQPY	Kasarda *et al.* (1983)
ω-2	*T. speltoides*	ARQLNPSNKELQSPQQSFY	Tatham and Shewry (1995)
ω-2	*T. monococcum*	ARQLNPPSDQELQSPQQLYPQQPY	Tatham and Shewry (1995)
ω-2	*T. aestivum*	ARELNPSNKELQSPQQSFS	Kasarda *et al.* (1983)
ω-1D^t gliadin	*T. tauschii* (D)	ARELNPSNKELQSPQQSFSHQQQPFPQQPY	Hassani *et al.* (2008)
ω-2D^t gliadin	*T. tauschii* (D)	ARELNPSNKE	Kasarda *et al.* (1983)
ω-1A	*T. aestivum* CS (A)	ARHLNPSDQELQSPQQQFLKKQSYPLQPYP	Masoudi-Nejad *et al.* (2002)
ω-1	*T. monococcum*	RQLNPSDQELQSPQQLYPQQPYQQPY	Kasarda *et al.* (1983)
ω-1	*T. durum*	KELQSPQQSFXHQQQPF	Kasarda *et al.* (1983)
ω-1	*T. aestivum*	KELQSPQQSFSHQQQPFPQQPYPQQPY	Kasarda *et al.* (1983)
ω-5	*T. aestivum*	SRLLSPRGKELHTPQQQFPQQ	Kasarda *et al.* (1983)
1B2-ω	*T. aestivum* (B)	SRLLSPRGKELHTPQEQFPQQQ	Dupont *et al.* (2000)
ω-5	*T. aestivum Norin 61* (B)	SRLLSPRGKELHTPQEQFPQQQQF	Matsuo *et al.* (2005)
Barley			
C-1 hordein	*H. vulgare*	RQLNPSSQELQSPQQSYLQQPYPQNPY	Shewry *et al.* (1980)
pBe14	*H. vulgare*	RQLNPSHQELQSPQQPFLKQQSYL	Entwistle (1988)
Pbr17	*H. vulgare*	RQLNPSSQELQSPQQSYLQQPYPQ	Entwistle *et al.* (1991)
Rye			
pSec2B	*S. cereale*	RQLNPSEQELQSPQQPVPKEQSYPQQPYSH	Hull *et al.* (1991)
R2	*S. cereale*	RQLNPSEQELQSPQQPVPKEESY	Rybalka *et al.* (1985)
ω-1 secalin	*S. cereale*	RQLNPSEQELQSPQQPV	Shewry *et al.* (1980)

ω-gli-2 is highly similar to the sequence of *ω-gli-3* (only 17 amino acid residues are different), while lower level of similarity was observed with *ω-gli-1* due to a 34 amino acid deletion and two insertions with QQSQQ and PQQPSIL in repetitive region.

The deduced amino acid sequence for *ω-gli-1*, *ω-gli-2* and *ω-gli-3* indicated high frequencies of glutamine (Q) (38-41%), proline (P) (28-30%) and phenylalanine (F) (7-9%) and included no cysteine (C) residue, which is characteristic of ω-gliadins (Kasarda *et al*., 1983).

N-Terminal Amino Acid Sequences

N-terminal amino-acid sequences derived from *ω-gli-1*, *ω-gli-2* and *ω-gli-3* are aligned in Table 2. The three deduced N-terminal sequences started with ARQ/AQE, belonging to ω-2 type ω-gliadins based on the N-terminal classify by Kasarda *et al.* (1983). The N-termial sequences were also compared with those of ω-gliadins from the hexaploid wheat and wild

Fig. 2: Repetitive motifs of *ω-gli-1* by DNA sequence

species, C-hordein from *H. vulgare* and ω-secalin from *S. cereale* (Table 2). These N-terminal sequences of the three new ω-gliadin are similar to that from 1A and 1D ω-gliadins, C-hordein and ω-secalin, while less homologous to that from 1B ω-gliadins.

Repeat Structure

The repetitive domain of the gliadins is composed of short peptide motifs. The DNA sequence of the repeat domain of *ω-gli-1* is arrayed vertically in Fig. 2. The DNA sequences of the repetitive domain can be considered as CCA TTT/C CCC CAG CAA CCC/A CAA CAA. The derived amino acid motif $PFPQ_{1-2}PQQ$ is shown at the bottom and is identical to that reported for other ω-gliadins (Hsia and Anderson, 2001). Extra one to three Q codons were found in several motifs. Large fragment insertion/deletion was frequent in the repetitive domain of ω-gliadin sequences (Fig. 2). 34 amino acid residues deletion and two insertions with QQSQQ and PQQPSIL were found in *ω-gli-1*compared to *ω-gli-2* and *ω-gli-3*.

DISCUSSION

Comparison of N-terminal sequences of *ω-gli-1*, *ω-gli-2* and *ω-gli-3* with ω-gliadins from A, B and D genome of wheat, C-hordein of barley and ω-secalin of rye (Table 2), the

Table 3: Comparison of N-terminal amino acid sequences deduced from all the ω-gliadin DNA sequences at GenBank

Organism	Gene type	Accession	Deduced N-terminal amino acids sequences
T. turgidum sp. *paleocolchicum*	Pseudogene	*ω-gli-1*[a]	ARELNPSNKELQSPQQSFSHQQQP
T. turgidum sp. *paleocolchicum*	Pseudogene	*ω-gli-2*[a]	ARQLNPSNKELQSPQQSFSHQQKL
T. turgidum sp. *paleocolchicum*	Putatively functional gene	*ω-gli-3*[a]	ARELNPSNKELQSPQQSFSHQQQP
T. aestivum	Pseudogene	GQ423432	ARHLNPSDQELQSPQRQFLKKQSYPLQPYP
T. aestivum	Pseudogene	GQ423431	ARHLNPSDQELQSPQQQFLKKQSYPLQPYPH
T. aestivum	Pseudogene	GQ423430	ARHLNPSDQELQSPQQQFLKKQSYPLQPYP
T. aestivum	Pseudogene	GQ423428	ARHLNPSDQELQSPQQQFLEK*SYPLQPYP
T. aestivum	Pseudogene	DQ307378	ARHLNPSDQELQSPRQQFLKKQSYPLQPYP
T. aestivum	Pseudogene	DQ287981	ARHLNPSDQELQSPRQQFLKKQSYPLQPYP
T. aestivum	Putatively functional gene	AY591334	ARHLNPSDQELQSPQQQFLEKTIISAATIS
Lophopyrum elongatum	Pseudogene	FJ598082	ARQLNPSNQELQSPQQPFAHE*QPFPKQQS
Lophopyrum elongatum	Pseudogene	FJ598081	ARQLNPSNQEKQSPQQSLSHQQQQPFPKQQ
Lophopyrum elongatum	Pseudogene	FJ598079	ARQLNPSNQELQSPQQSFSHQQQPFPKQQS
T. aestivum×*Lophopyrum elongatum*	Pseudogene	FJ598076	ARQLNPSEQELQSPQQPVPKEQSYPQQPYP
T. aestivum	Pseudogene	EF116277	ARQLNPNNKELQSPQRSFSHQQKLFPQQPYP
T. aestivum	Pseudogene	AF280606	ARQLNPSNKELQSPQQSFSHQQQPFP
T. aestivum×*Lophopyrum elongatum*	Putatively functional gene	FJ598078	ARQLNPSNKELQSPEQSFSH
T. aestivum×*Lophopyrum elongatum*	Putatively functional gene	FJ598077	ARQLNPSEQELQSPQQPVPKEQSYPQQ
T. aestivum×*Lophopyrum elongatum*	Putatively functional gene	FJ598075	ARQLNPNEQELQSPQQPVPKEQSYPQQ
T. aestivum×*Lophopyrum elongatum*	Putatively functional gene	FJ598084	ARQLNPSNRELQSPQQSFSHQQQPFPKQQS
T. aestivum×*Lophopyrum elongatum*	Putatively functional gene	FJ598083	ARQLNPSNQEIQSPQQSLSHQQQQPFPK
T. aestivum×*Lophopyrum elongatum*	Putatively functional gene	FJ598080	ARQLNPSNKEKQSPEQSFSHQQQQSYPLQPYPQQ
T. aestivum	Putatively functional gene	FJ598074	ARQLNPSEQELQSPQQAVPKEQSYPQQPQQPFPQPQQ
T. aestivum	Putatively functional gene	FJ598069	ARQLNPSEQELQSPQQAVPKEQSYPQQPYPSHQPF
T. aestivum	Putatively functional gene	FJ598073	ARELNPSEQELQSPQQPVPKEQSYPQQPYPSHQPFP
T. aestivum	Putatively functional gene	FJ598070	ARELNPSEQELQQQQPRFQKGQQPFPQQSYPQQPYP
Ae.tauschii	Putatively functional gene	AY667097	ARELNPSNKELQSPQQSFSHQQQPFPQQPY
T. aestivum	Putatively functional gene	AF280605	ARELNPSNKELQSPQQSFSYQQQPF
T. aestivum	Pseudogene	AB181301	SRLLSPRGKELHTPQEQFPQQQ
T. aestivum	Putatively functional gene	AB181300	SRLLSPRGKELHTPQEQFPQQQQFP
T. aestivum	Putatively functional gene	AJ937839	SRLLSPSDQQLQSPQQQFPEEQSYPQQPYPQQ

[a] This study

KEL types differ from the ARQ/E types in the absence of the first eight residues. Based on all the deduced N-terminal amino acid sequences from the ω-gliadin DNA sequences at GenBank (Table 3), ω-gliadin could be divided into ARH-/ARQ-/ARE- and SRL-type. No KEL- and RQ-type were found. It supported the suspicion by DuPont *et al.* (2004) that KEL-type proteins may be the result of post-translational cleavage of the ARQ- and RPL- type proteins between the asparagin (N) and lysine (K). But the sequence of ω-gliadin in GenBank is limited; more gene sequences are needed for a better comparison.

The repeat domain motif from this paper is identical to that reported for other 1A and 1D ω-gliadins (Hsia and Anderson, 2001; Anderson *et al.*, 2009) and is similar to that proposed for the C-hordeins and ω-secalins, PQQPFPQQ (Tatham and Shewry, 1995) (Table 4). It is also very similar to that proposed for γ-gliadin, $PFPQ_{1-2}(PQQ)_{1-2}$ (Anderson *et al.*, 2001). 1A and 1D ω-gliadins have a longer repeat motif patten than 1B, the reason is assumed by Anderson *et al.* (2009) that drifting happened in the repetitive domain after the B-genome ancestor separated from a common ancestor of the A- and D-genomes.

Table 4: Repeat domain motifs of ω-gliadins from A, B and D genome, C-hordein, ω-secalin and the major classes of the gliadin superfamily

Type	Amino acids	References
ω-gliadin	$PFPQ_{1-2}PQQ$	This study
ω-gliadin (A genome)	$PFPQ_{1-2}PQQ$	Hsia and Anderson (2001)
ω-gliadin (B genome)	FPQ_{2-4}	Anderson *et al.* (2009)
ω-gliadin (D genome)	$PFPQ_{1-2}PQQ$	Anderson *et al.* (2009)
C-hordein	PQQPFPQQ	Tatham and Shewry (1995)
ω-secalin	PQQPFPQQ	Tatham and Shewry (1995)
α-gliadin	$PFPQ_{3-6}$	Chen *et al.* (2008)
γ-gliadin	$PFPQ_{1-2}(PQQ)_{1-2}$	Anderson *et al.* (2001)
LMW-glutenin	$P_{1-2}FPSQ_{2-6}$	Cassidy *et al.* (1998)

The insertions and large section deletion occur in the repeat domain of *ω-gli-1* compare with *ω-gli-2* and *ω-gli-3*. It also happened in other ω-gliadin genes with high frequency (Hsia and Anderson, 2001; Hassani *et al.*, 2008). It is probably because the large repetitive domain of ω-gliadin gene with repeated units of various peptides is apparently unstable, the occurrence of insertion/deletion mutations occur during the cloning process (Hsia and Anderson, 2001; Hassani *et al.*, 2008). Another possible reason is the high CAA/CAG ratio in ω-gliadin. The CAA/CAG ratio for the ω-gliadin (7:1) is much higher than that for the other major wheat prolamine classes (2:1). One consequence of higher frequencies of one codon in homopolymer runs is increase opportunity for unequal recombination and/or slip-mismatching during replication (Gojobori *et al.*, 1982).

A large number of ω-gliadin pseudogenes have been reported (Hsia and Anderson, 2001; Altenbach and Kothari, 2007; Anderson *et al.*, 2009). In this report, *ω-gli-1* and *ω-gli-2* are assumed to be pseudogenes because of the in-frame stop codon within repetitive domain. Almost all of the nonsense mutations were resulted from the C to T change in glutamine codons CAA and CAG. The C to T change induced in the stop codons TAA and TAG. It was theorized to predominate because of the ability of 5-methyl-cytidine to be incorrectly replicated as a thymidine (Gojobori *et al.*, 1982). In this report, the nonsense mutations in both *ω-gli-1* and *ω-gli-2* were resulted from the C to T change. In addition, some T to A, C to A, C to G and G to A changes were also found in α-gliadin (Teun *et al.*, 2006).

The distribution of pseudogenes varies with each prolamins gene family. The α-gliadin family is estimated to comprise 50% pseudogenes (Anderson *et al.*, 1997); only a few pseudogenes were reported in both γ-gliadins and LMW-GS families. The ω-gliadin genes have large repetitive domain and high frequency of glutamine codons, so they were suspected to have more premature stop codon generation than other prolamins (Anderson *et al.*, 2009).

CONCLUSION

Three new ω-gliadin sequences were reported and their relationship with other 1A, 1B and 1D ω-gliadins, C-hordeins and ω- secalins were discussed. The sequence identity and repeat domain motifs of ω-gliadin, C-hordeins and ω-secalins confirm that 1A and 1D ω-gliadins are closely related to each other and to the C-hordeins and ω- secalins, but differ from 1B ω-gliadins. The interesting found is, based on the N-terminal protein sequences, ω-gliadins could be divided into KEL, ARQ, ARE, XRQ, or RQ and SRL types (Kasarda *et al.*, 1983), whereas, based on the DNA sequences, only the ARH-/ARQ-/ARE- and SLK-type ω-gliadins were deduced. No KEL- and RQ-type were found. It supported the suspicion by DuPont *et al.* (2004) that KEL- type proteins may be the result of post-translational cleavage of the ARQ- and RPL- type proteins between the asparagin (N) and lysine (K).

ACKNOWLEDGMENTS

We thank Dr. Jenny Fegent for reading the manuscript and making suggestions and corrections. This study was supported by the Key Technologies R and D Program (2006BAD01A02-23 and 2006 BAD13B02-06).

REFERENCES

Altenbach, S.B. and K.M. Kothari, 2007. Omega gliadin genes expressed in *Triticum aestivum* cv. Butte 86: Effects of post-anthesis fertilizer on transcript accumulation during grain development. J. Cereal Sci., 46: 169-177.

Altschul, S.F., F. Stephen, T.L. Maden, A.A. Shaffer and Z. Jinghui *et al*., 1997. Gapped blast and psi-blast a new generation of protein database search programs. Nucleic Acids Res., 25: 3389-3402.

Anderson, O.D., J.C. Litts, M.F. Gautier and F.C. Greene, 1984. Nucleic acid sequences and chromosome assignment of a wheat storage protein gene. Nucleic Acids Res., 12: 8129-8144.

Anderson, O.D., J.C. Litts and F.C. Greene, 1997. The alpha-gliadin gene family. I. Characterization of ten new wheat alpha-gliadin genomic clones, evidence for limited sequence conservation of flanking DNA and Southern analysis of the gene family. Theor. Applied Genet., 95: 50-58.

Anderson, O.D., C.C. Hsia, and V. Torres, 2001. The wheat γ-gliadin genes: Characterization of ten new sequences and further understanding of γ-gliadin gene family structure. Theor. Applied Genet., 103: 323-330.

Anderson, O., Y. Gu, X. Kong, G. Lazo and J. Wu, 2009. The wheat ω-gliadin genes: Structure and EST analysis. Func. Integr. Genomic, 9: 397-410.

Bartels, D., J. Altosaar, N.P. Harberd, R.F. Barker and R.D. Thompson, 1986. Molecular analysis of gamma-gliadin gene families at the complex Gli-1 locus of bread wheat (*T. aestivum* L.). Theor. Applied Genet., 72: 845-853.

Cassidy, B.G., J. Dvorak and O.D. Anderson, 1998. The wheat low-molecular-weight glutenin genes: Characterization of six genes and progress in understanding gene family structure. Theor. Applied Genet., 96: 743-750.

Chen, X., W. Li, Y. Wei, G. Chen and Y. Zheng, 2008. Cloning and molecular characterization of nine α-gliadin genes from *Triticum turgidum* ssp. *paleocolchicum*. J. Boil. Sci., 8: 542-548.

DuPont, F.M., W.H. Vensel, R. Chan and D.D. Kasarda, 2000. Characterization of the 1B-type ω-gliadins from *Triticum aestivum* cultivar Butte. Cereal Chem., 77: 607-614.

DuPont, F., W. Vensel, T. Encarnacao, R. Chan and D. Kasarda, 2004. Similarities of omega gliadins from Triticum urartu to those encoded on chromosome 1A of hexaploid wheat and evidence for their post-translational processing. Theor. Applied Genet., 108: 1299-1308.

Entwistle, J., 1988. Primary structure of a C-hordein gene from barley. Carlsberg Res. Commun., 53: 247-258.

Entwistle, J., S. Knudsen, M. Muller and V. Cameron-Mills, 1991. Amber codon suppression: The *in vivo* and *in vitro* analysis of two C-hordein genes from barley. Plant Mol. Biol., 17: 1217-1231.

Gojobori, T., W.H. Li and D. Graur, 1982. Patterns of nucleotide substiturion in pseudogenes and functional genes. J. Mol. Evo., 18: 360-369.

Hassani, M., M. Shariflou, M. Gianibelli and P. Sharp, 2008. Characterization of a ω-gliadin gene in *Triticum tauschii*. J. Cereal Sci., 24: 73-78.

Hsia, C.C. and O.D. Anderson, 2001. Isolation and characterization of wheat -gliadin genes. Theor. Applied Genet., 103: 37-44.

Hull, G., N. Halford, M. Kreis and P. Shewry, 1991. Isolation and characterization of genes encoding rye prolamins containing a highly repetitive sequence motif. Plant Mol. Biol., 17: 1111-1115.

Kasarda, D.D., J.C. Autran, E.J.L. Lew, C.C. Nimmo and P.R. Shewry, 1983. N-terminal amino acid sequences of ω-gliadins and ω-secalins; Implications for the evolution of prolamin genes. Biochim. Biophys. Acta, 747: 138-150.

Masoudi-Nejad, A., S. Nasuda, A. Kawabe and T. Endo, 2002. Molecular cloning, sequencing, and chromosome mapping of a 1A-encoded ω-type prolamin sequence from wheat. Genome, 45: 661-669.

Matsuo, H., K. Kohno and E. Morita, 2005. Molecular cloning of cDNA, recombinant protein expression and characterization of a buckwheat 16-kDa major allergen. FEBS J., 272: 4431-4438.

Metakovsky, E.V., A.Y. Novoselskaya and A.A. Sozinov, 1984. Genetic analysis of gliadin components in winter wheat using two-dimensional polyacrylamide gel electrophoresis. Theor. Applied Genet., 69: 31-37.

Mori, N., T. Moriguchi and C. Nakamura, 1997. RFLP analysis of nuclear DNA for study of phylogeny and domestication of tetraploid wheat. Genes Genet. Syst., 72: 153-161.

Pistóna, F., G. Doradob, A. Martína and F. Barro, 2006. Cloning of nine γ-gliadin mRNAs (cDNAs) from wheat and the molecular characterization of comparative transcript levels of γ-gliadin subclasses. J. Cereal Sci., 43: 120-128.

Rafalski, J.A., 1989. Structure of wheat gamma-gliadin genes. Gene, 43: 221-229.

Rybalka, A.I., D.D. Kasarda and A.A. Sozinov, 1985. Rye R-gliadins (prolamins) synthesized in wheat endosperm. Agric. Bio., 2: 34-42.

Scheets, K. and C. Hedgcoth, 1988. Nucleotide sequence of a γ gliadin gene: Comparisons with other gamma-gliadin sequences show the structure of γ gliadin genes and the general primary structure of γ gliadins. Plant Sci., 57: 141-150.

Seilmeier, W., I. Valdez, E. Mendez and H. Wieser, 2001. Comparative investigations of gluten proteins from different wheat species. Eur. Food Res. Tech., 212: 355-363.

Shewry, P.R., A.S. Tatham, J. Forde, M. Kreis and B.J. Miflin, 1986. The classification and nomenclature of wheat gluten proteins: A reassessment. J. Cereal Sci., 4: 97-106.

Shewry, P.R., J.C. Autran, C.C. Nimmo, J.L.L. Ellen and D.D. Kasarda, 1980. N-terminal amino acid sequence homology of storage protein components from barley and a diploid wheat. Nature, 286: 520-522.

Tatham, A. and P. Shewry, 1995. The S-poor prolamins of wheat, barley and rye. J. Cereal Sci., 22: 1-16.

Teun, W.J.M., van Herpen and S.V. Goryunova, 2006. α-gliadin genes from the A, B and D genomes of wheat contain different sets of celiac disease epitopes. BMC. Genomics, 7: 1-1.

Yan, Z.H., Y.F. Wan, K.F. Liu, Y.L. Zheng and D.W. Wang, 2002. Identification of a novel HMW-GS and comparison of its amino acid sequence with those of homologous subunits. Chin. Sci. Bull., 47: 220-225.