RESEARCH NOTE

Cloning and characterization of novel fast ω-gliadin genes in *Triticum monococcum*

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Introduction

Fast ω-gliadin is the major allergen to wheat-dependent exercise-induced anaphylaxis (WDEIA). It is generally accepted that the proteins were expressed in *Gli-B1* loci located on the short arm of chromosome 1B in wheat. The knowledge of the orthologous genes in A and D genomes is still limited. In this study, the novel fast ω-gliadin genes were cloned from genome A of diploid wheat *Triticum monococcum.* The deduced amino acid sequences of N-terminal domain showed that they referred to TRQ-type ω-gliadins. This type of ω-gliadins have been identified in genome A by using mass spectroscopy and amino acid sequencing in previous studies (DuPont *et al.* 2004), but their coding sequences were lacking. Further analysis of IgE-binding epitopes suggested that the TRQ-type ω-gliadins have potential to trigger WDEIA.

Wheat is one of the most important sources of food in the human diet. In the kernels of wheat, gliadins are the main components of the gluten. Traditionally, gliadins are further classified into α-gliadins, γ-gliadins and ω-gliadins on the basis of their mobility in electrophoresis at low pH (Wan *et al.* 2014). The ω-gliadins are minor components among wheat prolamins and they have not been studied well due to difficulties in cloning their genes caused by highly repetitive sequences (Anderson *et al.* 2009). In common wheat, most of the ω-gliadins were encoded by the *Gli-A1*, *Gli-B1* and *Gli-D1* on the short arm of chromosomes 1A, 1B and 1D, respectively (Pogna *et al*. 1990; Shewry *et al*. 1995; DuPont *et al*. 2000, 2004). The ω-gliadins have been categorized into ω1, ω2 and ω5 types (DuPont *et al.* 2004). The ω5-gliadin, also referred as fast ω-gliadin, are encoded by *Gli-B1* and the proteins can be distinguished by the amino acid sequences at the N-terminal domain, which are Ser-Arg-Leu (SRL) in ω5-gliadin, Ala-Arg-Gln (ARQ) or Lys-Glu-Leu (KEL) in ω1-gliadin and Ala-Arg-Glu (ARE) in ω2-gliadin (Wan *et al.* 2014).

The ω5-gliadins have received great attention because they have been identified as the major allergen of WDEIA, a distinct form of wheat allergy induced by exercise (Matsuo *et al*. 2004, 2005; Battais *et al*. 2005; Denery-Papini *et al*. 2007; Cai and Yin 2013). WDEIA is a life-threatening disease and the clinical symptoms include swelling, urticaria or even anaphylactic shock (Matsuo *et al*. 2004, 2005; Cai and Yin 2013). Therefore, the objectives of this work were to isolate the fast ω-gliadin genes from *T. monococcum* and to analyse the allelic variation, potential allergenicity of the proteins and evolutionary relationships with the other storage proteins.

Materials and methods

Plant material

T. monococcum accession DV92 was grown in the experimental station of Shandong Agricultural University in Taian, China. Seeds from 15 days postanthesis were collected and stored temporarily in liquid nitrogen.

RNA extraction and reverse-transcription PCR (RT-PCR)

Total RNA was isolated from young seeds using the RNAprep pure Plant Kit (Tiangen, Beijing, China) following the manufacturer's instructions. RT-PCR was carried out in use of PrimeScript TM II 1st strand cDNA Synthesis Kit (Takara, Dalian, China).

PCR amplification, cloning and sequencing

Fast ω-gliadin gene-specific primers (P1: 5′-ATGAAGACC TTCATCATATTTGTCCTC-3′ and P2: 5′-CATCGTTGTTAG

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TCAATGGAGCATAA-3′) were designed based on the published fast ω-gliadin gene sequences of GenBank AB181300 and AB181301. PCR was performed in a total volume of 50 μL containing 2.5 U of ExTaq DNA polymerase and GC buffer I (Takara, Dalian, China). The reactions were performed as per the following protocol: 95°C for 5 min, 35 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 1.5 min and a final extension at 72°C for 10 min. The PCR products were separated in 1% agarose gel and retrieved by Universal DNA Purification Kit (Tiangen, Beijing, China). The procedures of

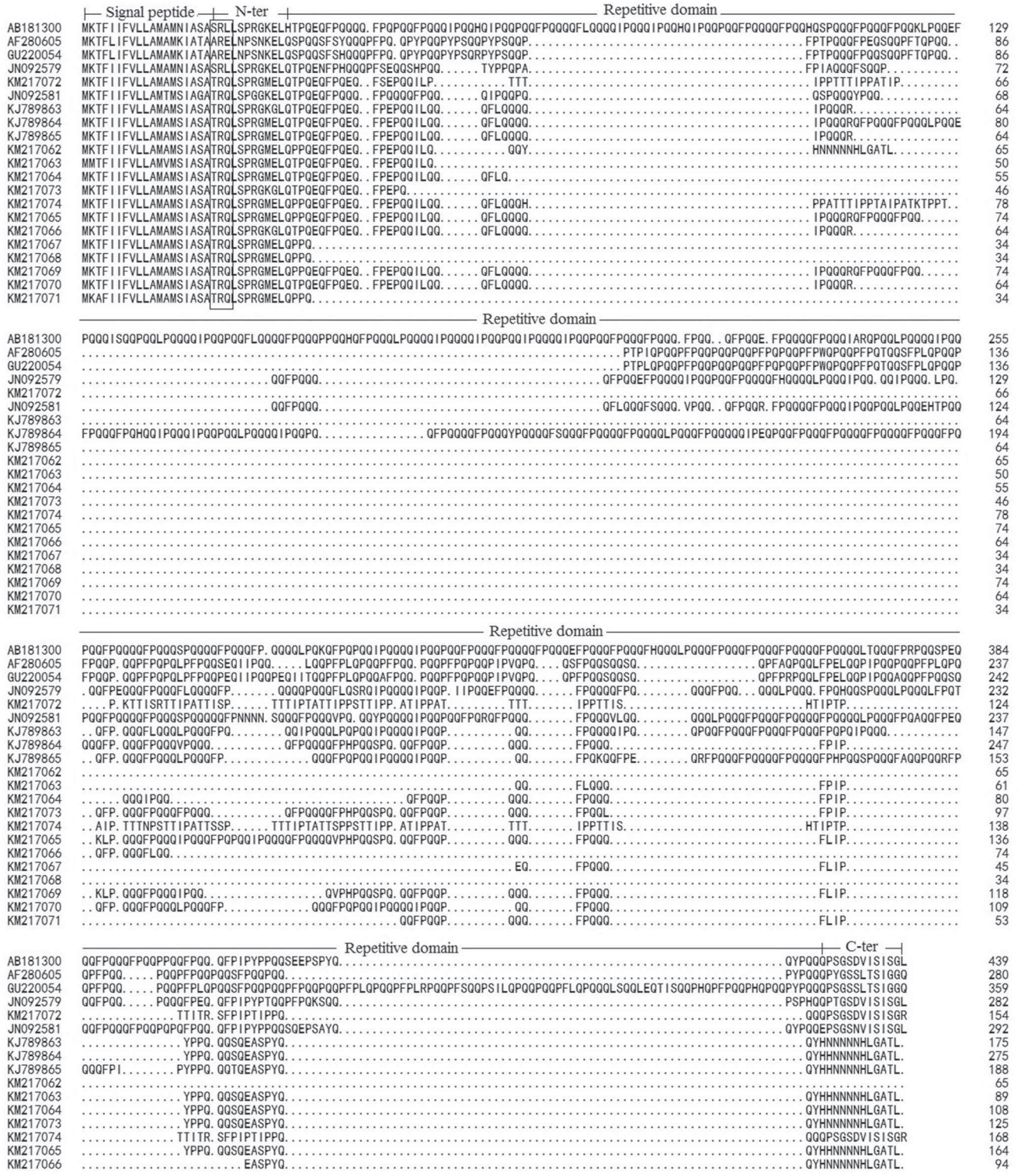

Figure 1. Multiple alignments of the deduced amino acid of ω-gliadins. The first three amino acids of the N-terminal domain are boxed.

cloning and sequencing were carried out in accordance with Li *et al.* (2012).

Sequence comparison and identification of IgE-binding epitopes

Complete amino acid sequences of fast ω-gliadins were used to create multiple sequence alignments by using DNAMAN 6.0.2 software. The sequences of IgE-binding epitopes were predicted according to Mastuo *et al*. (2004, 2005) and Battais *et al*. (2005), and only perfect matches were considered. A neighbour-joining (NJ) tree was constructed using the deduced amino acid sequences from wheat and other species according to Xie *et al.* (2010).

Results

Molecular cloning of 16 novel fast ω-gliadin genes

The size of major targeted DNA fragment was ∼1200 bp, but amplified fragments of 1000 and 500 bp were obtained (figure 1). A total of 16 full-ORF genes were identified after cloning and sequencing. The length of the genes ranged from 177 to 828 bp and the molecular weight of deduced amino acids ranged from 4.48 to 31.28 kDa (table 1). All the 16 complete gene sequences were deposited in GenBank (accession numbers KJ789863–KJ789865 and KM217062–KM217074).

Sequence comparison and phylogenetic analysis of fast ω-gliadin genes

Based on the deduced amino acid sequences, ω-gliadins in the present investigation were identified as TRQ type. Multiple alignments of deduced amino acid sequences showed that TRQ-type ω-gliadins had highly similar structures with SRL-type ω-gliadins (e.g. ω5-gliadin), but was different from ARE-type (e.g. ω2-gliadin) (figure 1). Comparison with ω5-gliadin AB181300 indicated that all the cloned genes contained frame-shift mutations. The insertion mutation of a single nucleotide (base A) at positions 184 and 151 of KM217072 and KM217074, respectively, lead to abnormal repetitive sequences. The other 14 sequences contained a frame-shift at the same position near the end of the repetitive domain and their amino acid sequences at the C-terminal domain were HHNNKNHLGATL (figure 1). The difference of C-terminal sequences in A and B genomes suggested that the characterization of C-terminal domain may provide a basis for chromosome location of fast ω-gliadin genes.

IgE-binding epitopes were present in almost all of the deduced amino acid sequences in this study and the quantity of QQFPQQQ is the most predominant among the IgE-binding epitopes. Four of the proteins did not contain any IgE-binding epitopes due to the small molecular weight or base mutation (table 2; figure 1).

To analyse the evolutionary relationships among different storage proteins, a NJ tree was constructed using the deduced amino acid sequences of 16 fast ω-gliadin genes obtained in this work and 36 other reported storage protein genes. As shown in figure 2, the proteins were apparently clustered into six groups. The TRQ-type ω-gliadins were clustered together (branch I) and separated from the clade of ARQ-/E-type ω-gliadins (branch V).

Table 1. Description of the ω-gliadin genes cloned in this study.

GenBank number	Sequence length (bp)	Amino acid (aa)	Molecular weight (kDa)	pI
KJ789863	528	175	18.75	6.63
KJ789864	828	275	31.28	5.58
KJ789865	567	188	20.56	6.84
KM217062	198	65	5.43	5.38
KM217063	270	89	8.31	5.34
KM217064	327	108	10.65	5.34
KM217065	495	164	17.48	6.35
KM217066	285	94	8.96	6.63
KM217067	222	73	6.33	5.96
KM217068	177	58	4.48	6.62
KM217069	441	146	15.25	6.21
KM217070	396	131	13.50	5.77
KM217071	246	81	7.30	6.62
KM217072	465	154	14.34	8.43
KM217073	378	125	12.63	6.20
KM217074	507	168	15.81	6.63

pI, isoelectric point.

Discussion

To date, four types of ω-gliadins have been identified and the TRQ-type ω-gliadin in *T. monococcum* had been reported by DuPont *et al.* (2004), but their DNA sequences are still lacking. This is partially attributed to the difficulty in gene isolation as highly repetitive DNA sequences present in the ω-gliadin genes (Zhang *et al.* 2007; Wang *et al.* 2008; Anderson *et al.* 2009; Li *et al.* 2012). Besides, the ω-gliadin genes were not stable in the host bacteria, the numerous occurrences of the sequence GCTGGGG within the ω-gliadin genes may be recognized by the RecBCD enzyme leading to recombination with the genes of bacteria (Li *et al.* 2012). In this study, we attempted cloning fast ω-gliadin genes using the cDNA from developing seeds as template and 16 complete ω-gliadin genes were derived. However, the complete gene with length of 1200 bp was not obtained. Possible reason was that the putative gene contained a large repetitive domain prone to recombination with the host bacteria and they could not be detected in the colony PCR.

The molecular weight of ω-gliadins usually ranged from 44 to 74 kDa (Denery-Papini *et al.* 2007). Compared with the previously studied ω-gliadins, the ω-gliadins identified in this work conserved N-terminal and C-terminal domains but showed much smaller molecular weight due to lose of internal repetitive motifs (figure 1). Similar result was also observed by Anderson *et al.* (2009) who reported a ω-gliadin gene which encoded a 13-kDa protein.

Table 2. Analysis of IgE-binding epitopes in TRQ-type ω-gliadins isolated from DV92.

	Number of IgE-binding epitopes									
GenBank number	QQFHQQQ	QQPPQQ	QQIPQQQ	QQLPQQQ	QQFPQQQ	QQSPEQQ	QQSPQQQ	QQYPQQQ	PYPP	Total
KJ789863	0	0	2	1	5	0	0	0	0	8
KJ789864	0	0	1	2	12	0	1	1	1	18
KJ789865	0	0	1	1	3	0	1	0	1	7
KM217062	0	0	0	0	0	0	0	0	0	0
KM217063	0	0	0	0	0	0	0	0	1	1
KM217064	0	0	0	0	1	0	0	0	1	2
KM217065	0	0	2	0	3	0	1	0	1	7
KM217066	0	0	1	0	0	0	0	0	0	1
KM217067	0	0	0	0	0	0	0	0	1	1
KM217068	0	0	0	0	0	0	0	0	0	0
KM217069	0	0	2	0	1	0	1	0	1	5
KM217070	0	0	2	1	3	0	0	0	0	6
KM217071	0	0	0	0	1	0	0	0	1	2
KM217072	0	0	0	0	0	0	0	0	0	0
KM217073	0	0	0	0	2	0	1	0	1	4
KM217074	0	0	0	0	0	0	0	0	0	0

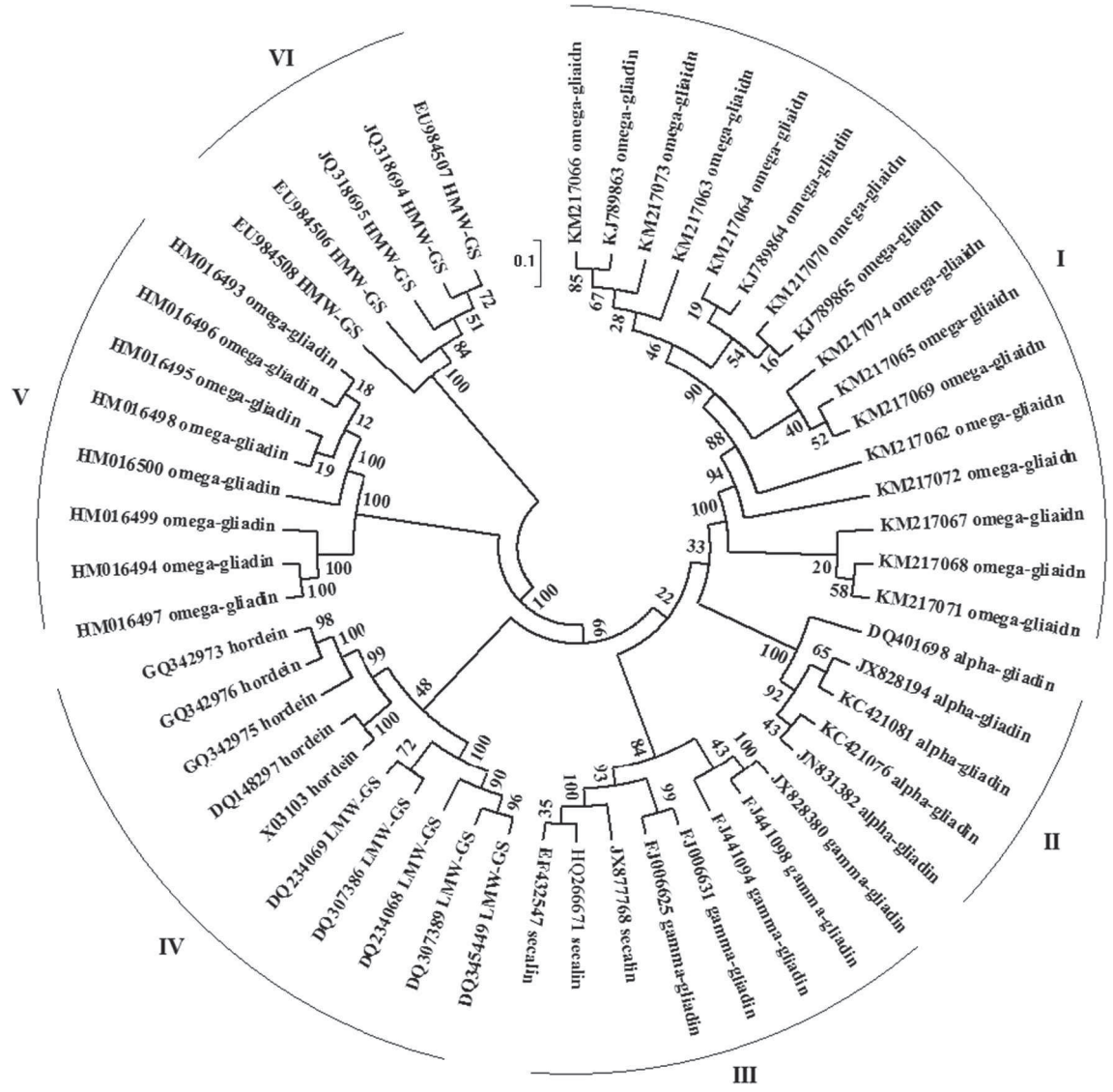

Figure 2. NJ tree based on the deduced amino acid sequences of 16 fast ω-gliadin genes cloned in this study and 36 other storage protein genes previously published.

It is generally accepted that the major allergen of WDEIA is ω-gliadin expressed by *Gli-B1* alleles and the IgE-binding epitopes have been identified (Matsuo *et al*. 2004, 2005; Battais *et al*. 2005), thus, Denery-Papini *et al.* (2007) suggested that the selection of wheat/rye translation lines or genotypes lacking the *Gli-B1* locus may reduce the allergenicity. In this paper, we confirmed that IgE-binding epitopes are not only present in ω5-gliadin, but also in TRQ-type ω-gliadins encoded by A genome. Whether the A genome of wheat/rye translation lines express the TRQ-type ω-gliadins, needs a further study. RNA inferference (RNAi) technology or traditional cross-breeding methods may be applied to eliminate these TRQ-gliadins in wheat (Altenbach *et al.* 2014; Waga and Skoczowski 2014).

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