MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS
OF ONE OMEGA-GLIADIN GENE FROM *Aegilops speltoides* L.

Wanqing WANG[1,4], Ke WANG[2,4], Xi CHEN[1], Slaven PRODANOVIC[3*],
Xiaohui LI[1], Xingguo YE[2*], Yueming YAN[1*]

[1]College of Life Science, Capital Normal University, Beijing, China
[2]Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China
[3]Faculty of Agriculture, University of Belgrade, Belgrade, Serbia
[4]Contributed equally to this work

Wang W., K. Wang, X. Chen, S. Prodanovic, X. Li, X.Ye, Y. Yan (2018): *Molecular characterization and phylogenetic analysis of one omega-gliadin gene from Aegilops speltoides* L..- Genetika, Vol 50, No.2, 503-517.
Gliadins, as the major components of wheat storage proteins, determine the extensibility properties of dough and have important effects on flour processing quality. Wheat related species carries potential storage protein gene resources for quality improvement. In this study, we isolated and characterized the first complete ω-gliadin gene *Omega-AS* from *Aegilops speltoides* L. (2n = 2x = 14, SS) by allelic-specific PCR and investigated its phylogenetic relationships among *Triticum* and *Aegilops* species. Molecular structure showed that *Omega-AS* gene consisted of 1122 bp encoding 373 amino acid residues with deduced molecular mass 41379.21 Da. *Omega-AS* gene was exceptionally rich in prolines and glutamines with fewer methionine and no cysteine. Sequence characterization and epitope analysis showed that three epitopes QQPIPVQPQQ, TQPQQPTPIQ and IQPQQPFPQQ were absent in *Omega-AS* gene encoded protein, indicating its potential value for wheat quality improvement with less toxic, or no toxic peptides . Phylogenetic analysis revealed that *Omega-AS* was closely related to gliadin genes of wheat and related species and its divergence from bread wheat was more recently (less than 1.243 MYA). Heterologous expression showed that *Omega-AS* gene could successfully express with a high level in *E. coli* under the control of T_7 promoter.

Corresponding author: Prof. Dr. Xingguo Ye, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R. China, Phone/fax: +86-10-82105173, E-mail: yexingguo@caas.cn
Prof. Dr. Yueming Yan, College of Life Science, Capital Normal University, Beijing, XisanhuanBeilu 105, 100048 Beijing, P.R. China, Phone/fax: +86-10-68902777, E-mail: yanym@cnu.edu.cn
Dr. Slaven Prodanović, Poljoprivredni fakultet, Univerzitet u Beogradu, Nemanjina 6, 11080, Beograd, Srbija, Phone: +381638687246, Fax: +381113161987, e-mail: slavenp@agrif.bg.ac.rs

The transcription expression pattern of *Omega-AS* gene during grain development detected by qRT-PCR revealed that the highest expression level occurred at 17 days post anthesis.

Key words Aegilops speltoides, ω-gliadins, phylogenetics, epitopes, heterologous expression

INTRODUCTION

Wheat is one of the most important grain crops in the world. Wheat seed contains abundant starch and proteins and serves as an important human food and vegetable protein sources. Wheat flour could be used to make a variety of food products, such as various breads, noodles, cookies, pasta, etc. According to different solubility, wheat seed proteins consist of four classes: water-soluble albumins, salt-soluble globulins, alcohol-soluble gliadins and acid- or base-soluble glutenins. The most important seed storage proteins are glutenins and gliadins according to their polymerization properties (SHEWRY and HALFORD, 2002). Polymeric glutenins consist of high and low molecular weight glutenin subunits (HMW-GS, LMW-GS) while the monomeric gliadins are classified into three groups: α/β-, γ- and ω-gliadins based on their electrophoretic mobility during polyacrylamide gel electrophoresis at low pH (SHEWRY *et al.*, 2003; WIESER, 2007). It is known that the viscoelasticity and extensibility, properties of gluten dough, are mainly determined by glutenins and gliadins, respectively (MA *et al.*, 2006). Thus, complex storage proteins in wheat endosperm are the major determinants of flour processing quality.

Gliadins and glutenins, as well as, proteins of the albumin/globulin fraction are involved in food allergy, of which gliadins are considered as important allergens and different classes seem to be related to the IgE response (BATTAIS *et al.*, 2005). Sequence characterization showed that a specific octapeptide and celiac disease (CD)-toxic epitope were present in the rich glutamine domain and C-terminal non-repetitive domain of gliadins, respectively (WANG *et al.*, 2012). According to ALTERBACH and KOTHARI (2007), ω-gliadins have been implicated, in particular, in food allergies and sensitivities, which are unusual proteins in terms of their repetitive sequences rich in glutamine and proline, lack of cysteine and alpha-helical structure. Their expression levels are changed in response to nitrogen and sulfur availability (DUPONT *et al.*, 2006a, b; WRIGLEY *et al.*, 1984). Among them, the ω-5 type gliadins were found to be associated with wheat-dependent exercise-induced anaphalaxis (WDEIA) and urticaria in adults and immediate-type wheat allergies in children (BATTAIS *et al.*, 2005; MATSUO *et al.*, 2004, 2005; PALOSUO *et al.*, 2001).

Genetic studies confirmed that all ω-gliadins are encoded by the *Gli-1* loci on the short arms of homoeologous chromosome 1, which are tightly linked to the *Glu-3* loci coding for LMW-GS (PAYNE *et al.*, 1984). The ω-gliadins could be divided into three characteristic types: ω-1, ω-2 and ω-5 based on their N-terminal sequences and mobility in lactate gel electrophoresis (KASARDA *et al.*, 1983; SHEWRY *et al.*, 1981). The ω-1 and ω-2 types have similar N-terminal sequences beginning with ARE, ARQ or KEL and a different repetitive motif PQQPFP. In comparison, the ω-5 types contain higher glutamine content and a different N-terminal sequences beginning with SRL and the repetitive motifs FPQQQ and QQIPQQ. BOUCHEZ *et al.* (2010) found that ω-5 chain-terminating gliadins contain single cysteine residues near the carboxyl termini. Although, omega gliadins are very important, but there are only very few gene sequences were obtained.

Aegilops and other wild species related to wheat are recognized as important sources of

novel genetic variations which could be used to improve bread wheat by recombination, introgression or genetic transformation (SCHNEIDER *et al.*, 2008; DOUSSINAULT *et al.*, 1983). *Aegilops speltoides* (2n = 2x = 14, SS) is a valuable reservoir for agronomically useful genes and is the source of the resistance genes, which have been transferred to common wheat, *Triticum aestivum* L. (2n =6x = 42, AABBDD) (FRIEBE *et al.*, 2000). So far, many HMW-GS, LMW-GS, α-gliadin and γ-gliadin genes from *Aegilops* have been cloned and characterized to provide genetic resources for wheat breeding (ZHU *et al.*, 2015; WANG *et al.*, 2012; ZHU *et al.*, 2010; OVIDIO and MASCI, 2004; ANDERSON and GREENE, 1989). However, few articles have been published on the cloning and characterization of ω-gliadin genes from *Aegilops* species.

The first ω-gliadin gene from *Aegilops speltoides* was cloned in this study, and its molecular characterization as well as, phylogenetic evolutionary relationships among *Triticum* and *Aegilops* species were investigated. Meanwhile, its expression features in *E. coli* and developing grains were also explored. Results provide new gene resource and new evidence for further understanding of the molecular structure, evolution and expression profiles of wheat ω-gliadin genes.

MATERIALS AND METHODS

Plant materials

Materials used in this study included *Aegilops speltoides* (2n = 2x = 14, SS) PI486264. *Triticum monococcum* (2n = 2x = 14, AA) PI428182 from Braunschweig, Germany and common wheat variety Chinese Spring (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) were used as controls.

DNA extraction and PCR amplification

Genomic DNA was extracted from leaves of adult plants by using plant DNA extract kit (Tiangen, Beijing, China). A pair of allelic-specific primers amplifying the complete open reading frame (ORF) of the ω-gliadin genes were designed and used for PCR amplifications. The sequences of the specific PCR primers designed were: 1F: 5'-CATCAAAGGCAAGCAAGCAGTA-3'and 1R: 5'-TCCATCCGTCTTGTATTAC AGGTC-3'. DNA polymerase KOD-Plus-Neo was purchased from TOYOBO. PCR reaction in a 20 μL volume with genomic DNA, dNTPs and buffer was performed in a S1000TM thermal cycler (Bio-Rad, USA) with the following program: an initial step of 94ºC for 5 min, 34 cycles of 94ºC for 1 min, 62ºC for 45 s and 72ºC for 1 min 30 s, and a final step of 10 min at 72ºC. Five recombined DNA clones were sequenced by Sangong Company (Shanghai) to avoid possible errors.

Sequence alignment, SNP and InDel identification

Multiple alignments of complete ω-gliadin nucleotide sequences were carried out by BioEdit 7.0 (Ibis Therapeutics, Carlsbad, California). Based on sequence comparison, single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) were identified.

Epitope identification

According to BATTAIS *et al.* (2005) and TANABE (2004), eight known CD epitopes present in ω-gliadins were identified in the deduced amino acid sequences of the cloned ω-gliadin gene.

Phylogenetic analysis

The deduced complete amino acid sequences of ω-gliadin genes were used for multiple alignment and phylogenetic analysis. The alignment data were converted to mega format using the MEGA (Molecular Evolutionary Genetics Analysis) program (KUMAR *et al.*, 2004). The detailed steps of phylogenetic tree construction and the estimated time of gene differentiation were referred to WANG *et al.* (2011). The evolutionary rate of 6.5×10^{-9} substitutions/site/year was used according to ALLABY *et al*. (1999).

Heterologous expression of ω-gliadin gene in E. coli

The cloned ω-gliadin gene was re-amplified to remove the signal peptides by designing new primer pairs: ω-1-2F (AAA<u>GGATCC</u>GGCAGCTAAACCCTAGC) and ω-1-2R (AAA<u>CTCGAG</u>TCATTGGCCACTGGTGCTTG). *BamHI* and *XhoI* sites (underlined) were incorporated into the 5' end of the ω-gliadin gene. After purification, the PCR products were ligated into the expression vector pET-28a (Novagen), and transformed into *E. coli* BL21 (DE3) plysS cells. The positive clone and control were selected and grown in the LB medium. When the samples reached an OD_{600} of 0.6, the 1 nmol/L IPTG was added into the medium. Subsequently, to extract the expressed proteins, 1.8 mL of bacterial culture was centrifuged at 10000 g for 5 min at room temperature and the proteins were collected in a 2.0 mL tube. The supernatant was discarded, and 50 µL H_2O were added, and then the sample was incubated at 100ºC for 10 min. Another 50 µL H_2O contained 2% (*w/v*) DTT was added and incubated at 65 ºC for 30 min. The expressed proteins were extracted and separated by SDS–PAGE according to LI *et al.* (2007).

mRNA extraction and qRT-PCR

The mRNA from seed endosperm was isolated by plant RNA extract kit (Tiangen, Beijing, China). cDNA synthesis was carried out with oligo (dT) from about 100 ng mRNA by using Superscript first strand synthesis system (Invitrogen, Beijing, China). The primers ROmegaF: ATGAAGACCTTTCTCATCTTTGTCC and ROmegaR: ATTGTAACTCTTTGTTGCTAGGG-TT were used to amplify the ω-gliadin genes. The reported best and most stable reference gene in different tissue and development stages of wheat, *ADP-ribosylation factor* (PAOLACCI *et al.*, 2009), was adopted to normalize gene expression (primers ADPF: GCTCTCCAACAACAT-TGCCAAC and ADPR: GCTTCTGCCTGTCACATACGC). Primers were synthesized by Sangon Biotech (Shanghai, China). Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted using an ABIPrism 7500 system (Applied Biosystems, Foster City, CA). Each PCR was repeated three times in total volumes of 20 µL containing 2×Taq PCR Master Mix (Tiangen). Validation experiments were performed to demonstrate that amplification efficiencies of the *Omega-AS*-specific primers were approximately equal to the amplification efficiency of the endogenous reference primers.

RESULTS AND DISCUSSION

Molecular cloning and characterization of ω-gliadin genes from Ae. speltoides

The specific primers 1F and 1R were used to amplify the ω-gliadin genes from *Ae. speltoides* (SS) PI 486264 and *T. monococcum* (AA) accession PI428182. One specific fragment around 1000 bp that was amplified from both species, well corresponds to the size of ω-gliadin genes (Fig. 1). Sequencing results showed that one complete ORF from *Ae. speltoides*, named as *Omega-AS* and two pseudogenes from *T. monococcum* were obtained. *Omega-AS* gene consisted

of 1122 bp encoding 373 amino acid residues and the deduced molecular mass was 41379.21 Da. The structural characterization of *Omega-AS* gene was similar to those of previously characterized ω-gliadin genes (Fig. 2). Four distinct domains occurred in all ω-gliadin genes: signal of 19 amino acid residues, a non-repetitive N-terminal domain, repetitive domain and a C-terminal domain. Similarly, there were no introns in the sequence. *Omega-AS* encoded protein was exceptionally rich in proline and glutamine with 3 methionines and no cysteine. The *Omega-AS* gene was deposited in GenBank with accession number MF370924.
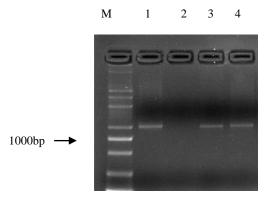

Fig. 1 PCR amplification of ω-gliadin genes from *Ae. speltoides* and *T. monococcum*. Legend: M. 5000 bp DNA Marker; 1. *T. monococcum* (AA) accession PI428182; 2. Chinese Spring; 3-4. *Ae. speltoides* (SS) accession PI486264. The amplification fragments are indicated.
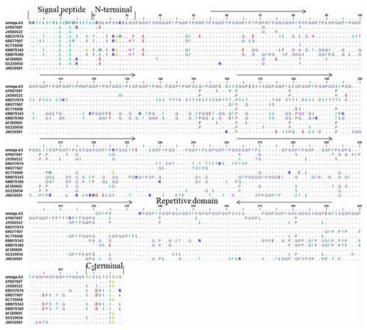

Fig. 2 Sequence aligment of *Omega-AS* gliadin gene from *Ae. speltoides* with other known ω-gliadin genes

Previously characterized ω-gliadins revealed three characteristic types based on N-terminal sequences and mobility in lactate gel electrophoresis: ω-1 type began KEL in N terminal sequences, ω-2 type began ARQ, ARE or XRQ, and ω-5 type began SRL (SHEWRY *et al.*, 1981; KASARDA *et al.*, 1983). DUPONT *et al.* (2000, 2004) demonstrated that Butte 86 kernels accumulated chromosome 1A-encoded ω-gliadins beginning with the N-terminal sequences of ARQ, RQL or KEL and chromosome 1D-encoded ω-gliadins with the N-terminal sequences of ARE and KEL. But the 1B encoded ω-gliadins only had ω-5 type. The *Omega-AS* encoded protein from *Ae. speltoides* had the N-terminal sequences of ARQ and belonged to ω-2 type gliadin. This is the first cloned ω-2 type gliadin gene from S genome, the progenitor of B genome of common wheat.

The *Omega-AS* gene was used to compare with 20 other ω-gliadin genes reported previously and SNP and InDel variations were detected (Table 1). In total, 7 SNPs were present in *Omega-AS* while two insertions (CCAGCAACC and AAC) occurred at the position 507-515, 584-586, respectively. Among 7 SNPs, there were 6 nonsynonymous substitutions. These point mutations included: Leu to Ile, Ala to Pro, Glu to Leu, Pro to Ser, Pro to Leu, and Ile to Thr. The CCAGCAACC insertion encoded three amino acid residues: two glutamine residues and one proline residue, while the AAC insertion encoded one glutamine residue.

Table 1. SNP and InDel identification in Omega-AS gliadin gene from Aegilops speltoides

SNP location	Bases in *Omega-AS*	Bases in other genes*	InDel location
28	A	C	507-515
37	C	G	Insert sequence
629	T	A or C	CCAGCAACC
702	A	C or G	
757	T	C	584-586
977	T	A or C	Insert sequence
1109	C	A or T	AAC

*Other 20 ω-gliadin genes include AB181300, AF280605, AY667097, GU220054, JX566521, JX566523, JX566524, JX566525, KF412579, KF412580, KF412582, KF412585, KC716065, KC716066, KC716067, KC716068, KM879343, KM879360, JN092577 and JN092583.

Identification of toxic epitopes related to different diseases in ω2-gliadins

The ω2-gliadins could induce different symptoms as atopic eczema/dermatitis syndrome (AEDS), urticaria and more severe reactions as wheat-dependent exercise-induced anaphylaxis (WDEIA) (BATTAIS *et al.*, 2005). In the previous studies, six toxic epitopes (QQPIPVQPQQ, PELQQPIPQQ, TQPQQPTPIQ, QQPQQPFPQQ, IQPQQPFPQQ and LQPQQPFPQQ) in ω2-gliadin for wheat allergic patients were identified (BATTAIS *et al.*, 2005). QQPFP and PQQPF were found to be responsible for the allergenicity of gliadin as epitope structures using sera of wheat-allergic patients with atopic dermatitis (TANABE, 2004). The stimulatory toxic epitopes in *Omega-AS* encoded gliadin were identified (Table 2). In *Omega-AS*, two previously reported toxic epitopes PELQQPIPQQ and QQPQQPFPQQ appeared one time. LQPQQPFPQQ occurred five times while IQPQQPFPQQ, TQPQQPTPIQ and QQPIPVQPQQ were absent. The occurrence frequency of the short peptides QQPFP and PQQPF was 19 and 17 times,

respectively. The less toxic epitopes are, the less likely they develop an allergic reaction. This would be helpful for screening ω-gliadin genes with few or no CD epitopes from wheat related species in wheat quality improvement program.

Table 2. Sequence alignment for IgE-binding epitopes in Omega-AS gliadin gene from Aegilops speltoides

Reported peptides	Location in *Omega-AS*	Number
LQPQQPFPQQ[a]	90-99,131-140,178-187,243-252,259-268	5
QQPQQPFPQQ[a]	340-349	1
PELQQPIPQQ[a]	227-236	1
QQPIPVQPQQ[a]	-	0
TQPQQPTPIQ[a]	-	0
IQPQQPFPQQ[a]	-	0
PQQPF[b]	92-96,103-107,110-114,118-122,133-137,141-145,172-176, 180-184,204-208,237-241,245-249,261-265,269-273,277-281,285-289, 319-323,342-346	17
QQPFP[b]	41-45,63-67,78-82,93-97,104-108,111-115,119-123,134-138,142-146,181-185, 205-209,216-220,238-242,246-250,254-258,262-266, 270-274,278-282,343-347	19

[a]BATTAIS F. *et al.* 2005

[b]TANABE S. *et al.* 2004

Phylogenetic relationships of ω-gliadin genes among wheat and related species

A phylogenetic tree was constructed using the complete coding sequence of the *Omega-AS* gene obtained in this work and 33 GenBank gliadin genes from common wheat and related species, including 4 α/β-gliadin genes (X02538, M16496, D84341 and AJ33612); 2 γ-gliadin genes (HQ286692 and JN587178); 27 ω-gliadin genes (AB181300, AB181301, AY667097, GU220054, JN092577, JN092578, JN092579, JN092580, JN092581, JN092582, JN092583, JX566521, JX566523, JX566524, JX566525, KC716065, KC716066, KC716067, KC716068, KM879343, KM879356, KM879357, KM879360, KF412579, KF412580, KF412582 and KF412585). Twenty gliadin genes were clustered into three subgroups, corresponding to α/β-, γ- and ω-gliadin genes, respectively (Fig. 3). Obviously, α/β- and γ-gliadin genes were gathered into a big branch and ω-gliadin genes formed a single branch. Furthermore, the ω-gliadin gene branch was clearly divided into two subgroups: ω2- and ω5-gliadin genes.

An estimation of divergence time (million years ago, MYA) among the 34 genes was shown in Table S1. The divergent times estimated between three subgroups ranged from 5.276 to 3.935MYA. α/β-gliadin and γ-gliadin had a close genetic relationship, and the divergence between ω-gliadin and α/β-, γ-gliadin is earlier. ω2- and ω5-gliadin genes diverged about 4 MYA, indicating that the ω2- and ω5-gliadins diverged later than subgroup divergence. *Omega-AS* gene from *Ae. speltoides* was diverged from bread wheat more recently, generally less than 1.243 MYA.
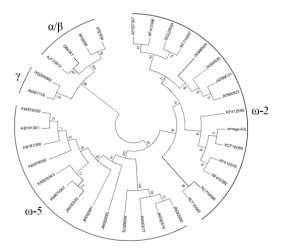
Fig. 3 Phylogenetic analysis of ω-gliadin genes among wheat and related species

Heterologous expression analysis

The cloned ORF without signal peptide of *Omega-AS* gene was ligated into expression vector pET-28a, and then transformed into *E. coli* BL21 (DE3) plysS cells. The positive hybrid plasmid expressed a new protein through the induction of IPTG. The protein encoded by *Omega-AS* could continuously expressed from 1 h to 7 h, and the expressed protein was clearly identified by SDS-PAGE (Fig. 4).
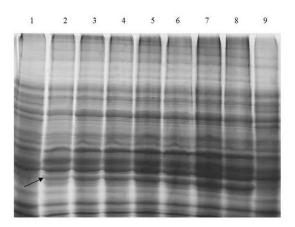

Fig. 4 Identification of the *Omega-AS* encoded protein expressed in *E. coli* by SDS-PAGE.
    Legend: The expressed proteins are indicated by arrow. 1. The positive hybrid plasmid without IPTG.
    2-8. The protein of *Omega-AS* expressed from 1 h to 7 h by the induction of IPTG. 9. vector pET-28a.

Dynamic transcriptional expression profiling of Omega-AS gene during grain development of Ae. speltoides

qRT-PCR was used to detect the dynamic transcriptional expression profiling of *Omega-AS* gene during grain development at 8, 11, 13, 15, 17, 19, 21, 23 and 26 days post-anthesis (DPA) in *Ae. speltoides* using specific primer pairs ROmegaF/ROmegaR. The expression of *Omega-AS* gene was rapidly increased from 8 to 17 DPA and then decreased from 17 to 26 DPA. The highest expression level occurred at 17 DPA (Fig. 5). This is well correspond to the accumulation trend of gliadins as well as HMW and LMW glutenins (LIU *et al.*, 2012).
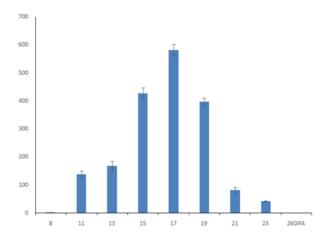

Fig. 5 Dynamic transcript expression patterns of the *Omega-AS* gene during grain development of *Ae. speltoides* detected by qRT-PCR. Abscissa represents the grain developmental stages (days postanthesis, DPA) and ordinate represents the dynamic transcriptional expression level of *Omega-AS* gliadin genes. Error bars indicate standard errors of three biological replicates.

Considerable storage protein genes encoded HMW-GS, LMW-GS, α-gliadins and γ-gliadins from *Triticum* and related *Aegilops* species were cloned and characterized, until now (DUPONT *et al.*, 2004; XIE *et al.*, 2010; ZHU *et al.*, 2010; WANG *et al.*, 2011; WANG *et al.*, 2012, 2013). However, the molecular characterization of ω-gliadin genes in *Ae. speltoides* is still unknown. This study cloned and characterized the first ω-2 gliadin gene *Omega-AS* from *Ae. speltoides*, which consisted of 373 amino acid residues with four distinct domains present in all ω-gliadin genes. Its encoded protein was exceptionally rich in proline and glutamine with little methionine and no cysteine (Fig. 2). Phylogenetic relationships of ω-gliadin genes among wheat and related species revealed that O*mega-AS* gene from *Ae. speltoides* was closely related to those from *Triticum* and other *Aegilops* species () and diverged from bread wheat more recently, generally less than 1.2 MYA (Fig. 3, Table S1).

Gliadins play important role in determining extensibility properties of gluten dough, but they also appear as important allergens and different classes seem to be involved in the IgE response. The α-gliadin genes have close relationships with the LMW-GS and γ-gliadin genes, but distinct from the ω-gliadin and HMW-GS genes (ZHU *et al.*, 2010). BATTAIS *et al.* (2003) found IgE

binding to α/β-, γ- and ω-gliadins for wheat allergic children and adults. Gliadins contain various celiac disease (CD, a T cell-mediated autoimmune disease of the small intestine) related toxic peptides such as four T cell stimulatory toxic epitopes (PFPQPQLPY, FRPQQPYPQ, QGSFQPSQQ and PQPQLPYPQ) in α-gliadins (VAN HERPEN *et al.*, 2006; XIE *et al.*, 2010), five (FLQPQQPFPQQPQQPYPQQPQQPFPQ, LQPQQPFPQQPQQPYPQQPQ, VQGQGIIQPQQP-AQL, FSQPQQQFPQPQ and QPQQSFPQQQ) in γ-gliadins (LOPONEN 2006), six (QQYPQQQ, QQLPQQQ, QQIPQQQ, QQFPQQQ, QQSPEQQ and QQSPQQQ) in ω5-gliadins (MATSUO *et al.*, 2004) and six (QQPIPVQPQQ, PELQQPIPQQ, TQPQQPTPIQ, QQPQQPFPQQ, IQPQQPFPQQ and LQPQQ- PFPQQ) in ω2-gliadins (BATTAIS *et al.*, 2005). Previous studies on α-gliadins and γ-gliadins in wheat related *Aegilops* species showed that the α- and γ-gliadin genes usually had extensive simple nucleic polymorphism (SNP) variations that let to their encoding proteins containing fewer toxic peptides (XIE *et al.*, 2010; ZHU *et al.*, 2010; WANG *et al.*, 2012). These results provide the possibility that desirable gliadin genes with fewer or no toxic peptides in the related *Aegilops* species could be discovered and used for wheat quality improvement.

The ω5-gliadins, components of fast ω-gliadins, have been considered as a major allergen in the anaphylaxis. The special roles of ω5-gliadins in WDEIA were demonstrated by several studies (LEHTO *et al.*, 2003; PALOSUO *et al.*, 1999; MORITA *et al.*, 2003). The ω2-gliadins were also found to have toxic epitopes in wheat allergic patients (BATTAIS *et al.*, 2005). Comparative analysis demonstrated that *Omega-AS* encoded gliadin lacked three toxic epitopes IQPQQPFPQQ, TQPQQPTPIQ and QQPIPVQPQQ (Table 2). The same type of QPQQPFP was found in γ- and ω2-gliadins after compared all of gliadin epitopes (BATTAIS *et al*., 2005). At least five residues were found to require for the IgE-binding, and QQPFP and PQQPF are responsible for the allergenicity of gliadins (TANABE, 2005). Therefore, γ- and ω2-gliadins may have same conserved epitopes. The present work found that *Omega-AS* gene encoded ω2-gliadin had fewer CD epitopes than other genes, which could be used as potential gene resource for the improvement of bread wheat cultivars with fewer even no gliadin toxic peptides. In addition, since *Omega-AS* gene contained different SNP and InDel variations, this could facilite its fast transfer and utilization through molecular-assisted selection during wheat quality improvement programs (Table 1).

CONCLUSION

The first complete ω-gliadin gene *Omega-AS* from *Aegilops speltoides*, was isolated and characterized in this study. . Sequence characterization showed that *Omega-AS* gene had similar structure to those in *Triticum* and related species. Epitope analysis indicated that *Omega-AS* gene had considerable SNP and InDel variations that resulted in fewer CD epitopes in the encoded ω2 type gliadin, particularly lacking three known epitopes IQPQQPFPQQ, TQPQQPTPIQ and QQPIPVQPQQ. Phylogenetic analysis revealed that *Omega-AS* was closely related to those from wheat and related species and it's divergence from bread wheat was more recently. *Omega-AS* gene could successfully express with a high level in *E. coli* under the control of T$_7$ promoter. The transcription expression pattern of *Omega-AS* gene during grain development detected by qRT-PCR revealed that the highest expression occurred at 17 days post anthesis.

ACKNOWLEDGMENTS

REFERENCES

ALLABY, R.G., M. BANERJEE, T.A. BROWN (1999): Evolution of the high molecular weight glutenin loci of the A, B, D, and R genomes of wheat. Genome, *42*: 296–307.

ANDERSON, O.D., F.C. GREENE (1989): The characterization and comparative analysis of high-molecular-weight glutenin genes from genomes A and B of a hexaploid bread wheat. TAG, *77*: 689–700.

ALTENBACH, S.B., 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* (2):169-177.

BATTAIS, F., F. PINEAU, Y. POPINEAU, C. APARICIO, G. KANNY, L. GUERIN, D.A. MONERET-VAUTRIN, S. DENERY-PAPINI (2003): Food allergy to wheat: identification of immunogloglin E and immunoglobulin G-binding proteins with sequential extracts and purified proteins from wheat flour. Clin. Exp. Allergy, *33* (7): 962-970.

BATTAIS, F., P. COURCOUX, Y. POPINEAU, G. KANNY, D.A. MONERET-VAUTRIN, S. DENERY-PAPINI (2005): Food allergy to wheat: differences in immunoglob in E-binding proteins as a function of age or symptoms. J. Cereal Sci., *42*: 109-117.

BATTAIS, F., T. MOTHES, D. A. MONERET-VAUTRIN, F. PINEAU, G. KANNY, Y. POPINEAU, M. BODINIER, S. DENERY-PAPINI (2005): Identification of IgE-binding epitopes on gliadins for patients with food allergy to wheat. Allergy, *60*: 815-821.

BOUCHEZ-MANHIOUT, I., J. SNEGAROFF, M. TYLICHOVA, C. PECQUET, G. BRANLARD, M. LAURIERE (2010): Low molecular weight glutenins in wheat-dependent, exercise-induced anaphylaxis: allergenicity and antigenic relationships with omega 5-gliadins. Int. Arch. Allergy Immunol., *153*: 35-45.

D'OVIDIO, R., C. MARCHITELLI, C.L. ERCOLI, E. PORCEDDU (1999): Sequence similarity between allelic *Glu-B3* genes related to quality properties of durum wheat. TAG, *98*:455–461.

DOUSSINAULT, G., A. DELIBES, R. SANCHEZ-MONGE, F. GARCIA-OLMEDO (1983): Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. Nature, *303*: 698–700.

DUPONT, F.M., W.H. VENSEL, R. CHAN, D.D. KASARDA (2000): Characterization of the 1B-type omega gliadins from *Triticum aestivum* 'Butte'. Cereal Chem., *77*: 607–614.

DUPONT, F.M., W. VENSEL, T. ENCARNACAO, R. CHAN, D.D. KASARDA (2004): Similarities of omega gliadins from *Triticum urartu* to those encoded on chromosome 1A of hexaploid wheat and evidence for their posttranslational processing. TAG, *108*: 1299–1308.

DUPONT, F.M., W.J. HURKMAN, W.H. VENSEL, R. CHAN, R. LOPEZ, C.K. TANAKA, S.B. ALTENBACH (2006a): Differential accumulation of sulfur-rich and sulfur-poor wheat flour proteins is affected by temperature and mineral nutrition during grain development. J. Cereal Sci., *44*: 101–112.

DUPONT, F.M, W.J. HURKMAN, W.H. VENSEL, C. TANAKA, K.M. KOTHARI, O.K. CHUNG, S.B. ALTENBACH (2006b): Protein accumulation and composition in wheat grains: effects of mineral nutrients and high temperature. Europ. J. Agron., *25*: 96–107.

FRIEBE, B., L.L.QI, S. NASUDA, P. ZHANG, N.A. TULEEN, B.S. GILL (2000): Development of a complete set of *Triticum aestivum - Aegilops speltoides* chromosome addition lines. TAG, *101*:51–58.

KASARDA, D.D., J.C. AUTRAN, J.L. LEW, C.C. NIMMO, P.R. SHEWRY (1983): N-terminal amino acid sequences of ω-gliadins and ω-secalins: Implications for the evolution of prolamin genes. Biochimica et Biophysica Acta-Protein Structure

and Molecular Enzymology, *747*: 138-150.

KUMAR, S., K. TAMURA, M. NEI (2004): MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics, *5*:150-63.

LEHTO, M., K. PALOSUO, E. VARJONEN, M.L. MAJURI, U. ANDERSSON, T. REUNALA *et al*. (2003): Humoral and cellular responses to gliadin in wheat-dependent, exercise-induced anaphylaxis. Clin. Exp. Allergy, *33*:90–95.

LI X.H., Y.Z. ZHANG, L.Y. GAO, A.L. WANG, K.M. JI, Z.H. HE, R. APPELS, W.J. MA, Y.M. YAN (2007): Molecular cloning, heterologous expression and phylogenetic analysis of a novel y-type HMW gluten in subunit gene from G genome of *Triticum timopheevii*. Genome, *50*:1130–1140.

LIU W., Y.Z. ZHANG, X. GAO, K. WANG, S.L. WANG, Y. ZHANG, Z.H. HE, W.J. MA, Y.M. YAN (2012): Comparative proteome analysis of glutenin synthesis and accumulation in developing grains between superior and poor quality bread wheat cultivars. J. Sci. Food Agric., *92*: 106–115.

LOPONEN, J. (2006): Prolamin degradation in sourdoughs. (dissertation) EKT series 1372 University of Helsinki, Yliopistopaino, Helsinki, Finland.

MA Y.Q., C.R. HAN, Z.Z. SHI (2006): Advance on the study of wheat gliadins. Food Sci., *27*: 813-817.

MATSUO H., E. MORITA, A.S. TATHAM, K. MORIMOTO, T. HORIKAWA, H. OSUNA, Z. IKEZAWA, S. KANEKO, K. KOHNO, S. DEKIO (2004): Identification of the IgE-binding epitope in ω-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. J. Biol. Chem., *279*: 12135–12140.

MATSUO H., K. KOHNO, E. MORITA (2005): Molecular cloning, recombinant expression and IgE-binding epitope of ω-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. FEBS J., *272*: 4431–4438.

MORITA E., H. MATSUO, S. MIHARA, K. MORIMOTO, A. SAVAGE, A. TATHAM (2003): Fast omega-gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis. J. Dermatol. Sci., *33*:99–104.

PALOSUO K., H. ALENIUS, E. VARJONEN, M. KOIVULUHTA, J. MIKKOLA, H. KESKINEN *et al*. (1999): A novel wheat gliadin as a cause of exercise-induced anaphylaxis. J Allergy Clin. Immunol., *103*:912–917.

PALOSUO K., E. VARJONEN, O.M. KEKKI, T. KLEMOLA, N. KALKKINEN, H. ALENIUS, T. REUNALA (2001): Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. J. Allergy Clin. Immunol., *108*:634–638.

PAOLACCI A.R., O.A. TANZARELLA, E. PORCEDDU, M. CIAFFI (2009): Identification and validation of reference genes for quantitative RT-PCR normalization in wheat. BMC Mol Biol, *10*:11.

PAYNE P.I., E.A. JACKSON, L.M. HOLT, C.N. LAW (1984): Genetic linkage between endosperm storage protein genes on each of the short arms of chromosomes 1A and 1B in wheat. TAG, *67*: 235–243.

SCHNEIDER A., M. ISTVÁN , M. MOLNÁR-LÁNG (2008): Utilisation of *Aegilops* (goatgrass) species to widen the genetic diversity of cultivated wheat. Euphytica, *163*: 1–19.

SHEWRY P.R., M.S. WOLFE, S.E. SLATER, S. PARMAR, A.J. FAULKS (1981): Barley storage proteins in relation to varietal identification, malting quality and mildew resistance. Barley Genetics Iv: Fourth International Barley Genetics Symposium, 22–29.

SHEWRY P.R., N.G. HALFORD (2002): Cereal seed storage proteins: structures, properties and role in grain utilization. J. Exp. Bot., *53*: 947–958.

SHEWRY P.R., N.G. HALFORD, A.S. TATHAM, Y. POPINEAU, D. LAFIANDRA, P.S. BELTON (2003): The high molecular weight subunits of wheat glutenin and their role in determining wheat processing properties. Adv. Food Nutr. Res., *45*: 219–302.

TANABE S. (2004): IgE-binding Abilities of Pentapeptides, QQPFP and PQQPF, in Wheat Gliadin, J. Nutr. Sci. Vitaminol., *50*: 367-370.

VAN HERPEN T., S.V. GORYUNOVA, J. VAN DER SCHOOT, M. MITREVA, E. SALENTIJN, O. VORST, M.F. SCHENK, P.A. VAN VEELEN, F. KONING, L.J.M. VAN SOEST, B. VOSMAN, D. BOSCH, R.J. HAMER, L. GILISSEN, M.J.M. SMULDERS (2006): Alpha-gliadin genes from the A, B, and D genomes of wheat contain different sets of celiac disease epitopes. BMC

Genomics, *7*:1.

WANG K., L.Y. GAO, S.L. WANG, Y.Z. ZHANG, X.H. LI, M.Y. ZHANG, Z.Z. XIE, Y.M. YAN, M. BELGARD, W.J. MA (2011): Phylogenetic relationship of a new class of LMW-GS genesin the M genome of *Aegilops comosa*. TAG, *122*: 1411–1425.

WANG S.L., X.X. SHEN, P. GE, J. LI, S. SUBBURAJ, X.H. LI, F. J. ZELLER, S. L. K. HSAM, Y.M. YAN (2012): Molecular characterization and dynamic expression patterns of two types of γ-gliadin genes from *Aegilops* and *Triticum* species. TAG, *125*:1371–1384.

WANG S.L., Z. YU, M. CAO, X. SHEN, N. LI, X. LI, W. MA, H. WEIBGERBER, F.J. ZELLER, S.L.K. HSAM, Y. YAN (2013): Molecular mechanisms of HMW glutenin subunits from 1S[l] genome positively affecting wheat breadmaking quality, PLoS ONE, *8*: e58947.

WIESER H. (2007): Chemistry of gluten proteins. Food Microbiol., *24* (2): 115-119.

WRIGLEY C.W., D.L. DU CROS, J.G. FULLINGTON, D.D. KASARDA (1984): Changes in polypeptide composition and grain quality due to sulfur deficiency in wheat. J. Cereal Sci., *2*: 15–24.

XIE Z.Z., C.Y. WANG, K. WANG, S.L. WANG, X.H. LI, Z. ZHANG, W.J. MA, Y.M. YAN (2010): Molecular characterization of the celiac disease epitope domains in α-gliadin genes in *Aegilops tauschii* and hexaploid wheats (*Triticum aestivum* L.). TAG, *121*: 1239–1251.

ZHU G., S. WANG, S. ZHEN, X. SHEN, S. PRODANOVIC, Y. YAN (2015): Molecular characterization and phylogenetic analysis of unusual x-type HMW glutenin subunits from 1S[l] genome of *Aegilops longissima*. Genetika, *47*: 185–203.

ZHU X.P., X. LI, Y.X. LI, Y.M. YAN (2010): Cloning and localization of α-gliadin genes from common wheat and *Aegilops tauschii* and their molecular evolution. Acta Agron. Sin., *36*: 580–589.

MOLEKULARNA KARAKTERIZACIJA I FILOGENETSKA ANALIZA OMEGA-GLIJADIN GENA IZ *AEGILOPS SPELTOIDES* L.

Wanqing WANG[1,], Ke WANG[2,], Xi CHEN[1], Slaven PRODANOVIC[3*],
Xiaohui LI[1], Xingguo YE[2*], Yueming YAN[1*]

[1]Koledž za prirodne nauke, Kapital Normal Univerzitet, Peking, Kina
[2]Institut za ratarstvo, Kineska Akademija poljoprivrednih nauka, Peking, Kina
[3]Poljoprivredni fakultet, Univerzitet u Beogradu, Beograd, Srbija

Izvod

Glijadini, kao glavne komponente skladišnih proteina kod pšenice, određuju svojstva rastegljivosti testa i imaju značajne efekte na kvalitativne osobine brašna. Srodnici pšenice predstavljaju potencijalne izvore gena skladišnih proteina, značajnih za poboljšanje kvalieta. U ovom radu smo izolovali i okarakterisali prvi kompletan ω-glijadin gen *Omega-AS* iz *Aegilops speltoides* L. (2n = 2x = 14, SS) alel-specifičnim PCR-om i izučavali njegove filogenetske veze sa rodovima *Triticum* i *Aegilops*. Molekulska struktura pokazala je da se gen *Omega-AS* sastoji od 1122 bp koje kodiraju 373 aminokiselinska ostatka sa molekulskom masom od 41379.21 Da. *Omega-AS* je posebno bogat prolinom i glutaminima, manje metioninom i ne sadrži cistein. Analiza sekvence i epitopa pokazala je da su tri epitopa QQPIPVQPQQ, TQPQQPTPIQ i IQPQQPFPQQ bila odsutna iz *Omega-AS* kodirajućeg proteina, ukazujući na njegov potencijalni značaj za popravku kvaliteta pšenice, manje toksičnim ili netoksičnim peptidima. Filogenetska analiza pokazala je da je gen *Omega-AS* blizak glijadinskim genima pšenice i srodnih vrsta i da je do njegove divergentnosti došlo skorije (pre manje od 1.243 miliona godina). Pod kontrolom T_7 promotora utvrđen je visok nivo ekspresije *Omega-AS* gena u *E. coli*. Transkripciona ekspresija *Omega-AS* gena, utvrđena primenom qRT-PCR tokom formiranja zrna, ukazala je da je najviši nivo ekspresije bio 17 dana posle cvetanja.

Table S1. Divergent times of gliadin genes from wheat and related species. The divergent times are specified by the numbers, and the unit was million years ago (MYA). Below the diagonal: divergent time of genes; above the diagonal: standard error.