



Analysis of Structural Homology and Evolutionary Relationships among Gamma- and Omega-gliadins in Different Wheat Species and Varieties

Anna Vadimovna Tretyakova¹, Ludmila Vladimirovna Derevshikova¹, Pavel Andreevich Krylov¹ 10.18805/ajdfr.DRF-437

ABSTRACT

Background: Gamma- and omega-gliadins are major components of gluten in wheat and can trigger allergic reactions and gluten intolerance. An increase in the number of motifs in their sequences correlates with a rise in toxic epitopes, leading to a stronger immune response. Evaluating the structural homology of gliadins across different wheat species and varieties can help identify toxic epitopes, which may be edited to neutralize their effects. This study aimed to evaluate the structural homology, evolutionary relationships and presence of toxic epitopes in various wheat species and varieties.

Methods: Multiple sequence alignment using the ClustalW algorithm (BLOSUM62 matrix) in the Ugene program was used to identify structural differences between gamma- and omega-gliadins. The alignment results were evaluated based on percentage identity, scores and the presence of homologous regions. Phylogenetic analysis was performed using the maximum likelihood method with the JTT+G+F model for gamma-gliadins and the LG+G+F model for omega-gliadins, using the MEGA11 software with bootstrap support.

Result: Gamma-gliadins had a high degree of similarity across different wheat species and varieties, while omega-gliadins showed more variability and were less conserved. Toxic epitopes linked to celiac disease were most commonly found in gamma-gliadins of *T. aestivum* and *T. dicoccoides* Atlit2015, with fewer in *T. monococcum* clone 45-L024585. The unique amino acid composition of omega-gliadins was associated with diverse toxic epitope variations. *T. aestivum* Chinese Spring D2 and D3 contained more toxic epitope segments related to celiac disease, whereas no presumed toxic epitopes were detected in *T. monococcum* DV80. The obtained data can be used for genetic editing aiming to create wheat varieties that include allergen-neutral gliadins by reducing the repetitive motifs of celiac disease epitopes.

Key words: Gliadins, Multiple alignment, Phylogenetic analysis, Toxic epitopes, Wheat.

INTRODUCTION

Currently, there is a global trend toward strengthening food safety controls (Steinhausova *et al.*, 2015; Wang *et al.*, 2016). Simultaneously, there is a focus on implementing advanced agricultural technologies that can enhance crop quality and safety (Kumaraswamy *et al.*, 2016; Karthik *et al.*, 2020). The consumption of wheat products raises safety concerns due to the presence of gluten proteins, which can provoke allergic reactions and exacerbate chronic conditions such as celiac disease (Elli *et al.*, 2015; Caio *et al.*, 2019; Sharma *et al.*, 2020; Cabanillas, 2020). Gluten, a key quality indicator of flour, comprises a complex of monomeric and polymeric proteins known as gliadins and glutenins, which are critical for the rheological properties of dough (Murakami *et al.*, 2018; Chaudhary *et al.*, 2022; Schuster *et al.*, 2023). Glutenins, the polymeric fraction, consist of high and low molecular weight subunits (Sihmar *et al.*, 2020; Wieser *et al.*, 2023). Soluble in alcohol solutions, glutenins have a monomeric structure. To date, five gliadin monomers have been identified: alpha, beta, gamma, omega and delta, each containing both similar and unique structural elements. Gliadins account for 30% to 50% of the total grain protein content, a substantial portion (Juhász *et al.*, 2015).

¹Federal State Budget Scientific Institution, Federal Scientific Centre of Agroecology, Complex Melioration and Protective Afforestation of the Russian Academy of Sciences, Russia.

Corresponding Author: Pavel Andreevich Krylov, Federal State Budget Scientific Institution, Federal Scientific Centre of Agroecology, Complex Melioration and Protective Afforestation of the Russian Academy of Sciences, Russia.
Email: krylov-p@vfanc.ru

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Gamma- and omega-gliadins are major gluten components in wheat, significantly impacting dough texture, elasticity and baking properties. However, these gliadins can also trigger allergic reactions and gluten intolerance. The structure of gamma-gliadins includes a domain with repetitive motifs P(Q/L/S/T/I/V/R/A) F(S/Y/V/Q/I/C/L) P(R/L/S/T/H/C/Y) Q1-2(P(S/L/T/A/F/H)QQ), which can be repeated 7 to 22 times (Qi *et al.*, 2009). An increase in the number of these motifs corresponds with an increase in toxic epitopes,

enhancing the immune response (Narwal *et al.* 2020; Hailegiorgis *et al.*, 2022). Therefore, evaluating structural homology is crucial to identifying wheat varieties and species containing toxic epitopes. This knowledge can facilitate the genomic editing of these epitopes to neutralize their effects (Juhász *et al.*, 2012; Songstad *et al.*, 2017; Camerlengo *et al.*, 2020; Brackett *et al.*, 2022). This study primarily focuses on gamma- and omega-gliadins across various wheat species and varieties. The objective is to evaluate structural homology, evolutionary relationships and identify toxic epitopes in different wheat species and varieties.

MATERIALS AND METHODS

Studies were conducted on the basis of the FSC of agroecology RAS in the laboratory of genomic and postgenomic technologies from December 2022-February 2024.

Data on the amino acid sequences of the studied wheat proteins were obtained from open-access databases including UniProtKB, NCBI Protein and NCBI Conserved Domain. Information on the amino acid sequences of toxic epitopes was sourced from the AllergenOnline database. Sequences were selected based on the degree of annotation and the availability of complete sequences. In cases where multiple clones and isolates of a protein were available, a single representative sequence was chosen. The selected sequences and their identifiers are listed in Table 1.

To identify structural differences between gamma- and omega-gliadins, a multiple sequence alignment was

performed using the Ugene software (UNIPRO, Russia) with the ClustalW algorithm (BLOSUM62 matrix). The alignment results were evaluated based on percentage identity, scores and the presence of conserved regions. A phylogenetic tree was constructed from the amino acid sequences using the maximum likelihood method, employing the JTT+G+F model for gamma-gliadins and the LG+G+F model for omega-gliadins, with bootstrap support, in the MEGA11 software (MEGA, Japan). Nodes with bootstrap support values greater than 70 were considered significant.

RESULTS AND DISCUSSION

Evaluation of the structural homology of gamma-and omega-gliadins

The results of the multiple sequence alignment of gamma- and omega-gliadins revealed both similarities and differences in structure among various wheat species and varieties (Table 2-5, Fig 1 and 2). Gamma-gliadins across all wheat species and varieties had over 62% similarity. In *T. aestivum*, the proteins demonstrated more than 90% sequence similarity. The gamma-gliadins of *T. urartu* showed over 73% similarity, with the sequences of *T. urartu* G1812 and *T. urartu* PI428198 allele Gli-gamma 3 being completely identical, despite belonging to different varieties. This indicates that gamma-gliadins are quite conserved across different wheat types and varieties.

The similarity among omega-gliadins ranged from 11% to 94%. Omega-gliadins from *T. aestivum* Chinese Spring variety had 92% similarity, while omega-gliadins D from the same variety showed the highest similarity, ranging

Table 1: Identifiers of amino acid sequences of gamma-and omega-gliadins in different species and varieties of wheat.

Wheat	Identifier
Gamma-gliadins	
<i>Triticum aestivum</i> L.	AAA34289.1
<i>Triticum aestivum</i> × <i>Thinopyrum elongatum</i> II-13-11	ACI04107.1
<i>Triticum spelta</i> L.	Q9XEW0
<i>Triticum monococcum</i> L. clone 45-L024585	ACJ76947.1
<i>Triticum dicoccoides</i> (Asch. and Graebn.) Schweinf Atlit2015	XP_037445787.1
<i>Triticum macha</i> Dekapr. and Menabde clone Gli-Mr1	ACM41415.1
<i>Triticum turgidum</i> L. clone 4	ACJ03441.1
<i>Triticum urartu</i> Thumanjan ex Gandilyan G1812	EMS45054.1
<i>Triticum urartu</i> Thumanjan ex Gandilyan PI428198 allele Gli-gamma 1, 2, 3	AKB95611.1, AKB95612.1, AKB95613.1
Omega-gliadins	
<i>Triticum dicoccoides</i> Fast omega gliadin clone ES9-41	AKA88516.1
<i>Triticum aestivum</i> L. Chinese Spring B3, B6, D1, D2, D3	ATY51554.1, ATY51555.1, ATY51569.1, ATY51568.1, ATY51570.1
<i>Triticum aestivum</i> L. Jinan 177	AAT01617.1
<i>Triticum aestivum</i> L. Yumai34 clone 4	AGZ20259.1
<i>Triticum aestivum</i> L. Zhengfeng5 clone 1	AGO17771.1
<i>Triticum monococcum</i> L. DV80	AIU64844.1
<i>Triticum urartu</i> Thumanjan ex Gandilyan PI428198 allele Gli-omega-1	AKB95614.1
<i>Triticum timopheevii</i> Zhuk	Q0GK30

from 94% to 100%. Omega-gliadins B and D from the Chinese Spring variety are markedly different from each other and from other *T. aestivum* varieties. The most significant differences were observed between the Jinan 177 variety and other *T. aestivum* varieties. The lower similarity between omega-gliadins and gamma-gliadins may be due to differences in their sequence lengths and amino acid compositions. It is also possible that these proteins are encoded by different genes (Altenbach *et al.*, 2007; Goryunova *et al.*, 2012), although this information may not be available for some *T. aestivum* varieties and species.

The multiple sequence alignment also identified a highly conserved signal peptide in gamma-gliadins, consisting of

19 amino acids. However, no distinct signaling peptide was found in the gamma-gliadin structure of the hybrid *T. aestivum* × *T. elongatum*. Repeats of amino acids, primarily glutamine (Q) and proline (P), were clearly visible. The protein sequences contain two domains: the AAI_SS domain and the Gliadin domain. The AAI_SS domain is involved in protecting plants from insects, fungi, bacteria and viruses, by inhibiting alpha-amylase and proteinases, which play roles in starch digestion and gluten absorption (Franco *et al.*, 2002). Proteins with this domain are often associated with allergic reactions (Breiteneder *et al.*, 2004). The role of the Gliadin domain is not fully understood, but it is rich in cysteine, which contributes to the formation of strong disulfide bonds that maintain protein structure (Ma *et al.*, 2020).

Table 2: The similarity percentage and scores obtained from multiple alignment of gamma-gliadins in different species and varieties of wheat.

Wheat	%, score										
	1	2	3	4	5	6	7	8	9	10	11
1											
2	93,333										
3	81,291	77,275									
4	75,269	70,253	69,248								
5	74,267	69,249	76,273	75,271							
6	98,351	93,335	82,294	75,268	74,266						
7	67,239	62,221	75,270	68,245	82,294	66,238					
8	71,256	67,240	64,231	86,310	72,257	71,255	64,230				
9	74,266	69,248	78,279	79,284	86,308	74,265	84,301	75,271			
10	72,258	67,240	75,271	77,276	84,302	72,257	85,306	73,263	97,350		
11	71,256	67,240	64,231	86,310	72,257	71,255	64,230	100,359	75,271	73,263	

Notes: 1- *T. aestivum*, 2- *T. aestivum* × *T. elongatum* II-13-11, 3- *T. spelta*, 4- *T. monococcum* clone 45-L024585, 5- *T. dicoccoides* Atlit2015, 6- *T. macha* clone Gli-Mr1, 7- *T. turgidum* clone 4, 8- *T. urartu* G1812, 9- *T. urartu* PI428198 allele Gli-gamma 1, 10- *T. urartu* PI428198 allele Gli-gamma 2, 11- *T. urartu* PI428198 allele Gli-gamma 3.

Table 3: The similarity percentage and scores obtained from multiple alignment of omega-gliadins in different species and varieties of wheat.

Wheat	%, score											
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	56,278											
3	49,244	92,454										
4	41,203	53,262	46,228									
5	40,199	54,269	48,235	94,464								
6	40,199	54,269	48,235	94,464	100,494							
7	38,186	18,87	11,56	23,115	23,115	23,115						
8	70,347	39,193	32,160	62,304	65,319	65,319	41,202					
9	63,310	45,221	38,188	75,372	73,361	73,361	35,172	69,342				
10	73,361	30,149	24,117	35,174	34,167	34,167	37,183	68,337	57,282			
11	50,246	49,241	42,208	76,376	78,384	78,384	32,160	70,347	76,377	44,217		
12	54,267	34,167	27,134	36,178	36,180	36,180	35,176	53,264	48,238	48,237	47,234	

Notes: 1- *T. dicoccoides* fast omega gliadin clone ES9-41, 2- *T. aestivum* Chinese Spring B3, 3- *T. aestivum* Chinese spring B6, 4- *T. aestivum* Chinese spring D1, 5- *T. aestivum* Chinese spring D2, 6- *T. aestivum* Chinese spring D3, 7- *T. aestivum* Jinan 177, 8- *T. aestivum* Yumai 34 clone 4, 9- *T. aestivum* Zhengfeng 5 clone 1, 10- *T. monococcum*, 11- *T. urartu* PI428198 allele Gli-omega-1, 12- *T. timopheevii*.

Table 4: Toxic epitopes of gamma-gliadins in wheat species and varieties.

Celiac disease epitopes	Region	Wheat
ATANMQVDPGQVQWPQQP	17-23	<i>T. dicoccoides</i> Atlit2015 <i>T. turgidum</i> clone 4 <i>T. urartu</i> PI428198 allele Gli-gamma 1, 2
QVDPSGQVQWPQ	22-33	<i>T. urartu</i> G1812 <i>T. urartu</i> PI428198 allele Gli-gamma 3
QQPFCQQPQRTIPQPHQTFH	42-61	<i>T. dicoccoides</i> Atlit2015
QQTYPHQPQQQFPQTQQPQQ	72-91	<i>T. dicoccoides</i> Atlit2015
PFPQPQQTFPQ	92-113	<i>T. dicoccoides</i> Atlit2015
SQQPQQQFSQPQQQFPQPQQ	168-190	<i>T. spelta</i>
QQPQQSFPQQQ	189-199	<i>T. aestivum</i> × <i>T. elongatum</i> Il-13-11 <i>T. aestivum</i> <i>T. dicoccoides</i> Atlit2015 <i>T. macha</i> clone Gli-Mr1 <i>T. spelta</i> <i>T. turgidum</i> clone 4 <i>T. urartu</i> PI428198 allele Gli-gamma 1, 2
PFPQPQQPF	84-93; 102-111; 120-147	<i>T. aestivum</i> × <i>T. elongatum</i> Il-13-11 <i>T. aestivum</i> <i>T. macha</i> clone Gli-Mr1 <i>T. dicoccoides</i> Atlit2015 <i>T. turgidum</i> clone 4 <i>T. urartu</i> PI428198 allele Gli-gamma 1, 2, 3
QQPAQYEVIRSLVRLTPNM	322-341	<i>T. turgidum</i> clone 4 <i>T. urartu</i> PI428198 allele Gli-gamma 1, 2
QYEVIRSLVRLTPNM	326-340	<i>T. turgidum</i> clone 4 <i>T. urartu</i> PI428198 allele Gli-gamma 1, 2
SQQPQQPFPQPQQQFPQPQQ	168-190	<i>T. dicoccoides</i> Atlit2015 <i>T. turgidum</i> clone 4
PQQPFPQPQQQFPQPQPQQ	171-193	<i>T. dicoccoides</i> Atlit2015 <i>T. turgidum</i> clone 4
VQGQGIIQPQQPAQL	313-327	<i>T. aestivum</i> × <i>T. elongatum</i> Il-13-11 <i>T. aestivum</i> <i>T. macha</i> clone Gli-Mr1 <i>T. spelta</i> <i>T. urartu</i> G1812 <i>T. urartu</i> PI428198 allele Gli-gamma 3
IHVAHSIIMQQEQQGGVPI	264-297	<i>T. dicoccoides</i> Atlit2015
LGIIQPQQPAQLEGIRSLVL	316-335	<i>T. dicoccoides</i> Atlit2015
QFPQLQQPQQPLQPQQPQ	173-192	<i>T. urartu</i> G1812 <i>T. urartu</i> PI428198 allele Gli-gamma 3
QFPQLQQPQQP	163-174	<i>T. aestivum</i> × <i>T. elongatum</i> Il-13-11 <i>T. aestivum</i>
QFPQLQQPQQP	173-183	<i>T. monococcum</i> clone 45-L024585
FLQPQQPFPQPQQPYPQQPQQPFPQ	78-149	<i>T. spelta</i>
FSQPQQQFPQPQ	175-189	<i>T. spelt</i>
PQQPFPQPQQP	81-91; 99-109; 117-127	<i>T. aestivum</i> × <i>T. elongatum</i> Il-13-11 <i>T. aestivum</i> <i>T. macha</i> clone Gli-Mr1 <i>T. spelta</i> <i>T. monococcum</i> clone 45-L024585 <i>T. urartu</i> G1812 <i>T. urartu</i> PI428198 allele Gli-gamma 3

Based on the results of multiple amino acid sequence alignments, omega-gliadins are composed primarily of glutamine (Q), proline (P) and phenylalanine (F), making up 80% of their structure. The identified motif PFPQ₁₋₂PQQ₁₋₂ appears in three variations: |PEPQQPQ|, |PFPQQPQ| and |PFPQPQQ|, repeated 24 times. Due to the overlapping nature of these motifs, it is challenging to detect distinct domains in these regions. The repetitive motif |PEPQQPQ|, characteristic of omega-gliadins, is also found in gamma-gliadins. Through bioinformatics analysis, segments of toxic epitopes associated with celiac disease were identified and analyzed in various wheat species and varieties (Table 3, 4).

Epitopes such as |PFPQPQQTFPQ|, |SQPQQPFPQPQQFPQPQQ| and |PQQPFPQPQQFPQPQQPQQ| contain the repetitive motif |PEPQQPQ|, while the epitope |PFPQPQQPF| contains the motif |PFPQPQQ|, a variant of |PEPQQPQ|. This motif is present in 8 out of the 11 wheat species and varieties. Gamma-gliadin contains multiple sequences of toxic epitopes that interact with human

T-helper cells, triggering an immune response, likely due to the conserved regions within gamma-gliadin that include several repetitive motifs such as PFPQ1-2PQQ1-2 (Qi *et al.*, 2009). The highest number of toxic epitopes was detected in the gamma-gliadins of *T. aestivum* and *T. dicoccoides* Atlit2015, while only two epitopes were found in *T. monococcum* clone 45-L024585 (Table 4).

A repetitive motif, |PFPQPQ|, is present in omega-gliadin epitopes such as |QQPQQPFPQPQQPFP| and |PLQPQQPFPQPQQPFPQPQ|. The epitopes |PQQPFPQPQQP| and |PLQPQQPFPQPQQPFPQPQ| share similar motifs |PFPQQPQ| and |PFPQPQQ|. Two epitopes, |QIPQQPQQF| and |QQQQIPQQPQQF|, are also similar and likely homologous, being present in similar regions of *T. aestivum* Chinese Spring B3 and B6. Additionally, these epitopes are found in other positions in *T. aestivum* Chinese Spring B3, at 251-262 and 302-313, due to a single amino acid substitution in the sequence. Thus, the variations in toxic epitopes in omega-gliadins are related to their unique amino acid composition, particularly the abundance of

Table 5: Toxic epitopes of omega-gliadins in wheat species and varieties.

Celiac disease epitopes	Region	Wheat
QIPQQPQQF	103-111; 135-143	<i>T. dicoccoides</i> Fast omega gliadin clone ES9-41
		<i>T. aestivum</i> Chinese Spring B3, B6
	200-208; 254-262; 305-313; 357-365	<i>T. aestivum</i> Chinese Spring B3
	200-208; 357-365	<i>T. aestivum</i> Chinese Spring B6
		<i>T. aestivum</i> Chinese Spring B3
QQQQIPQQPQQF	197-208; 251-262; 302-313; 354-365	<i>T. aestivum</i> Chinese Spring B3
	197-208; 354-365	<i>T. aestivum</i> Chinese Spring B6
PQQPFPQPQQP	140-164 366-377; 374-385	<i>T. aestivum</i> Chinese Spring D1
	140-164; 278-289; 342-353; 366-377; 382-393	<i>T. aestivum</i> Chinese Spring D2
	140-164; 278-289; 342-353; 366-377; 374-385	<i>T. aestivum</i> Chinese Spring D3
	140-164	<i>T. aestivum</i> Zhengfeng5 clone 1
	140-167; 301-329; 334-345	<i>T. urartu</i> PI428198 Gli-omega-1
QQPQQPFPQPQQPFP	162-176	<i>T. aestivum</i> Chinese Spring D1, D2, D3
		<i>T. aestivum</i> Yumai34 clone 4
		<i>T. aestivum</i> Chinese Spring D1, D2, D3
PFPWQPQQPFPQ	174-185	<i>T. aestivum</i> Chinese Spring D1, D2, D3
		<i>T. aestivum</i> Zhengfeng5 clone 1
PLQPQQPFPQPQQPFPQPQ	194-213; 275-293	<i>T. aestivum</i> Chinese Spring D2
	194-213	<i>T. aestivum</i> Chinese Spring D3
		<i>T. aestivum</i> Zhengfeng5 clone 1
		<i>T. urartu</i> PI428198 Gli-omega-1
		<i>T. aestivum</i> Chinese Spring D1
SHQQPFPQPQPYPQQPYPS	79-100	<i>T. aestivum</i> Chinese Spring D1, D2, D3
		<i>T. aestivum</i> Yumai34 clone 4
		<i>T. aestivum</i> Chinese Spring D1
LQPQQPFPQ	138-159; 195-203; 340-348; 364-372	<i>T. aestivum</i> Chinese Spring D2, D3
	138-159; 195-203; 276-284; 340-348	<i>T. aestivum</i> Yumai34 clone 4
	138-159	<i>T. aestivum</i> Zhengfeng5 clone 1
	138-159; 195-203	<i>T. urartu</i> PI428198 Gli-omega-1
IIPQQPQQPFL	254-276	<i>T. aestivum</i> Chinese Spring D2
	182-195	<i>T. aestivum</i> Chinese Spring D3
		<i>T. urartu</i> PI428198 Gli-omega-1

glutamine, proline and phenylalanine. *T. aestivum* Chinese Spring D2 and D3 have the highest number of identified celiac epitope regions.

Phylogenetic analysis

Phylogenetic analysis revealed significant genetic diversity among the studied wheat proteins. Fig 3 presents a

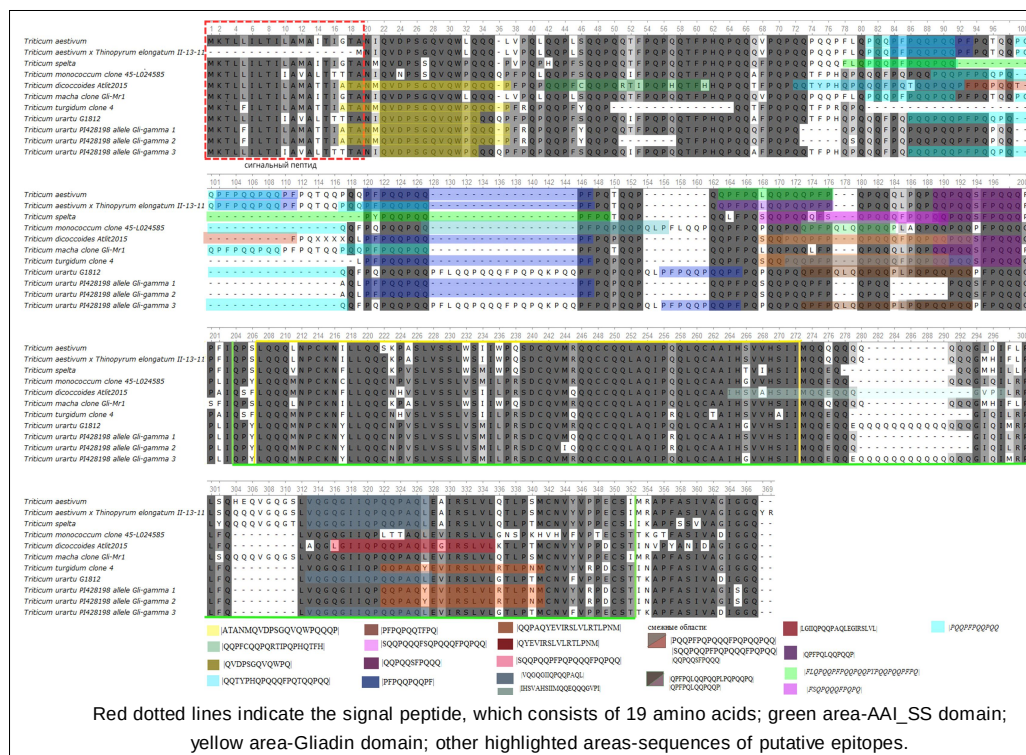


Fig 1: Multiple alignment of gamma-gliadins of different species and varieties of wheat.

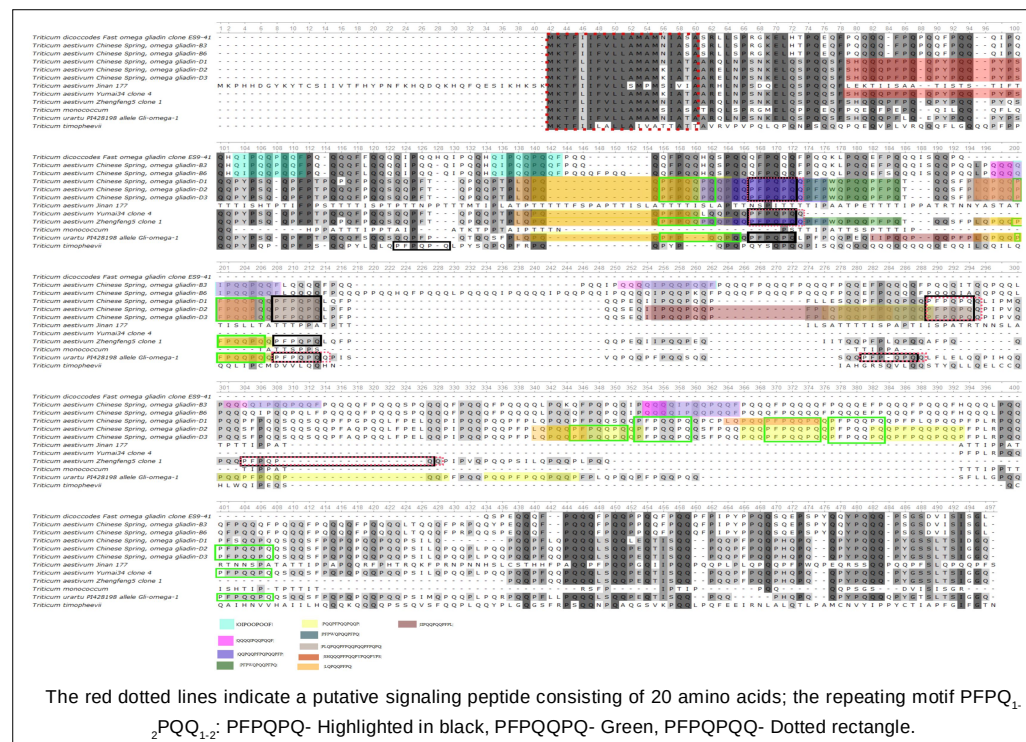


Fig 2: Multiple alignment of omega-gliadins of different species and varieties of wheat.

phylogram illustrating the distribution of gamma-gliadin proteins across the wheat species and varieties, grouping them into distinct clades. The proteins from *T. urartu* G1812, *T. urartu* PI428198 alleles Gli-gamma 1, 2 and 3 are closer to the common ancestor, while those from *T. dicoccoides* Atlit2015, *T. macha* clone Gli-Mr1, *T. aestivum* and *T. aestivum* × *Thinopyrum elongatum* II-13-11 are more distantly related. Almost all clades have very high bootstrap support. However, the clade containing *T. dicoccoides* Atlit2015 and *T. turgidum* clone 4, as well as the clade with *T. aestivum* and *T. aestivum* × *Thinopyrum elongatum* II-13-11, have bootstrap support values below 70, indicating less reliable branching in these parts of the phylogenetic tree. Notably, *T. urartu* does not form a single clade; gamma-gliadins *T. urartu* G1812 and *T. urartu* PI428198 allele Gli-gamma 3 are more similar to the protein from *T. monococcum* clone 45-L024585 than to *T. urartu* PI428198 alleles Gli-gamma 1 and 2, suggesting significant allelic diversity within this species.

Fig 4 shows the phylogenetic tree of omega-gliadin proteins. The proteins from *T. aestivum* Jinan 177, *T. monococcum* and *T. timopheevii* are the most divergent from the common ancestor. In contrast, the proteins from *T. aestivum* Chinese Spring B3, B6, *T. aestivum* Chinese Spring D1, D2 and *T. urartu* are closer to the common ancestor. Only one clade has a bootstrap support value below 70, which includes *T. aestivum* Zhengfeng5, *T. urartu* PI428198 allele Gli-omega-1, *T. aestivum* Chinese Spring D1 and D3 and *T. aestivum* Yumai34 clone 4. This clade also appears

to be more conserved compared to the others. The omega-gliadins D2 and D3 from the Chinese Spring variety show greater similarity to the Yumai34 clone 4 than to the D1 omega-gliadin of the same variety. Additionally, the omega-gliadins B3 and B6 of the Chinese Spring variety differ significantly from the omega-gliadins D1, D2 and D3. These findings suggest that each of these species may possess unique functional characteristics.

The results of the multiple alignment and phylogenetic analysis of gamma-gliadin proteins demonstrate a high degree of homology among the amino acid sequences across the studied wheat species and varieties. In contrast, omega-gliadins have a lower degree of conservation, suggesting they may be more prone to evolutionary changes. It is important to note that these gliadins are encoded by different genes, which may not always be fully represented in databases due to incomplete annotation. The phylogenetic analysis of gamma- and omega-gliadins across various wheat species and varieties enabled the tracing and establishment of their evolutionary relationships and relatedness.

The gamma- and omega-gliadin proteins in the studied wheat varieties are rich in repeating motifs, many of which are part of epitopes associated with celiac disease. The highest number of such epitopes was identified in gamma-gliadins from *T. aestivum* and *T. dicoccoides* Atlit2015, while only two epitopes were found in *T. monococcum* clone 45-L024585. The unique

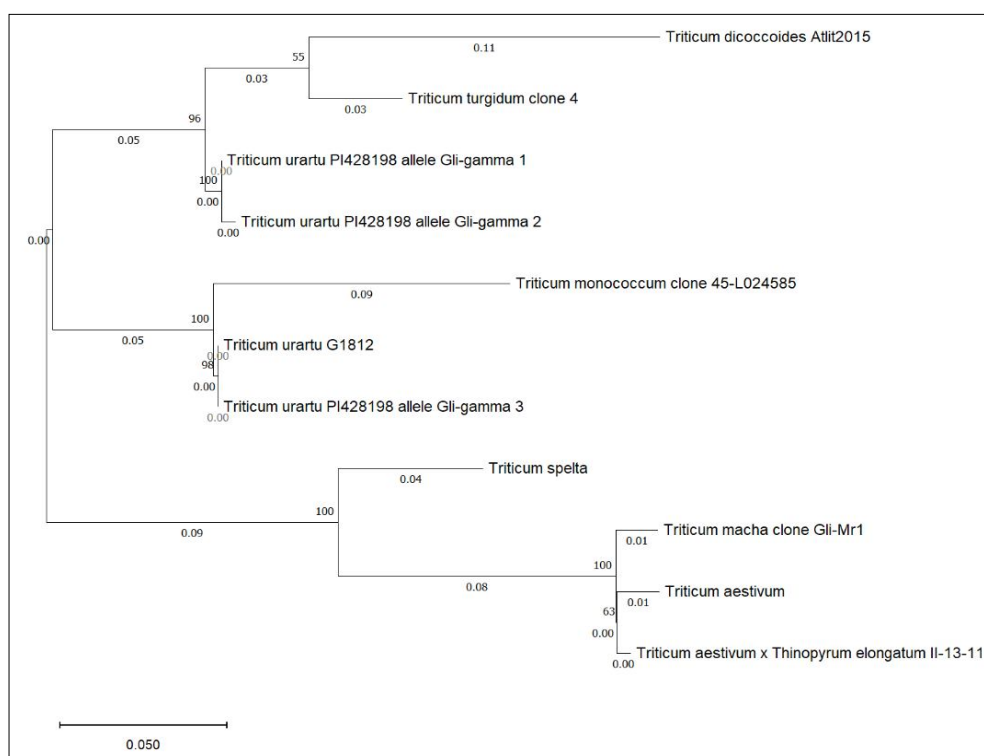


Fig 3: Phylogenetic analysis of wheat gamma-gliadins of different species and varieties.

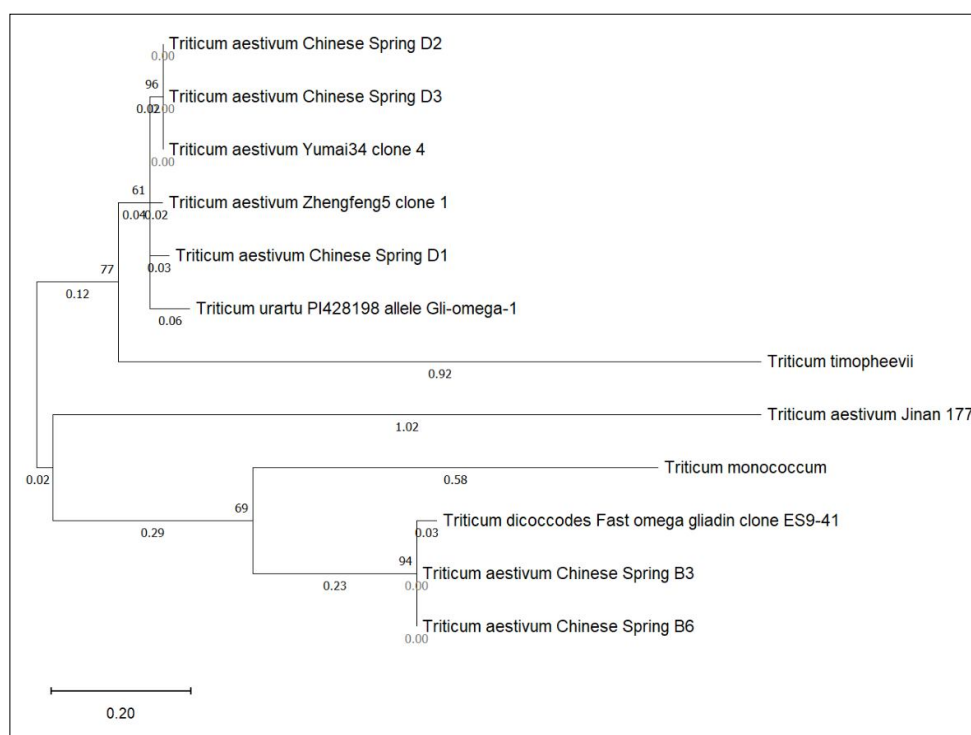


Fig 4: Phylogenetic analysis of wheat imega-gliadins of different species and varieties.

amino acid composition of omega-gliadins, characterized by high levels of glutamine, proline and phenylalanine, is linked to variations in their toxic epitopes. *T. aestivum* Chinese Spring D2 and D3 have the highest number of celiac disease-associated toxic epitopes. However, no putative toxic epitopes were found in *T. monococcum* DV80, which has previously been used in breeding programs to develop zinc-rich varieties (Kaur *et al.*, 2023).

CONCLUSION

The obtained data can be used for genetic editing, aiming to create wheat varieties that contain allergen-neutral gliadins through the reduction of repetitive epitope motifs associated with celiac disease. Moreover, altering the amino acid sequences of gliadins may lead to improved rheological properties of dough. However, further research is required to evaluate potential risks and undesirable side effects of genetic editing of these proteins.

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Conflict of interest

The authors declare no conflict of interest financial or otherwise.

REFERENCES

- Altenbach, S.B., Kothari, K.M. (2007) Omega gliadin genes expressed in *Triticum aestivum* cv. Butte 86: Effects of post-anthesis fertilizer on transcript accumulation during grain development. *Journal of Cereal Science*. 46(2): 169-177. doi: 10.1016/j.jcs.2007.02.001.
- Brackett, N.F., Pomés, A., Chapman, M.D. (2022). New frontiers: precise editing of allergen genes using CRISPR. *Frontiers in Allergy*. 2: 821107. doi: 10.3389/falgy.2021.821107.
- Breiteneder, H., Radauer, C. (2004). A classification of plant food allergens. *J. Allergy Clin Immunol*. 113(5): 821-831. doi: 10.1016/j.jaci.2004.01.779.
- Cabanillas, B. (2020). Gluten-related disorders: Celiac disease, wheat allergy and nonceliac gluten sensitivity. *Critical Reviews in Food Science and Nutrition*. 60(15): 2606-2621. doi: 10.1080/10408398.2019.1651689.
- Caio, G., Volta, U., Sapone, A., Leffler, D.A., De Giorgio, R., Catassi, C., Fasano, A. (2019). Celiac disease: A comprehensive current review. *BMC Medicine*. 17(1): 142. doi: 10.1186/s12916-019-1380-z.
- Camerlengo, F., Frittelli, A., Sparks, C., Doherty, A., Martignago, D., Larré, C., Lupi, R., Sestili, F., Masci, S. (2020). CRISPR-Cas9 multiplex editing of the α -Amylase/Trypsin inhibitor genes to reduce allergen proteins in durum wheat. *Front. Sustain. Food Syst*. 4: 104. doi: 10.3389/fsufs.2020.00104.
- Chaudhary, N., Viridi, A.S., Dangi, P., Khatkar, B.S., Mohanty, A.K., Singh, N. (2022). Protein, thermal and functional properties of α -, γ - and ω -gliadins of wheat and their effect on bread making characteristics. *Food Hydrocoll*. 124: 107212. doi: 10.1016/j.foodhyd.2021.107212.

- Elli, L., Branchi, F., Tomba, C., Villalta, D., Norsa, L., Ferretti, F., Roncoroni, L., Bardella, M.T. (2015). Diagnosis of gluten related disorders: Celiac disease, wheat allergy and non-celiac gluten sensitivity. *World Journal of Gastroenterology*. 21(23): 7110-7119. doi: 10.3748/wjg.v21.i23.7110.
- Franco, O.L., Rigden, D.J., Melo, F.R., Grossi-De-Sá, M.F. (2002). Plant alpha-amylase inhibitors and their interaction with insect alpha-amylases. *European Journal of Biochemistry*. 269(2): 397-412. doi: 10.1046/j.0014-2956.2001.02656.x.
- Goryunova, S.V., Salentijn, E.M., Chikida, N.N., Kochieva, E.Z., van der Meer, I.M., Gilissen, L.J., Smulders, M.J. (2012). Expansion of the gamma-gliadin gene family in *Aegilops* and *Triticum*. *BMC Evolutionary Biology*. 12: 215. doi: 10.1186/1471-2148-12-215.
- Hailegiorgis, D., Seid, E., Lee, C.A., Yun, S.J. (2022). Variations in immunodominant epitope and molecular conformation of alpha-gliadins in elite Ethiopian durum wheat cultivars. *J. Crop sci. Biotechnol.* 25: 325-336. doi: 10.1007/s12892-021-00134-0.
- Juhász, A., Békés, F., Wrigley, C.W. (2015). Wheat proteins. *Applied Food Protein Chemistry*. 219-303. doi: 10.1002/9781118860588.ch11.
- Juhász, A., Gell, B., Békés, F., Balázs, E. (2012). The epitopes in wheat proteins for defining toxic units relevant to human health. *Functional and Integrative Genomics*. 12: 585-598. doi: 10.1007/s10142-012-0302-3.
- Karthik, A., Uma, M.M. (2020). Smart fertilizer strategy for better crop production. *Agricultural Reviews*. 42(1): 12-21. doi: 10.18805/ag.R-1877.
- Kaur, K., Mavi, G.S., Bhagat, I., Sharma, A., Srivastava, P., Kaur, H., Sohu, V.S. (2023). Phenotypic evaluation of grain zinc enhanced wheat lines for agronomic and quality traits. *Indian Journal of Agricultural Research*. 57(1): 30-34. doi: 10.18805/IJArE.A-5553.
- Kumaraswamy, S., Shetty, P.K. (2016). Critical abiotic factors affecting implementation of technological innovations in rice and wheat production: A review. *Agricultural Reviews*. 37(4): 268-278. doi: 10.18805/ag.v37i4.6457.
- Ma, Y., Lee, C.J., Park, J.S. (2020). Strategies for optimizing the production of proteins and peptides with multiple disulfide bonds. *Antibiotics (Basel, Switzerland)*. 9(9): 541. doi: 10.3390/antibiotics9090541.
- Murakami, T., Nishimura, T., Ogawa, T., Kitabatake, N., Tani, F. (2018). Molecular analysis of polymeric glutenins in wheat gluten with gliadin-like characteristics by ammonia dispersion. *Food Science and Technology Research*. 24(6): 1049-1058. doi: 10.1111/1750-3841.13221.
- Narwal, S., Sharma, B., Saini, R., Singh, R.B., Gupta, O.P., Pandey, V., Ram, S., Singh, G.P. (2020). Exploring indian wheat genotypes for less celiac disease toxic epitopes. *Journal of Cereal Research*. 12(1): 79-82. doi: 10.25174/2582-2675/2020/89975.
- Qi, P.F., Wei, Y.M., Ouellet, T., Chen, Q., Tan, X., Zheng, Y.L. (2009). The gamma-gliadin multigene family in common wheat (*Triticum aestivum*) and its closely related species. *BMC Genomics*. 10: 168. doi: 10.1186/1471-2164-10-168.
- Schuster, C., Huen, J., Scherf, K.A. (2023). Comprehensive study on gluten composition and baking quality of winter wheat. *Cereal Chem*. 100: 142-155. doi: 10.1002/cche.10606.
- Sharma, N., Bhatia, S., Chunduri, V., Kaur, S., Sharma, S., Kapoor, P., Kumari, A., Garg, M. (2020). Pathogenesis of celiac disease and other gluten related disorders in wheat and strategies for mitigating them. *Frontiers in Nutrition*. 7: 6. doi: 10.3389/fnut.2020.00006.
- Sihmar, M., Sharma, J.K., Santal, A.R., Singh, N.P. (2020). Electrophoretic Evaluation of major seed storage protein fraction, gliadins and glutenins of eighty-six Indian wheat genotypes. *Agricultural Science Digest*. 40(2): 115-121. doi: 10.18805/ag.D-5085.
- Songstad, D.D., Petolino, J.F., Voytas, D.F., Reichert, N.A. (2017). Genome editing of plants. *Crit. Rev. Plant Sci*. 36: 1-3. doi: 10.1080/07352689.2017.1281663.
- Steinhauserova, I., Borilova, G. (2015). New trends towards more effective food safety control. *Procedia Food Science*. 5: 274-277. doi: 10.1016/j.profoo.2015.09.078.
- Wang, S., Weller, D., Falardeau, J., Strawn, L.K., Mardones, F.O., Adell, A.D., Moreno, S.A.I. (2016). Food safety trends: From globalization of whole genome sequencing to application of new tools to prevent foodborne diseases. *Trends Food Sci. Technol*. 57: 188-198. doi: 10.3390/foods12132535.
- Wieser, H., Koehler, P., Scherf, K.A. (2023). Chemistry of wheat gluten proteins: Qualitative composition. *Cereal Chem*. 100: 23-35. doi: 10.1002/cche.10572.