Research Article Open Access

Antigenic and genetic characterization of gluten of several wheat varieties

Manvel Badalyan[1], Tatevik Aloyan[1*], Satenik Kharatyan[2], Varya Dilanyan[3], Arayik Vardanyan[1], Hamlet Martirosyan[1], Armine Abrahamyan[1], Lusine Avagyan[3], Andreas Melikyan[1]

[1]Agrobiotechnology Scientific Center, Branch of Armenian National Agrarian University, Etchmiadzin, Republic of Armenia (RA); [2]Scientific Centre for Risk Assessment and Analysis in Food Safety Area, Yerevan, RA; [3]Armenian National Agrarian University, Yerevan, RA

***Corresponding Author**: Tatevik Aloyan, PhD," Agrobiotechnology Scientific Center" Branch of ANAU, 1 Isi–Le–Mulino Str., Etchmiadzin 1101, Republic of Armenia

Submission Date: October 30th, 2024; **Acceptance Date:** November 19th, 2024; **Publication Date:** November 27th, 2024

Please cite this article as: Badalyan M., Aloyan T., Kharatyan S., Dilanyan S., Vardanyan A., Martirosyan H., Abrahamyan A., Avagyan L., Melikyan A. Antigenic and genetic characterization of gluten of several wheat varieties. *Functional Food Science* 2024; 4(11): 452-465. DOI: https://www.doi.org/10.31989/ffs.v4i11.1507

ABSTRACT

Background: At this stage of agricultural development, the demand for modern wheat varieties have risen considerably. Beyond achieving maximum yield, it is now essential to produce grain that is both high-quality and safe. It is evident that addressing these challenges is nearly impossible through classical selection methods, which rely on a limited set of phenotypic indicators. Obtaining new varieties and enhancing the existing varieties of wheat to meet the needs of both the population and industry can only be achieved through advanced biotechnological methods, including molecular markers and genetic transformations. In this context, protein or biochemical markers are widely employed. Analyzing the electrophoresis spectrum and protein formula of gliadin enables the identification of key selection-genetic parameters of different wheat varieties. This approach also helps pinpoint the fractions and genotypes causing gluten intolerance or celiac disease.

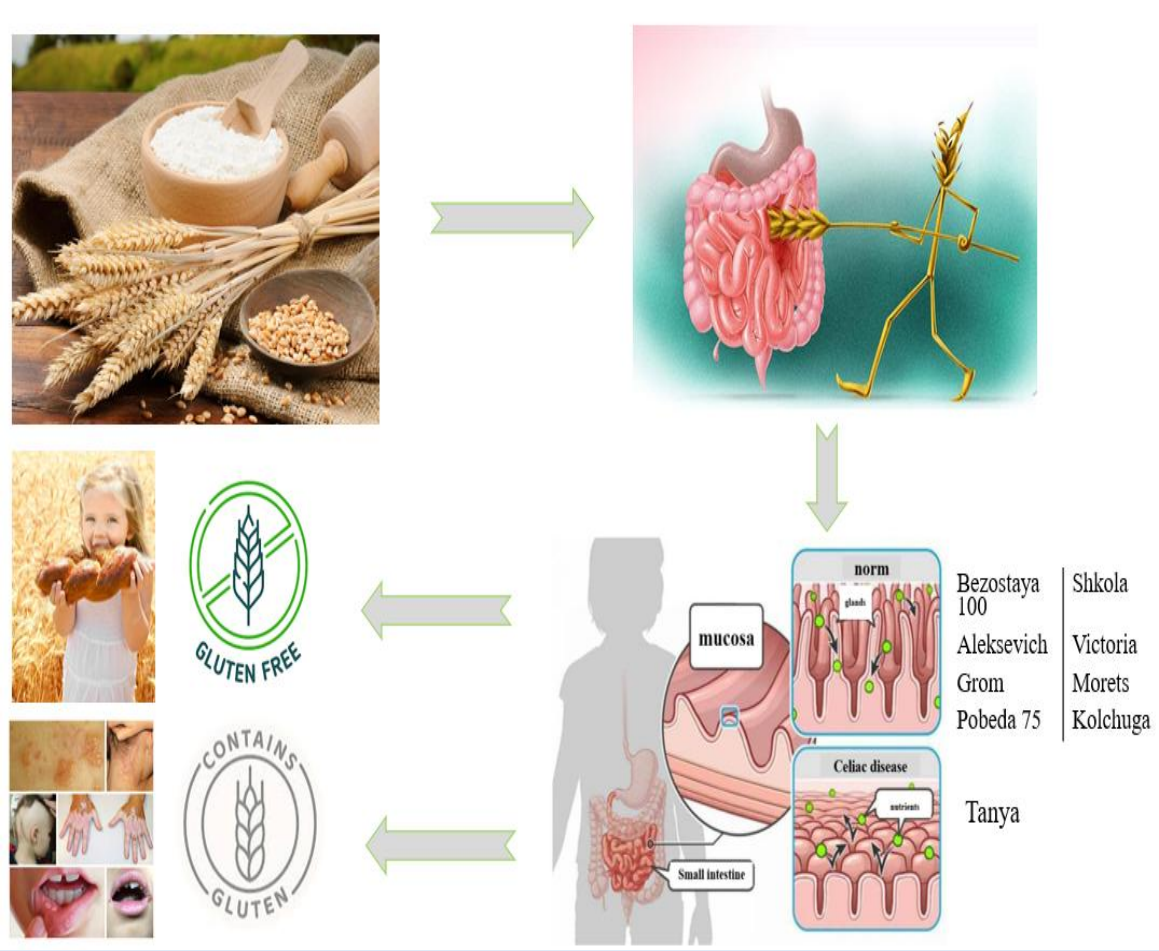
Objective: The objective of this study is to analyze the electrophoretic spectrum in gliadin of several valuable wheat varieties cultivated in Armenia. This includes identifying their protein profiles, conducting genomic analysis, and clarifying the genetic regulation and toxic epitopes that exhibit antigenic activity responsible for gluten intolerance.

Methods: The experimental studies were conducted during 2023-2024 at the Biological Research Laboratory of the "Agrobiotechnology Scientific Center," a branch of ANAU. The study utilized wheat varieites commonly grown in Armenia, including Bezostaya 100, Aleksevich, Grom, Pobeda 75, Shkola, Victoria, Morets, Tanya and Kolchuga varieties of. Electrophoresis was performed on 8% polyacrylamide gel.

Results: In the electrophoresis spectrum of the wheat varieties studied, the α, β, γ and ω regions were identified. The genes responsible for synthesizing the different fractions in these regions are located in 6A, 6B (S), 1A (S), 1 B (S) and 1D (S) chromosomes. Among the studied wheat varieties, only the Tanya was found to have an antigenic gliadin fraction (fraction 7), which can trigger gluten intolerance and allergy.

Conclusion*:* The results obtained can be used as genetic markers in the breeding process to develop new high-yield, antigen-free wheat varieties and improve existing ones.

Keywords: Celiac disease, gliadin, protein, electrophoresis, genome, protein formula.



INTRODUCTION

The demand for functional foods is growing worldwide as consumers seek to improve their health without making drastic change to their diet. Bioactive compounds in functional foods provide benefit beyond basic nutrition, such as antioxidative properties and disease prevention. Although these compounds are present in small quantities across various foods, they have the potential to reduce the risk of developing disease [1-2]. Functional foods not only offer essential nutrients but also play a role in promoting overall health and reducing the likelihood of chronic illnesses. [3–5]. Wheat (*Triticum* L.), one of the world's primary crops, contains numerous phytochemicals and nutraceutical compounds. It provides significant amounts of the dietary fiber, protein, energy, vitamins, antioxidants, and minerals essential for a healthy life. Additionally, wheat protein is unique in its properties and has diverse potential food applications as a food ingredient [6-7].

The protein content of the grain and its physicochemical properties are critical economic factors, as they directly impact the production of quality bakery and pasta products. The versatility of wheat protein in various food applications makes it an essential ingredient in the development of nutritional and functional foods [8]. The protein in wheat grain endosperm constitutes 12-14% of its mass. They proteins are highly diverse but can be classified based on their physiological functions into storage (residual) and protoplast (functional) proteins [9].

The storage proteins in the wheat grain are primarily gliadins and glutenin, which together form gluten. The highest concentration of gluten is highest is found in the cells of the sub-aleurone layer of the endosperm [10-11]. For over 250 years, gluten has been a focus of research for technologists, biochemists, breeders, and geneticists.

Gluten was first discovered in 1728 by the Italian scientist J.B. Beccari [12-13]. In 1819, G. Taddei demonstrated that it is composed of two different proteins, identifying one are soluble in alcohol and naming it gliadin. Later, American researcher T.B. Osborne identified the second component of gluten, which is soluble in a base, and named it glutenin [18-19]. Gliadin and glutenin together make up 80-85% of the total protein content in the endosperm [20-21]. Gluten plays a unique role in the baking process. By absorbing large amounts of water, it forms a continuous protein matrix, that encapsulates starch granules, creating a network essential for fermentation. During this process, carbon dioxide is released, which relaxes the dough, gives it a porous structure, and increases the volume [22–24].

Research has shown quantitative and qualitative traits of grain storage proteins are specific to each variety, are genetically determine, and are inherited in a codominant manner [25-26]. Each population within a species or variety has a unique genetic structure, distinguishing it from others. Gliadin and glutenin polymorphisms are now widely used as genetic markers to identify the genetic characteristics of different cultivars [27-28]. These markers provide valuable insights into the population-genetic and nutritional properties of both cultivated and wild wheat species.

Currently, the separation of residual protein fractions and the identification of wheat varieties are carried out using polyacrylamide gel electrophoresis and

chromatography. Gliadin electrophoresis was first performed by R.W. Jones, in 1959 [28-29]. Subsequent research revealed that, under a constant electric field, gliadin separates into 4 zones: α1234567, β12345, γ12345, ω12345678 [30, 31]. This diversity is governed by a multigenic system, with the genes regulating the synthesis of different sections of α, β, γ and ω regions of gliadin located on different chromosomes (6A, CD (a), CB (S), 1 A (S), 1 B (S), 1D (S)) – a trait characteristic of the species [32]. Each allele encodes two or more gliadin polypeptides (electrophoretic fraction) that exhibit linked inheritance patterns (Figure 1) [33

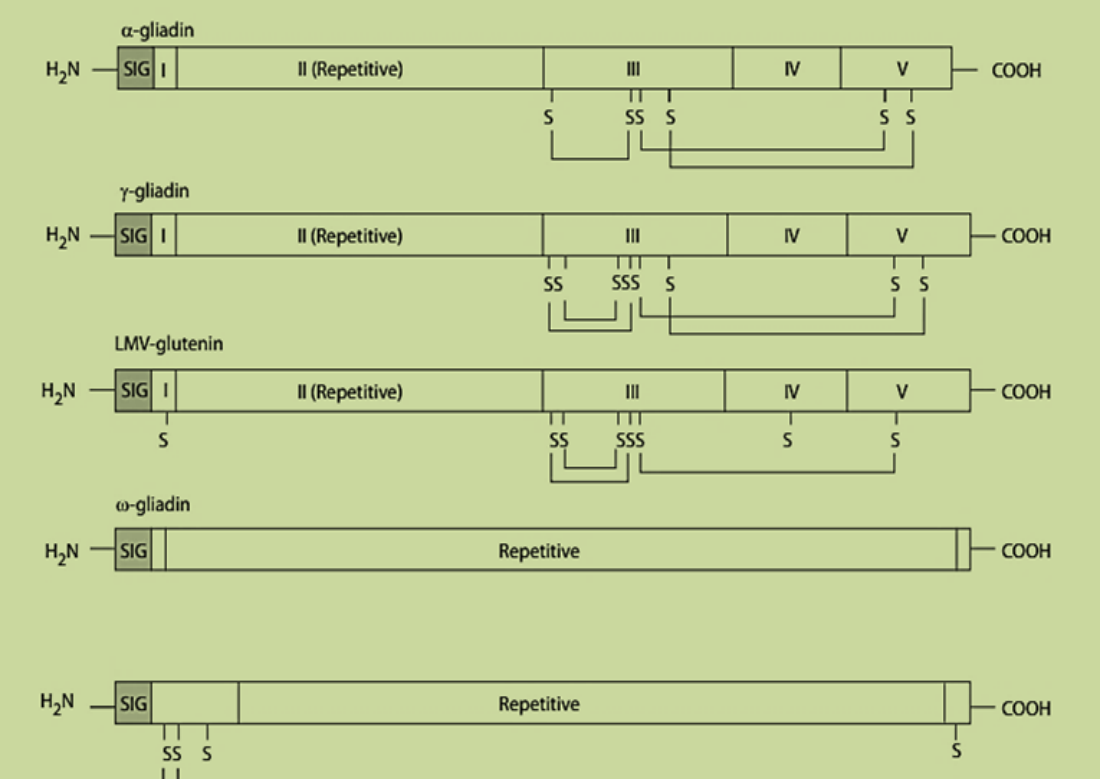


Figure 1. The general structure of the wheat prolamin classes is diagrammed showing the main sequence domains, conserved cysteine residues (S), and intramolecular disulfide bonds (lines connecting Ss). The signal peptides (SIG) are shown in blue [34].

The molecular mass of the α, β, and γ fractions of gliadin ranges from 31,000 to 34,700 Da and constitutes 80 to 90% of the total fractions [35, 36]. These fractions are rich in glutamic acid and glutamine, which makeup 35-45% of their composition, followed by proline (15-20%), cystine (2.5%), methionine (1%) and lysine (0.5-0.7%) [37-38]. The α and β gliadins share a similar primary structure, consisting of approximately 250 and 300 amino acid residues, respectively. Their primary structure includes a signal peptide (20 amino acids), a stable N-terminal domain (5 amino acid residues), a double proline-rich domain, and repeated glutamine sequences. These sequences contain characteristic heptapeptides (P-Q-P-Q-P-F-P) and pentapeptides (P-Q-Q-P-Y) [37-38]. γ-gliadin polypeptides, on the other hand, consist of a series of domains (Figure 1). This includes a signal sequence of 20 amino acids, an N-terminal peptide of 12 amino acid residues, and a subsequent domain primarily composed of proline and gluten [39].

In the electrophoresis spectrum of gliadin, the ω region accounts for 18-20% of the total. ω-gliadin has a very large molecular mass, ranging from 64,000-80,500 Da. Its key characteristics include a very weak charge of molecules and a low or absent content of methionine and cystine, along with the presence of intramolecular S-S bonds (Figure 1). The overall structure of ω-gliadin consists of a signal peptide (19 amino acid residues), an N-terminal domain (10-11 residues), a repeat domain that occupies 90-96% of the protein, and a C-terminal domain composed of 10-11 amino acid residues [40-41].

While cereals play a vital role in the human diet, they can also pose health risks causing conditions like celiac disease (CD) or gluten intolerance and allergy. Celiac disease is a chronic genetic disease in which gluten-containing foods damage the mucosa of the small intestine, where the main absorption of nutrients occurs. In the case of this disease, the organism does not absorb enough proteins, fats, carbohydrates and vitamins, which leads to weight loss and exhaustion [42–46].

Gluten intolerance affects 0.5-1% of the population and develops in individuals with genetic abnormalities in their immune system. In these cases, the body recognizes gluten as an allergen, which leads to the activation of an immune response reaction [47].

CD-stimulating gliadin peptides typically contain one or more antigenic determinants (epitopes) that can bind to T cells. These epitopes, which are usually rich in proline and glutamine, are resistant to degradation by protoplasm [48-49]. Such epitopes bind to specific haplotypes of the human leukocyte antigen (HLA) , forming leukocyte antigens HLA-DQ2 and HLA-DQ8. This binding triggers complex biochemical and biological changes in the cell, leading to the destruction of epithelial cells and atrophy of the small intestinal glands, which causes various diseases that may mask the true cause of the condition [40, 50–52].

Allergy to gluten is an acquired condition that can develop at any stage of life. For various reasons, the immune system od the patient begins to recognize the proteins in cereals as foreign and harmful, triggering a protective reaction against them [53].

It is proven that intestinal proteolytic dysfunction and allergy are due to gliadin function and genome [54-55].

The purpose of this study is to examine the electrophoretic spectrum of gliadin from several valuable varieties of wheat cultivated in Armenia, decode their protein formulas, and perform genomic analysis.

The results of the research, as genetic markers, will enable the control of the wheat breeding process through the genetic regulation of gliadin loci. This will help create new wheat varieties free of toxic (allergenic) gliadin fractions or improving existing ones for the population affected by celiac disease.

MATERIALS AND METHODS

Sampling: Experimental studies were conducted in 2023-2024 at the Biological Research Laboratory of the "Agrobiotechnology Scientific Center" branch of ANAU.

The Armenian cultivated wheat varieties used in the study were Bezostaya 100, Aleksevich, Grom, Pobeda 75, Shkola, Victoria, Morets, Tanya and Kolchuga. All the varieties studied are soft winter wheat cultivars of Russian production.

Experimental studies: The wheat seeds were powdered, and the powder was then mixed with 10-fold of 0.2 M NaCl buffer and incubated at 45°C for 1 h. Afterward, the mixture was centrifuged at 8000× g for 30 min to remove the precipitation, and the pH of the supernatant was

adjusted to 6.4 using 2 M HCl. Following overnight storage at 4°C, the precipitate was collected by centrifugation (6000× g, 20 min, 4°C) and frozen at 24 °C for 24 h. Next, approximately 10 g of gliadin and 90 g of deionized water (10°C) were mixed using a glass rod, and the solution was homogenized twice. The supernatant was discarded, and the precipitate was dissolved in a minimal volume of a buffer solution containing 0.4 g of Tris, 3 ml of 1 M HCl,1 g of Sodium Dodecyl Sulfate (SDS), 5 g of sucrose, 18 g of urea, 2.5 mL of mercaptoethanol and 0.25 g of bromophenol blue in 100 mL of water, centrifuged and used for electrophoresis.

Electrophoresis was performed on an 8% polyacrylamide gel using a Multigel-long electrophoresis apparatus (Biometra, Germany) (Table 1).

Table 1. Conditions necessary for electrophoresis of gliadin protein.

Protein	Gel, %	Gel length, cm	Sample titer	Buffer		Power voltage, V	Phoresis duration, hours
				gel	electrode		
Gld	8	12	1:1	0.05 *M* tris *HCl*, pH=8,8	0.016 *M* aluminum-lactose, pH=8,7	280	3,0

After electrophoresis, the gel was fixed for 60 minutes in a solution of ethanol, acetic acid, distilled water (40:10:60), then stained with Kummas G-250 dye for 30-60 minutes. The gel was then washed 3 times with washing buffer (10% acetic acid solution). Visualization of the gel was performed using the GelDoc Go (Bio-Rad, USA) system. Image analysis of the electrophoresis image was performed using GelAnalyzer 23.1.1 software. To decipher the gliadin spectrum and protein formulas of the samples studied, the research results were compared with the reference spectrum of gliadin (Figure 2) [30] Genomic analysis of gliadin was performed according to gliadin genetic regulations (Figure 3).

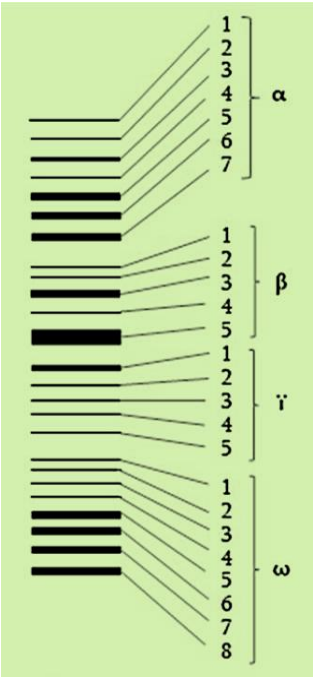


Figure 2. Reference spectrum of gliadin.

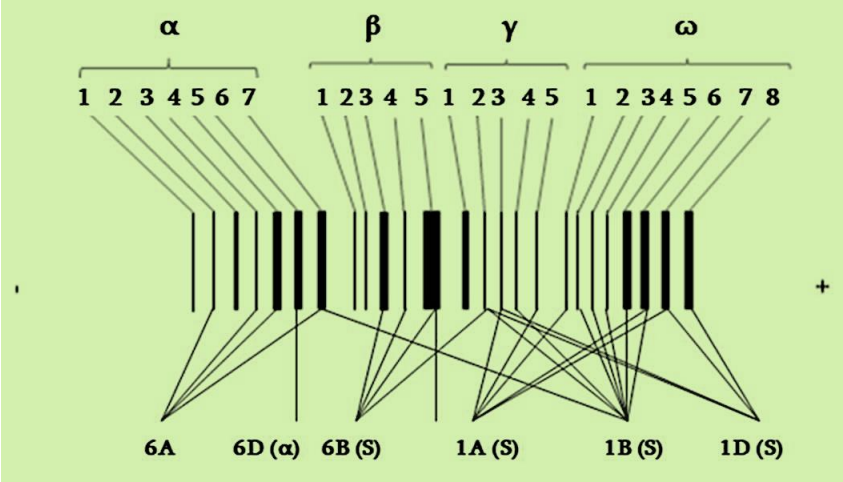


Figure 3. Genetic regulations of gliadin.

Statistical data processing was performed using SPSS and MS Excel programs, using standard methods of population-genetic research.

RESULTS

In the current conditions of agricultural intensification, the requirements for modern wheat varieties have changed significantly. In addition to maximum yield, it is also necessary to produce high-quality grain free of foreign genes and allergens, which is nearly impossible with classical breeding methods. Currently, molecular-genetic markers are used to address these challenges, with the electrophoresis spectrum of wheat storage proteins proving irreplaceable for several key indicators [56-57].

The protein formulas and genetic regulations of gliadin for the studied wheat varieties are shown in Tables 2 and 3. The obtained results are statistically significant (P>0.99).

Table 2. Protein formula of gliadin of different wheat varieties.

№	Varieties of wheat	Protein formula and fractions of gliadin (Gld)			
		α	β	γ	ω
1	Bezostaya 100	0	3 5	1 2	1 3 4 7
2	Aleksevich	0	5	3 5	2 4 5
3	Grom	1 2 7	1 4	3 5	3 7 8
4	Pobeda 75	2	1 3 5	2 3 4	1 2 3 6 8
5	Shkola	0	1 2 4 5	1 2 5	1 2 3 4 6 7
6	Victoria	2 4 6	5	3 5	1 2 3 7
7	Morets	0	1 3 4	3 4 5	1 2 3 4
8	Tanya	7	1 5	2 3 4 5	2 3 4 6 7 8
9	Kolchuga	0	2 5	1 3 4	2 3 7

According to the data in the table, the Bezostaya 100 variety of wheat is distinguished by the sparse gliadin spectrum and bands in the foregram. There are 8 polypeptides, including 2 β, 2 γ and 4 ω bands. The α-

gliadin is missing. The genes regulating the synthesis of the β-gliadin fragments 3 5 are located in the 6B(S) and D chromosomes. The genes for the γ-gliadin fragments 1 and 2 are located on the – 1A(S) and 1D(S) chromosomes, and the genes for ω-gliadin fragments 1, 3, 4, and 7 are located on the 1A(S), 1B(S) and 1D(S) chromosomes.

The Aleksevich variety of wheat also does not show the abundant electrophoresis spectrum of gliadin, making it the variety with the lowest number of polypeptides. The α-gliadin is absent, and the genes regulating the synthesis of the β-gliadin fragment 5 are located on the 6B(S), D5 chromosomes. The genes γ-gliadin fragments 3 and 5 are located on the 1A(S) chromosome, and the genes for the ω-gliadin fragments 2, 4, and 5 are located on the 1B(S) chromosome (Table 3).

Table 3. Genome analysis of different wheat varieties by protein markers

№	Varieties of wheat	6A	6D(a)	6B(S)	1A(S)	1B(S)	1D(S)
1	Bezostaya 100	-		β 3 D 5	γ 1 2 ω 1 7	ω 3 4	γ 2 ω 7
2	Aleksevich	-		β 5 D 5	γ 3 5	ω 2 4 5	-
3	Grom	α 1 2 7		β 1 4	γ 3 5 ω 7	α 7 ω 3	ω 7 8
4	Pobeda 75	α 2		β 1 3 5 D 5	γ 2 3 ω 1 6	γ 4 ω 2 3	γ 3 4 ω 8
5	Shkola	-		β 1 2 3 4 5 D 5	γ 1 2 5 ω 1 7	ω 2 3 4 6	γ 2 ω 7
6	Victoria	α 2 4 6		β 5 D 5	γ 3 5 ω 1 7	ω 2 3	γ 3 ω 7
7	Morets	-		β 1 3 4	γ 3 4 5 ω 1	ω 2 3 4	γ 4
8	Tanya	α 7		β 1 D 5 γ 2	γ 3 5 ω 7	γ 4 ω 2 3 4 6	α 7 γ 2 3 ω 7 8
9	Kolchuga	-		β 2 5 D 5	γ 1 4 ω 1 7	ω 3	γ 4 ω 7

The formation of α 1 2 7, β 1 4, γ 3 5, ω 3 7 8 fragments is characteristic of the gliadin electrophoresis spectrum of wheat variety Grom (Table 2). There are ten polypeptides. The genes synthesizing the specified fragments of α-gliadin are located on the 6A and 1B(S) chromosomes; the β-gliadin fragments are on the 6B(S) chromosome; the γ-gliadin fragments are on the 1A(S) chromosome; and the ω-gliadin fragments are on the 1A(S), 1B(S), and 1D(S) chromosomes (Table 3).

All 4 bands are also present in the electrophoresis spectrum of gliadin of the Pobeda 75 wheat variety. The genes responsible for the synthesis of fragment 2 of α-gliadin are located on the 6A chromosome, fragments 1 3 5 of β-gliadin on the 6B(S) and D5 chromosome,

fragments 2 3 4 of γ-gliadin on the 1A(S), 1B(S), 1D(S) chromosome; and fragments 1 2 3 6 8 of ω-gliadin are located on 1A(S), 1B(S), 1D(S) chromosomes. There are twelve polypeptides in the electrophoresis spectrum of gliadin (Tables 23).

According to the analysis of the electrophoretic spectrum of Shkola wheat variety, gliadin formed β, γ and ω bands, with α-gliadin is absent. The total number of polypeptides is thirteen. The genes regulating the synthesis of fragments 1, 2, 4, and 5 of β-gliadin are located on the 6B(S) and D5 chromosomes; fragments 1, 2, and 5 of γ-gliadin are located on the 1A(S), 1D(S) chromosomes; and fragments 1 2 3 4 6 7 of ω-gliadin are located on the 1A(S), 1B(S), 1D(S) chromosomes (Tables 2, 3).

According to the electrophoretic formula of gliadin of the Victoria wheat variety, there are α 2 4 6, β 5, γ 3 5, and ω 1 2 3 7 bands, with corresponding fragments. The genes responsible for their synthesis are located as follows: α-gliadin on the 6A chromosome, β-gliadin on the 6B(S) chromosome, γ-gliadin on the 1A(S) and 1D(S) chromosome, and ω-gliadin on the 1A(S), 1B(S), 1D(S) chromosomes. The total number of polypeptides is ten (Tables 2, 3).

The α-gliadin is absent in the electrophoresis spectrum of Morets wheat variety. The distribution of ten protein polypeptides in the other bands is following: β 1 3 4, with synthesis genes located on the 6B(S) chromosome, γ 3 4 5 with genes located on the 1A(S) and 1D(S) chromosome, ω 1 2 3 4 with genes located on the 1A(S) and 1B(S) chromosomes (Tables 2, 3).

The electrophoretic spectrum of gliadin from the Tanya wheat variety includes all four bands. The genes regulating the synthesis of fragment 7 of α-gliadin are located on the 6A and 1D(S) chromosomes, fragments 1 5 of β-gliadin are located on the 6B(S) and D5 chromosomes, fragments 2 3 4 5 of γ-gliadin are located on the 1A(S), 1B(S), and 1D(S) chromosomes, and fragments 2 3 4 6 7 8 of ω-gliadin are located on the 1A(S), 1B(S), 1D(S) chromosomes (Tables 2, 3).

The α-gliadin is absent in the electrophoretic spectrum of gliadin from the Kolchuga wheat variety. There are two fragments in the β-gliadin, and three fragments each in the γ- and ω-gliadins. The genes responsible for the synthesis of fragments of β-gliadin are located on the 6B(S) and D5 chromosomes, the fragments 1 3 4 of γ-gliadin are located on the 1A(S) and 1D(S) chromosomes, the fragments 2 3 7 of ω-gliadin are located on the 1A(S) and 1D(S) chromosomes (Table 2, 3).

It should be noted that the genes responsible for gliadin synthesis are not located on 6D(a) chromosome in any of the studied wheat varieties.

DISCUSSION

Modern cultivated wheat varieties belong to two amphidiploid species: *Triticum durum* Desf and *Triticum aestivum* L. The first, *T. durum,* is tetraploid (2n=28) with the genomic formula AABB, while the second, *T. aestivum,* is hexaploid (2n=48) with the genomic formula AABBDD [56]. However, electrophoresis results of residual proteins from both hard and soft wheat indicate that these forms share identical genomes. The differences between varieties are attributed to the genetic diversity of the same genome. Furthermore, interspecific and intervarietal polymorphism of proteins and enzymes has been observed [59-60].

Regarding the genetic regulation of residual proteins of wheat, several researchers have noted that the genetic control of different gliding bands and fractions is associated with six chromosomes: 1A, 1B, 1D, 6A, 6B, 6D, within the respective groups of the first and sixth chromosome sets [31, 61–63].

Based on their analysis of the gliadin electrophoresis spectrum of the Kubanka wheat variety, T.I. Peneva and O.A. Lyapunova concluded that the α-gliadin is exclusively represented by four main fractions:

α 2 4 6 7, α 5 7, α 3 4 6 7, α 3 5 6 7. According to the authors, this structure is attributed to the ancient *durum-turgidum* biotype of wheat. The genes responsible for synthesizing these fragments of α-gliadin are located on 1A, 1B and 6B chromosomes [64].

Through their study of alleles and loci of residual proteins of soft winter wheat varieties, E.V. Zaika and colleagues reported that new alleles (Gli-Alx, Gli-Ble) are formed during intervarietal crosses. However, these alleles are found at very low frequencies and are associated with low baking quality [65].

Professional literature highlights a correlation between beneficial economic characteristics and gliadin fractions. According to researchers, wheat varieties where gliadin synthesis occurs on the Gld 1A, 1B, and 1D chromosomes show greater resistant to cold and drought. Furthermore, gliadin synthesized in Gld $1A_5$, $1A_4$, and $1B_1$ chromosomes increases the baking quality.11, 21].

As a result of studying the electrophoretic spectrum of gliadin of wild relatives and local cultivars of wheat cultivated in Armenia and deciphering the protein formula, it was observed that gliadin separates into 4 bands: α, β, γ and ω. Among these, α gliadin is found relatively rare. The genes responsible for the synthesis of β-gliadin are in 6B(S) and D5 chromosomes, γ-gliadin on the 1A(S) and 1B(S) chromosomes, and ω-gliadin on the 1D(S) and 1A(S) chromosomes. Notably, no genes regulating the synthesis of gliadin fragments were found on 6D(a) chromosome in the studied samples [28]. Overall, there are few varieties with genes for synthesizing different gliadin bands and fragments located on 6D(a) chromosome [27].

Gliadin peptides that contribute to celiac disease or gluten intolerance typically contain one or more epitopes capable of binding to human T cells [45, 46]. Additionally, it is well established that different types of gliadins differ significantly in the epitope content associated with celiac disease [36].

Wheat-dependent exercise-induced anaphylaxis (WDEIA) and baker's asthma are two common IgE-induced wheat allergens with an estimated frequency up to 2% [66-67].

D.W. Wang and his colleagues, studying the electrophoresis spectrum of gliadin of Xiaoyan 81 wheat variety, reported the presence of 24 CD epitopes. Among these, the toxic peptide of α-gliadin is composed of 33 amino acid residues and includes three epitopes: DQ2.5-glia-a1a, DQ2.5-glia -a1b and DQ2.5-glia-a2 encoded by the D genome [40].

A scientist studying the presence of celiac disease and WDEIA epitopes in the gliadin spectrum of various varieties of wheat reported that α-gliadin contains a significant number of toxic epitopes, the genes responsible for the synthesis of its fractions are located on the D chromosome, such as α-D4, α-D5, α-D6, α-D8, and α-D9. In contrast, the B genome-encoded ω-B3 and ω-B6 fractions of ω-gliadin do not contain celiac disease epitopes. However, the D genome-encoded fractions of ω-gliadin – ω-D1, ω-D2, ω-D3-- contain 12-18 CD epitopes, while the ω-D4 fraction contains 33 WDEIA epitopes [54].

It is evident that gluten sensitivity and allergy are influenced by the presence of specific spectra of α and ω gliadins, determined by the chromosomal location of the genes synthesizing them. Only four of the wheat varieties studied by us contain α-gliadin: Grom, Pobeda 75, Victoria, Tanya. However, none of them have α-gliadin synthesis genes on 6D(a) chromosome. Additionally, the genes responsible for the synthesis of the fragment 7 of α-gliadin in the Tanya wheat variety are located on the 1D(S) chromosome and included four epitopes associated with gluten intolerance. Of the nine wheat varieties studied, only Tanya was identified as a potential cause of celiac disease.

Currently, improving flour quality while reducing the amount of toxic gluten fractions is a critical challenge. One potential solution involves a molecular genetic approach, where the synthesis of unwanted gliadin is stopped due to RNA interference.

F. Barro and colleagues successfully suppressed the high expression of the genes responsible for the synthesizing α-, γ-, and ω-gliadins by employing RNA constructs. This method targeted CD epitopes from the highly immunogenic α- and ω-gliadins while preserving the total protein and starch content of the grain [68].

CONCLUSION

In the electrophoresis spectrum of gliadin from the wheat varieties studied, all bands are present except for α-gliadin, which appeared with a frequency of 44.4%. The genes responsible for synthesizing the different fractions of α-, β-, γ-, and ω-gliadins are located on 6A, 6B(S), 1B(S), and 1D(S) chromosomes. However, no genes for gliadin fragment synthesis were found on the 6D(a) chromosome.

Among the wheat varieties studied, Tanya is the only one where the genes responsible for the synthesis of fragment 7 of α-gliadin are located on the 1D(S) chromosome. This type of gluten can function as an antigen, potentially triggering celiac disease or gluten intolerance.

These findings can serve as genetic markers for developing new wheat varieties with desirable economic and biological characteristics, free from the gliadin fractions that cause celiac disease. Additionally, they can aide in improving existing varieties of wheat.

The results of a similar research conducted for the first time in Armenia would contribute to the production of safe food and the proper and efficient utilization of genetic resources.

Acknowledgment: The authors thank the journal's editorial board and reviewers, whose constructive comments and suggestions improved the quality of the article.

Abbreviations: CD: Celiac Disease, HLA: Human Leukocyte Antigen, SDS: Sodium Dodecyl Sulfate, WDEIA: Wheat-dependent exercise-induced anaphylaxis.

Competing interests: The authors declare that are no conflicts competing interests.

Authors' contributions: MB - conceptualization, methodology, validation, resources, data curation, writing-original draft preparation, writing-review and editing; TA - methodology, resources, data curation, writing-original draft preparation, writing-review and editing, SKh - methodology, data curation, resources, writing-review and editing; VD - resources, writing-review and editing; AV - resources, writing-review and editing; HM - resources, writing-review and editing; AA - resources, writing-review and editing; LA - resources, writing-review and editing; AM - conceptualization, methodology, validation, resources, data curation, writing-original draft preparation, writing-review, editing, supervision. All authors read and approved the final version of the manuscript.

REFERENCES:

1. Martirosyan, D.; Lampert, T.; Ekblad, M. Classification and Regulation of Functional Food Proposed by the Functional Food Center. Functional Food Science 2022, 2, 25. DOI: https://doi.org/10.31989/ffs.v2i2.890
2. Martirosyan, D.; Stratton, S. Quantum and Tempus Theories of Function Food Science in Practice. Functional Food Science 2023, 3, 55. DOI: https://doi.org/10.31989/ffs.v3i5.1122
3. Martirosyan, D.; Stratton, S. Advancing Functional Food Regulation. Bioact Compd Health Dis 2023, 6, 166. DOI: https://doi.org/10.31989/bchd.v6i7.1178

4. Martirosyan, D.; Miller, E. Bioactive Compounds: The Key to Functional Foods. Bioact Compd Health Dis 2018, 1, 36. DOI: https://doi.org/10.31989/bchd.v1i3.539
5. Avagyan, G.; Daveyan, S.; Eloyan, A.; Sahakyan, S.; Asatryan, A.; Martirosyan, H. Influence of Water-Absorbing Polymers on Grain Crop Yield, Disease Incidence, and End-Use Quality. Functional Food Science - Online ISSN: 2767-3146 2024, 4, 261–276. DOI: https://doi.org/10.31989/ffs.v4i7.1391
6. Hanuman, B.; Harmandeep, K.; Deep Narayan, Y. Application of Wheat and Its Constituents in Diverse Functional Food Products. In Wheat Science; CRC Press, 2023; p. 34.
7. Otles, S.; Nakilcioglu-Tas, E. Cereal-Based Functional Foods. In Functional Foods; Wiley, 2022; pp. 55–90. DOI: https://doi.org/10.1002/9781119776345.ch3
8. Shewry, P.R.; Hey, S.J. The Contribution of Wheat to Human Diet and Health. Food Energy Secur 2015, 4, 178–202. DOI: https://doi.org/10.1002/fes3.64
9. Khalid, A.; Hameed, A.; Tahir, M.F. Wheat Quality: A Review on Chemical Composition, Nutritional Attributes, Grain Anatomy, Types, Classification, and Function of Seed Storage Proteins in Bread Making Quality. Front Nutr 2023, DOI: https://doi.org/10.3389/fnut.2023.1053196
10. Konarev, V.G. Wheat Proteins; Kolos: Moskow, Russia, 1980; 351 p. (in Russian)
11. Khrunov, A.A.; Fisenko, A.V.; Beletsky, S.L.; Dragovich, A.Yu. Study of the Relationship between the Composition of Gliadins and Economically Valuable Traits of Soft Wheat. Izvestiya of Timiryazev Agricultural Academy 2011, 2, 11–19. (in Russian)
12. Bailey, C.H. A Translation of Beccaria's Lecture Concerning Grain (1728). Cereal Chem. 1941, 18, 555–561.
13. This vo Kientza, H. Who Discovered the Gluten and Who Discovered Its Production by Lixiviation? Notes Académiques de l'Académie d'agriculture de France / Academic Notes of the French Academy of Agriculture 2018, 6, 1–11. DOI: https://doi.org/10.58630/pubac.not.a435750
14. Taddei, G. Ricerchesulglutine Del Frumento. Giornale Di Fisica, Chimica, e Storianaturale. Brugnatelli 1819, 2, 360–361.
15. Urade, R.; Sato, N.; Sugiyama, M. Gliadins from Wheat Grain: An Overview, from Primary Structure to Nanostructures of Aggregates. Biophys Rev 2018, 10, 435–443. DOI: https://doi.org/10.1007/s12551-017-0367-2
16. Wrigley, C.W.; Békés, F.; Bushuk, W. Chapter 1 Gluten: A Balance of Gliadin and Glutenin. In Gliadin and Glutenin: The Unique Balance of Wheat Quality; AACC International, Inc.: 3340 Pilot Knob Road, St. Paul, Minnesota 55121, U.S.A., 2006; pp. 3–32.
17. Singh, S.; Singh, N.; MacRitchie, F. Relationship of Polymeric Proteins with Pasting, Gel Dynamic- and Dough Empirical-Rheology in Different Indian Wheat Varieties. Food Hydrocoll 2011, 25, 19–24. DOI: https://doi.org/10.1016/j.foodhyd.2010.05.001
18. Osborne, T.B. The Proteins of the Wheat Kernel; Carnegie Inst: Washington, 1907; 124 p.
19. Asri, N.; Rostami-Nejad, M.; Anderson, R.P.; Rostami, K. The Gluten Gene: Unlocking the Understanding of Gluten Sensitivity and Intolerance. Appl Clin Genet 2021, Volume 14, 37–50. DOI: https://doi.org/10.2147/TACG.S276596
20. Pourmohammadi, K.; Abedi, E. Hydrolytic Enzymes and Their Directly and Indirectly Effects on Gluten and Dough Properties: An Extensive Review. Food Sci Nutr 2021, 9, 3988–4006. DOI: https://doi.org/10.1002/fsn3.2344
21. Pourmohammadi, K.; Abedi, E.; Hashemi, S.M.B. Gliadin and Glutenin Genomes and Their Effects on the Technological Aspect of Wheat-Based Products. Curr Res Food Sci 2023, 7, 100622. DOI: https://doi.org/10.1016/j.crfs.2023.100622
22. Istudor, A.; Voicu, G.; Muscalu, G.; Tudor, P. Evaluation of Carbon Dioxide Released by Bread Dough during Proving Stage. E3S Web of Conferences 2020,180, 03012. DOI: https://doi.org/10.1051/e3sconf/202018003012
23. Ye, L.; Zheng, W.; Li, X.; Han, W.; Shen, J.; Lin, Q.; Hou, L.; Liao, L.; Zeng, X. The Role of Gluten in Food Products and Dietary Restriction: Exploring the Potential for Restoring Immune Tolerance. Foods 2023, 12, 4179. DOI: https://doi.org/10.3390/foods12224179
24. Zhang, M.; Jia, R.; Ma, M.; Yang, T.; Sun, Q.; Li, M. Versatile Wheat Gluten: Functional Properties and Application in the Food-Related Industry. Crit Rev Food Sci Nutr 2023, 63, 10444–10460. DOI: https://doi.org/10.1080/10408398.2022.2078785
25. Zobova, N.V.; Bogdanov, V.V.; Ibragimova, M.Z. Gliadin Component Composition of Siberian Varieties of Spring Soft Wheat Referred to Various Grain Quality Groups. The Bulletin of KrasGAU 2018, 6, 61–65. (in Russian)
26. Yu, S.; Assanga, S.O.; Awika, J.M.; Ibrahim, A.M.H.; Rudd, J.C.; Xue, Q.; Guttieri, M.J.; Zhang, G.; Baker, J.A.; Jessup, K.E.; et al. Genetic Mapping of Quantitative Trait Loci for End-Use Quality and Grain Minerals in Hard Red Winter Wheat. Agronomy 2021, 11, 2519. DOI: https://doi.org/10.3390/agronomy11122519
27. Konarev, V.G. Molecular-Biological Studies of the Gene Pool of Cultural Plants in VIR (1967-2007).; 2nd ed.; VIR: Saint Petersburg, 2007; 134 p. (in Russian)

28. Badalyan, M.V.; Melikyan, A.Sh.; Aloyan, T.B.; Sahakyan, A.J. Study of Genetic Diversity of Cereals Using Protein Markers. AgriScience and Technology 2023, 1, 24–30. (in Armenian) DOI: https://doi.org/10.52276/25792822-2023.1-24
29. Jones, R.W.; Taylor, N.W.; Senti, F.R. Electrophoresis and Fractionation of Wheat Gluten. Arch Biochem Biophys 1959, 84, 363–376. DOI: https://doi.org/10.1016/0003-9861(59)90599-5
30. Konarev V.G. Plant Proteins as Genetic Markers; Kolos: Moscow, Russia, 1983; 320 p. (in Russian)
31. Markgren, J.; Hedenqvist, M.; Rasheed, F.; Skepö, M.; Johansson, E. Glutenin and Gliadin, a Piece in the Puzzle of Their Structural Properties in the Cell Described through Monte Carlo Simulations. Biomolecules 2020, 1095. DOI: https://doi.org/10.3390/biom10081095
32. Yu, Z.; Yunusbaev, U.; Fritz, A.; Tilley, M.; Akhunova, A.; Trick, H.; Akhunov, E. CRISPR-based Editing of the Ω- and Γ-gliadin Gene Clusters Reduces Wheat Immunoreactivity without Affecting Grain Protein Quality. Plant Biotechnol J 2024, 22, 892–903. DOI: https://doi.org/10.1111/pbi.14231
33. Metakovsky, E.; Pascual, L.; Vaccino, P.; Melnik, V.; Rodriguez-Quijano, M.; Popovych, Y.; Chebotar, S.; Rogers, W.J. Heteroalleles in Common Wheat: Multiple Differences between Allelic Variants of the Gli-B1 Locus. Int J Mol Sci 2021, 22, 1832. DOI: https://doi.org/10.3390/ijms22041832
34. Anderson, O.D.; Dong, L.; Huo, N.; Gu, Y.Q. A New Class of Wheat Gliadin Genes and Proteins. PLoS One 2012, 7, e52139. DOI: https://doi.org/10.1371/journal.pone.0052139
35. Barak, S.; Mudgil, D.; Khatkar, B.S. Biochemical and Functional Properties of Wheat Gliadins: A Review. Crit Rev Food Sci Nutr 2015, 55, 357–368. DOI: https://doi.org/10.1080/10408398.2012.654863
36. Wang, D.; Li, F.; Cao, S.; Zhang, K. Genomic and Functional Genomics Analyses of Gluten Proteins and Prospect for Simultaneous Improvement of End-Use and Health-Related Traits in Wheat. Theoretical and Applied Genetics 2020, 133, 1521–1539. DOI: https://doi.org/10.1007/s00122-020-03557-5
37. Noma, S.; Kawaura, K.; Hayakawa, K.; Abe, C.; Tsuge, N.; Ogihara, Y. Comprehensive Molecular Characterization of the α/β-Gliadin Multigene Family in Hexaploid Wheat. Molecular Genetics and Genomics 2016, 291, 65–77. DOI: https://doi.org/10.1007/s00438-015-1086-7
38. Kajendran, K.; Chandrasekharan, N.V.; Hettiarachchi, C.M.; Sulochana Wijesundera, W.S. Molecular Characterization and Expression of α-Gliadin Genes from Wheat Cultivar Dacke in Bg 250 Rice Variety. GM CropsFood2019,10,102–114. DOI: https://doi.org/10.1080/21645698.2019.1622990
39. Metakovsky, E.; Melnik, V.A.; Pascual, L.; Wrigley, C.W. Gliadin Genotypes Worldwide for Spring Wheats (*Triticum aestivum* L.) 2. Strong Differentiation of Polymorphism between Countries and Regions of Origin. J Cereal Sci 2019, 87, 311–317. DOI: https://doi.org/10.1016/j.jcs.2019.04.015
40. Wang, D.-W.; Li, D.; Wang, J.; Zhao, Y.; Wang, Z.; Yue, G.; Liu, X.; Qin, H.; Zhang, K.; Dong, L.; et al. Genome-Wide Analysis of Complex Wheat Gliadins, the Dominant Carriers of Celiac Disease Epitopes. Sci Rep 2017, 7, 44609. DOI: https://doi.org/10.1038/srep44609
41. Urade, R.; Sato, N.; Sugiyama, M. Gliadins from Wheat Grain: An Overview, from Primary Structure to Nanostructures of Aggregates. Biophys Rev 2018, 10, 435–443. DOI: https://doi.org/10.1007/s12551-017-0367-2
42. Catassi, C.; Fasano, A. Celiac Disease. Curr Opin Gastroenterol 2008, 24, 687–691. DOI: https://doi.org/10.1097/MOG.0b013e32830edc1e
43. Auricchio, R.; Tosco, A.; Piccolo, E.; Galatola, M.; Izzo, V.; Maglio, M.; Paparo, F.; Troncone, R.; Greco, L. Potential Celiac Children: 9-Year Follow-Up on a Gluten-Containing Diet. American Journal of Gastroenterology 2014, 109, 913–921. DOI: https://doi.org/10.1038/ajg.2014.77
44. Akhondi, H.; Ross, A.B. Gluten-Associated Medical Problems; StatPearls. Treasure Island (FL): StatPearls Publishing, 2022;
45. Gómez, C.; Colina, J. Formulation of Gluten-Free Cupcake with Hydrocolloids for People with Celiac Disease. Bioact Compd Health Dis 2019, 2, 134. DOI: https://doi.org/10.31989/bchd.v2i6.630
46. Thatsanasuwan, N.; Duangjai, A.; Suttirak, P.; Phanthurat, N. Proximate Composition and Sensory Attributes of Gluten-Free Pasta Made from Jackfruit Seeds. Functional Foods in Health and Disease 2023, 13, 11. DOI: https://doi.org/10.31989/ffhd.v13i1.1039
47. Posner, E.B.; Haseeb, M. Celiac Disease; StatPearls. Treasure Island (FL): StatPearls Publishing, 2023;
48. Escarnot, E.; Gofflot, S.; Sinnaeve, G.; Dubois, B.; Bertin, P.; Mingeot, D. Reactivity of Gluten Proteins from Spelt and Bread Wheat Accessions towards A1 and G12 Antibodies in the Framework of Celiac Disease. Food Chem 2018, 268, 522–532. DOI: https://doi.org/10.1016/j.foodchem.2018.06.094

49. Ruiz-Carnicer, Á.; Comino, I.; Segura, V.; Ozuna, C. V.; Moreno, M. de L.; López-Casado, M.Á.; Torres, M.I.; Barro, F.; Sousa, C. Celiac Immunogenic Potential of α-Gliadin Epitope Variants from Triticum and Aegilops Species. Nutrients 2019, 11, 220. DOI: https://doi.org/10.3390/nu11020220
50. Stamnaes, J.; Sollid, L.M. Celiac Disease: Autoimmunity in Response to Food Antigen. Semin Immunol 2015, 27, 343–352. DOI: https://doi.org/10.1016/j.smim.2015.11.001
51. Khan, T.; Rahman, M.; Ahmed, I.; Al Ali, F.; Jithesh, P.V.; Marr, N. Human Leukocyte Antigen Class II Gene Diversity Tunes Antibody Repertoires to Common Pathogens. Front Immunol 2022, 13. DOI: https://doi.org/10.3389/fimmu.2022.856497
52. Aljada, B.; Zohni, A.; El-Matary, W. The Gluten-Free Diet for Celiac Disease and Beyond. Nutrients 2021, 13, 3993. DOI: https://doi.org/10.3390/nu13113993
53. Oxentenko, A.S.; Murray, J.A. Celiac Disease: Ten Things That Every Gastroenterologist Should Know. Clinical Gastroenterology and Hepatology 2015, 13, 1396–1404. DOI: https://doi.org/10.1016/j.cgh.2014.07.024
54. Altenbach, S.B.; Chang, H.-C.; Simon-Buss, A.; Mohr, T.; Huo, N.; Gu, Y.Q. Exploiting the Reference Genome Sequence of Hexaploid Wheat: A Proteomic Study of Flour Proteins from the Cultivar Chinese Spring. Funct Integr Genomics 2020, 20, 1–16. DOI: https://doi.org/10.1007/s10142-019-00694-z
55. Cenni, S.; Sesenna, V.; Boiardi, G.; Casertano, M.; Russo, G.; Reginelli, A.; Esposito, S.; Strisciuglio, C. The Role of Gluten in Gastrointestinal Disorders: A Review. Nutrients 2023, 15, 1615. DOI: https://doi.org/10.3390/nu15071615
56. Dai, Y.; Li, J.; Shi, J.; Gao, Y.; Ma, H.; Wang, Y.; Ma, H. Molecular Characterization and Marker Development of the HMW-GS Gene from *Thinopyrum elongatum* for Improving Wheat Quality. Int J Mol Sci 2023, 24, 11072. DOI: https://doi.org/10.3390/ijms241311072
57. Song, L.; Wang, R.; Yang, X.; Zhang, A.; Liu, D. Molecular Markers and Their Applications in Marker-Assisted Selection (MAS) in Bread Wheat (*Triticum aestivum* L.). Agriculture 2023, 13, 642. DOI: https://doi.org/10.3390/agriculture13030642
58. Feldman, M.; Levy, A.A. *Triticum* L. In Wheat Evolution and Domestication; Springer International Publishing: Cham, 2023; pp. 365–526.
59. Brown, J.W.S.; Law, C.N.; Worland, A.J.; Flavell, R.B. Genetic Variation in Wheat Endosperm Proteins: An Analysis by Two-Dimensional Electrophoresis Using Intervarietal Chromosomal Substitution Lines. Theoretical and Applied Genetics 1981, 59, 361–371. DOI: https://doi.org/10.1007/BF00276450
60. Giancaspro, A.; Giove, S.L.; Zacheo, S.A.; Blanco, A.; Gadaleta, A. Genetic Variation for Protein Content and Yield-Related Traits in a Durum Population Derived From an Inter-Specific Cross Between Hexaploid and Tetraploid Wheat Cultivars. Front Plant Sci 2019, 10. DOI: https://doi.org/10.3389/fpls.2019.01509
61. Waga, J.; Zientarski, J.; Szaleniec, M.; Obtułowicz, K.; Dyga, W.; Skoczowski, A. Null Alleles in Gliadin Coding Loci and Wheat Allergenic Properties. Am J Plant Sci 2013, 04, 160–168. DOI: https://doi.org/10.4236/ajps.2013.41021
62. Metakovsky, E.; Melnik, V.; Rodriguez-Quijano, M.; Upelniek, V.; Carrillo, J.M. A Catalog of Gliadin Alleles: Polymorphism of 20th-Century Common Wheat Germplasm. Crop J 2018, 6, 628–641. DOI: https://doi.org/10.1016/j.cj.2018.02.003
63. Jang, Y.-R.; Cho, K.; Kim, S.; Sim, J.-R.; Lee, S.-B.; Kim, B.-G.; Gu, Y.Q.; Altenbach, S.B.; Lim, S.-H.; Goo, T.-W.; et al. Comparison of MALDI-TOF-MS and RP-HPLC as Rapid Screening Methods for Wheat Lines With Altered Gliadin Compositions. Front Plant Sci 2020, 11. DOI: https://doi.org/10.3389/fpls.2020.600489
64. Peneva, T.I.; Lyapunova, O.A. Electrophoretic Patterns of Gliadin as Markers of Genotypes in the Analysis of the Durum Wheat Landrace Kubanka. Proceedings on applied botany, genetics and breeding 2021, 181, 127–135. (in Russian) DOI: https://doi.org/10.30901/2227-8834-2020-4-127-135
65. Zaika, E.V.; Kozub, N.A.; Sozinov, I.A.; Sozinov, A.A.; Starichenko, V.N. Analysis of Genotypes of Winter Soft Wheat Varieties of the NSC "Institute of Agriculture of the National Academy of Agrarian Sciences" by Alleles of Storage Protein Loci. Bulletin of the Belarusian State Agricultural Academy 2014, 4, 53–57. (in Russian)
66. Vinichenko N.A.; Salina E.A. Gliadins: Structure and Toxic Epitopes. Letters to Vavilov Journal of Genetics and Breeding 2021, 7, 163–171. (in Russian) DOI: https://doi.org/10.18699/LettersVJ2021-7-21
67. Cabanillas, B. Gluten-Related Disorders: Celiac Disease, Wheat Allergy, and Nonceliac Gluten Sensitivity. Crit Rev Food Sci Nutr 2020, 60, 2606–2621. DOI: https://doi.org/10.1080/10408398.2019.1651689
68. Barro, F.; Iehisa, J.C.M.; Giménez, M.J.; García-Molina, M.D.; Ozuna, C. V.; Comino, I.; Sousa, C.; Gil-Humanes, J. Targeting of Prolamins by RNAi in Bread Wheat: Effectiveness of Seven Silencing-fragment Combinations for Obtaining Lines Devoid of Coeliac Disease Epitopes from Highly Immunogenic Gliadins. Plant Biotechnol J 2016, 14, 986–996. DOI: https://doi.org/10.1111/pbi.12455