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# Comparison of Alleles at *Gli-2* Loci of Common Wheat by Means of Two-Dimensional Electrophoresis of Gliadin

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**Abstract**—Electrophoretic mobility (EM) and molecular weight (MW) of some allelic variants of α- and β-gliadins contrlled by *Gli-2* loci were compared by means of two-dimensional (APAGE × SDS) electrophoresis. Comparison of α-gliadins of the alleles *Gli-A2b* and *Gli-A2p*, of β-gliadins of the *Gli-B2b* and *Gli-B2c*, and of β-gliadins of the *Gli-D2b*, *Gli-D2c*, *Gli-D2j*, and *Gli-D2r* indicated that a gliadin with lower EM had, as a rule, bigger MW which is known to depend on the length of the polyglutamine domain of gliadin of α-type. However, allelic variants of the α-gliadin encoded by *Gli-D2b* and *Gli-D2e* differ in EM but not in apparent MW. It might be caused by a substitution of some charged/uncharged aminoacids in the polypeptide of gliadin. Allele *Gli-B2o* which is very frequent in up-to-date common wheat germplasm originated probably by means of unequal cross-ingover. Some alleles at *Gli-A2* is found to control completely different blocks of gliadins and therefore might come to common wheat from different genotypes of the polymorphic diploid donor of the A genome. The results indicate that the reason of the known more vast polymorphism of gliadins controlled by *Gli-2* loci as compared with *Gli-1* loci is the considerable difference of the structure, first, of *Gli-1* and *Gli-2* loci (*Gli-2* loci have more expressed genes per locus) and, second, of genes encoding gliadins of α- and γ-types (α-gliadins are shown to contain a long polyglutamine sequences highly variable in their length).

*Keywords:* gliadin, alleles, mutations, common wheat
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## INTRODUCTION

Storage protein of wheat seeds—gliadin—is a convenient object for genetic studies due to its exceptionally high level of polymorphism [1]. Each common weat genotype is characterized by a unique set of several dozen synthesized gliadin polypeptides that differ in the primary sequence of amino acids and, consequently, in amino acid composition, electric charge, and molecular weight (MW). Synthesis of almost all gliadin polypeptides (gliadins) is controlled by six gliadin-encoding *Gli* loci (*Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2*, and *Gli-D2*). Multiple allelism was described for each of those loci [2–4]. Each allele controls synthesis of several polypeptides (block) inherited together. The alleles of the same locus differ in their electrophoretic mobility (EM) and the number of polypeptides encoded by the block [2–4]. The number of alleles at the *Gli-2* loci is significantly higher than the number of known alleles at the *Gli-1* loci [4]. New alleles develop as a result of spontaneous mutations in *Gli* loci. This causes EM alterations in gliadin polypeptides [5]. Blocks encoded by mutant variants of *Gli* loci are inherited [6].

According to EM in one-dimensional polyacrylamide gel (PAGE) in acid buffer, all gliadin polypeptides are classified in four groups: α- (the highest EM), β-, γ-, and ω-gliadins [1]. Not only polypeptides of gliadin but also amino acid sequences and nucleotide sequences encoding gliadins are classified in accordance with EM of gliadins. Almost all gliadins from the β-zone of electrophoregrams have α-type amino acid sequences [7, 8]. Thus, there are α-, γ-, and ω-gliadins on the one hand and α-, γ-, and ω-amino acid sequences and/or nucleotide sequences on the other hand [9].

Repetitive domains, as well as two polyglutamine domains, which are intragenic microsatellites at the level of DNA, are characteristic of genes encoding gliadins of the α-type [10, 11]. Genes of the *Gli-2* loci control synthesis of α-type gliadins (i.e., α- and β-gliadins on PAGE electrophoregrams). They vary in nucleotide substitutions, small deletions/insertions, length of microsatellites, and a number of repeats [10–13]. Large deletions within the domain of repeats were described [10].

Changes in length of polyglutamine region or a number of intragenic repeats cause changes in gliadin polypeptide MW. Mutation at the *Gli* locus can be detected via PAGE, since mutations cause changes in polypeptide EM [5]. Comparison of γ-gliadins encoded by alleles at *Gli-A1* or *Gli-D1* loci demonstrated that a

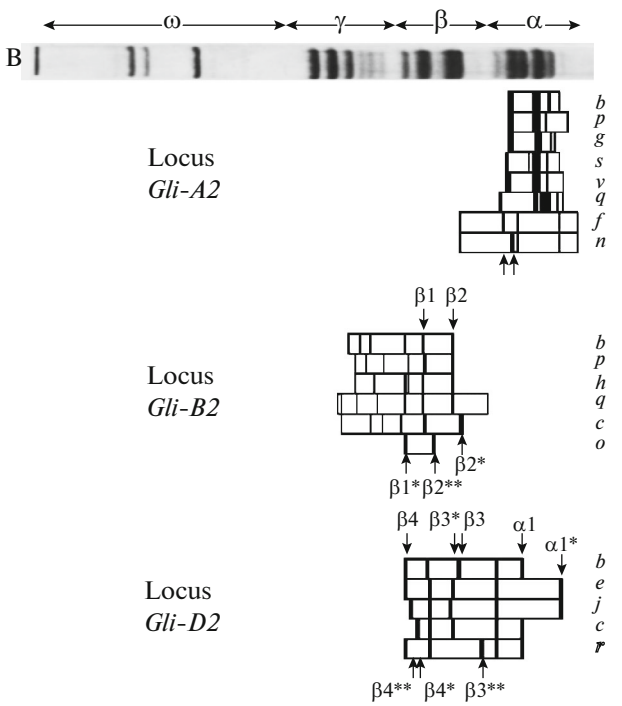


**Fig. 1.** Blocks of common weat gliadin components inherited together (PAGE). They are controlled by allelic variants from *Gli-2* loci. α- and β-gliadins mentioned in the text are emphasized. B—electrophoregram of variety Bezostaya 1 (standard). Gliadin zones (α, β, γ, and ω) are represented.

polypeptide with higher EM has a lower MW [14]. Mutations might induce substitution of a polar amino acid by a neutral one (or vice versa) in a polypeptide. This could induce EM alteration identifiable by PAGE, while MW remained the same.

Could EM alteration of gliadin polypeptide, which had an α-type amino acid sequence, change MW of the polypeptide? Why is the level of polymorphism higher at *Gli-2* loci than at *Gli-1* loci? To answer these questions, we compared EM and MW values for polypeptide blocks encoded by alleles at the *Gli-2* loci and several α- and β-gliadins controlled by known alleles at the *Gli-2* loci.

## MATERIAL AND METHODS

Common weat (*Trtiticum aestivum* L.) seeds from collections of the Science-Research Agricultural Institutes of Odessa, Ukraine, and Sant'Angelo Lodigiano, Italy, were used in our investigation.

It is well known that separation of gliadin polypeptides in one-dimensional electrophoresis in acid buffer (PAGE) is based on the electric charge of polypeptides [9]. Two-dimensional electrophoresis was performed as follows: first dimension resolution was done in PAGE in acid buffer, and second dimension resolution was performed in SDS-PAGE [14]. The relative gliadin MW could be evaluated using SDS-PAGE adequately, although absolute values could be incorrectly higher [9].

## RESULTS

PAGE analysis of gliadin was carried out for 939 varieties of common weat from 20 countries. In total, more than 150 alleles were identified at the *Gli* loci, and more than 90 of them were identified at the *Gli-2* loci [1, 9].

**Alleles at *Gli-A2* locus.** Two families of alleles at the *Gli-A2* locus are well known [7]: Bezostaya 1 type (*Gli-A2b*, *Gli-A2p*, *Gli-A2s*, and *Gli-A2q* alleles) and Chinese Spring type (*Gli-A2f* and *Gli-A2n*) (Fig. 1). Within each family, the blocks differ in EM for several α-gliadins [4]. In blocks encoded by alleles at the first family, 4–5 electrophoretic components were detected by PAGE. Evidently, some of these components are mixtures of two or more polypeptides characterized by similar EM, since two-dimensional patterns of the same blocks have up to ten α-gliadins for which EM and/or MW vary. Although PAGE components of blocks, encoded by alleles of the first family, differ only in details (Fig. 1), two-dimensional patterns of the same blocks differ in EM and MW for several α-gliadins simultaneously. For example, *Gli-A2p* (Fig. 2a), *Gli-A2b* (Fig. 2b), *Gli-A2q* (Figs. 2c, 2d), *Gli-A2s* (Fig. 2e), *Gli- A2g* (Fig. 2f), *Gli-A2v* (Fig. 2h), and *Gli-A2d* (Fig. 2j).

Analysis of two-dimensional electrophoregrams of the same block from different varieties demonstrated differences in relative staining intensity and/or EM for several α-gliadins from this block (Figs. 2c, 2d). These differences may be associated with experimental variation of the method [15].

Several alleles at the first family differ in EM of the most mobile α-gliadin [4]. For example, *Gli-A2p* allele encodes a fast-moving α-gliadin having higher EM in comparison with the *Gli-A2b* allele (Figs. 1, 4a). Two-dimensional electrophoresis of gliadin from Rusalka (allele *Gli-A2p*, Fig. 2a) and Bezostaya 1 (*Gli-A2b*, Fig. 2b) varieties demonstrated that higher EM of α-gliadin (allele *Gli-A2p*) is accompanied by a decrease in its MW by 0.5 kDa. In the course of analysis of individual seeds of variety Skorospelka Uluchshennaya, a spontaneous mutant was discovered. That mutant had altered EM in one of the α-gliadins (indicated by the arrow (Fig. 2j). SDS-PSGE analysis showed that a decrease in the EM of this α-gliadin in the mutant (Fig. 2l) is accompanied by an increase in its MW by approximately 0.5 kDa versus control.

Blocks of gliadin polypeptides, encoded by second family alleles at *Gli-A2n* and *Gli-A2f*, differ in EM of one electrophoretic component. It was located in the middle of the α-zone (Figs. 1, 4b, 4c). Two-dimensional electrophoresis of gliadin demonstrated that, instead of one α-gliadin of medium staining intensity

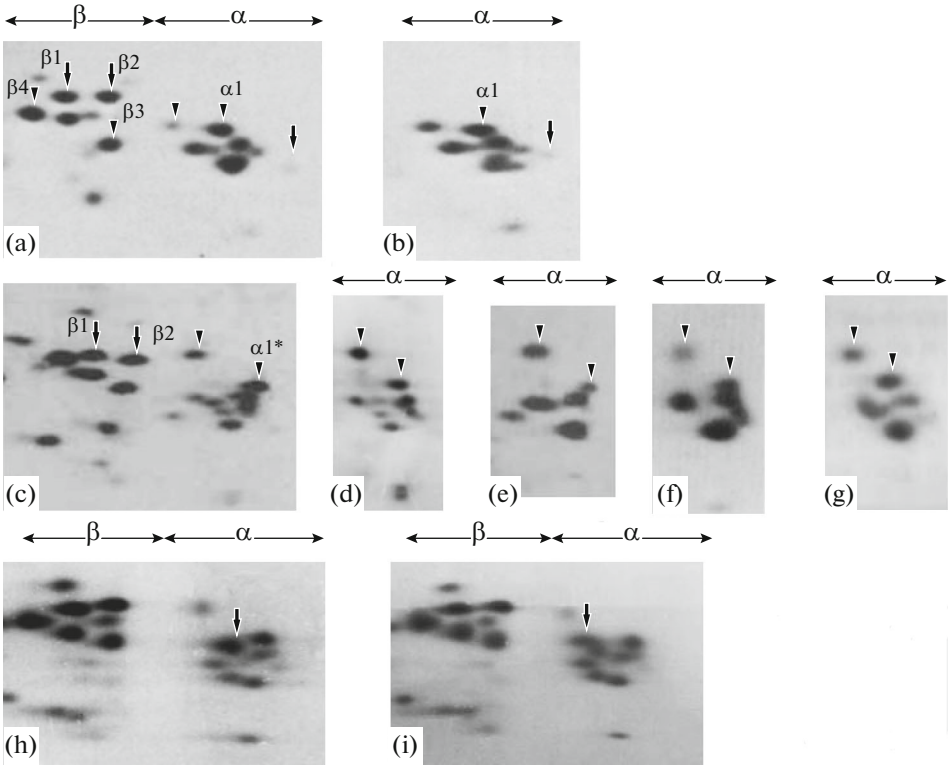


**Fig. 2.** Two-dimensional electrophoregrams of gliadin from common weat varieties (alleles present in the varieties are in parenthesis): (a) Rusalka (*Gli-A2p*, *Gli-B2p*, and *Gli-D2b*), (b) Bezostaya 1 (*Gli-A2b*), (c) Saratovskaya 29 (biotype *Gli-A2q*, *Gli-B2q*, *Gli-D2e*), (d) Kharkovskaya 6 (*Gli-A2q*), (e) Pandas (*Gli-A2g*), (f) Saratovskaya 29 (biotype *Gli-A2s*), (h) Perzivan 1 (*Gli-A2v*), (j, l) Skorospelka Uluchshennaya (biotype *Gli-A2d*), (j) control and (l) spontaneous mutant. Only (b, d, e, f, h) α-zone or (a, c, j, l) α- and β-zones. Long-tail arrows indicate gliadin polypeptides encoded by alleles at the *Gli-A2* locus and discussed in the text; short-tail arrows indicate *Gli-B2*; no-tail arrows indicate *Gli-D2*.

encoded by *Gli-A2f* allele (Fig. 3d), a pair of lightly stained components was detected in the block encoded by the *Gli-A2n* allele (Fig. 3f). It is noteworthy that the blocks encoded by alleles of different families, for example, *Gli-A2b* (Fig. 3e) and *Gli-A2n* (Fig. 3f), do not have the same components.

**Alleles at the *Gli-B2* locus.** Alleles of the *Gli-B2* locus encode blocks consisting, as a rule, of 5–8 weakly colored PAGE components located in the γ- and β-zones on electrophoregrams (Fig. 1). In the course of analysis of gliadin via two-dimensional electrophoresis, it was detected that many alleles at the *Gli-B2* locus encode synthesis of a specific pair of α-gliadins that have, according to SDS-PAGE data, the same MW of 36.5 kDa. This pair is present in blocks controlled, for example, by the following alleles: *Gli-B2b* (variety Bezostaya 1, Fig. 3e), *Gli-B2h* (variety Promin, Fig. 3d), *Gli-B2p* (variety Rusalka, Fig. 2a), *Gli-B2q* (variety Saratovskaya 29, Fig. 2e), etc. These gliadins are designated as β1 and β2.

Analysis of progeny segregation [16] demonstrates that gliadin β2* controlled by *Gli-B2c* from Siete Cerros has a higher EM (Figs. 1, 4d) in comparison to gliadin β2 controlled by *Gli-B2b* alleles [4]. Analysis of two-dimensional electrophoregrams showed that the polypeptide of β2* allele from *Gli-B2c* locus has a lower MW (36.0 kDa) as compared with gliadin β2 (Figs. 3c, 3e).

It was found that, instead of polypeptides β1 and β2, the *Gli-B2o* allele produces two other β-gliadins designated as β1* and β2** (Figs. 1, 4e). Comparison of two-dimensional electrophoregrams of gliadins from varieties Bezostaya 1 (allele *Gli-B2b*, Fig. 3e), Pandas (*Gli-B2o*, Fig. 3a), and Costantino (*Gli-B2o*, Fig. 3c) demonstrated that EM of gliadin β1* is lower than EM of β1, whereas its MW is approximately 41.0 kDa, and MW of gliadin β2** is approximately 30.0 kDa.

**Alleles at *Gli-D2* locus.** Blocks controlled by alleles of the *Gli-D2* locus contain mostly 3–5 polypeptides located in β- and α-zones on PAGE electrophoregrams and differ in EM (Fig. 1) [3, 4]. Blocks controlled by alleles at *Gli-D2b* in Bezostaya 1 and *Gli-D2e* in Mironovskaya Yubileynaya differ by EM of two gliadins. One of them (designated as β3 for allele *Gli-D2b* and β3* for allele *Gli-D2e*) is located at the boundary between β- and α-zones on electrophoregrams, and the other one is located in the α-zone (α1 and α1*, respectively). Gliadin β3 has higher EM than β3*, and

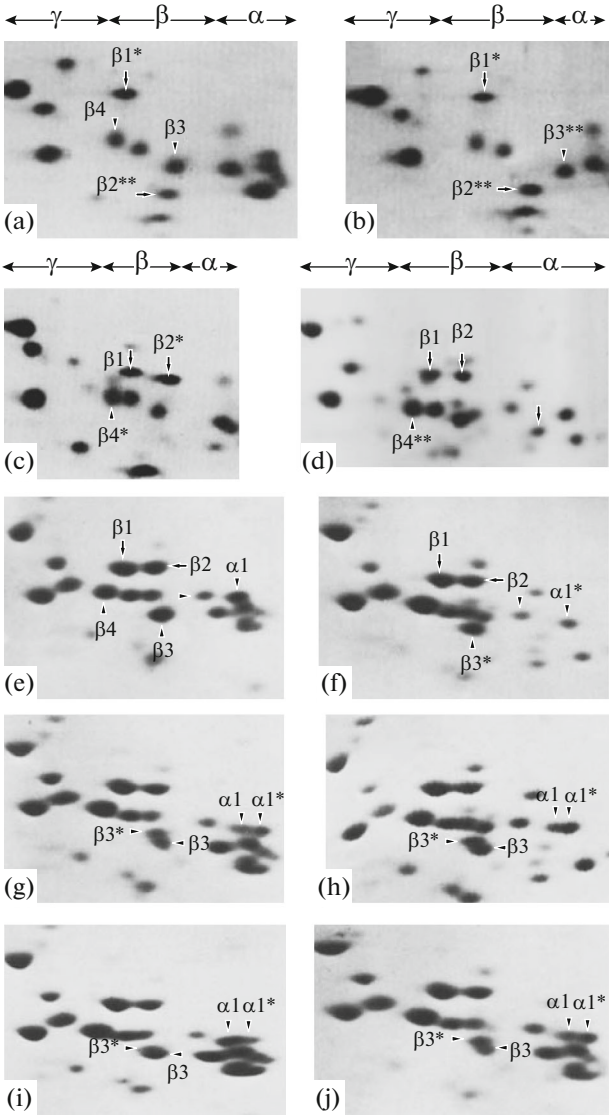


**Fig. 3.** Two-dimensional electrophoregrams of gliadin from common weat variety (alleles present in the varieties are in parenthesis): (a) Pandas (*Gli-A2g*, *Gli-B2o*, *Gli-D2b*), (b) Costantino (*Gli-A2g*, *Gli-B2o*, *Gli-D2r*), (c) Siete Cerros 66 (*Gli-B2c*, *Gli-D2c*), (d) Promin (*Gli-A2f*, *Gli-B2h*, *Gli-D2j*), (e) Bezostaya 1 (*Gli-A2b*, *Gli-B2b*, *Gli-D2b*), (f) Mironovskaya Yubileynaya (*Gli-A2n*, *Gli-B2b*, *Gli-D2e*), (h, j, l, m) gliadins from F2 seed obtained from crosses Bezostaya 1 and Mironovskaya Yubileynaya. α-, β-, and γ-zones of (a, d, l) electrophoregrams or (b, c) β- and γ-zones are represented. Long-tail arrows indicate gliadin polypeptides encoded by alleles at *Gli-A2* locus; short-tail arrows indicate *Gli-B2*; no-tail indicate *Gli-D2*. Long-tail arrow in (d) indicates α-gliadin different in *Gli-A2f* as compared to *Gli-A2n*.

α1 has lower EM in comparison with α1* (Figs. 1, 3e, 3f). In the course of two-dimensional electrophoretic analysis of gliadin in F2 seeds obtained from crosses between varieties Bezostaya 1 and Mironovskaya Yubileynaya, the following genotypes were detected: heterozygous for *Gli-D2* locus and homozygous for *Gli-A2b* locus (Figs. 3f, 3k), heterozygous for *Gli-D2* locus and homozygous for *Gli-A2n* (Fig. 3j), heterozygous for *Gli-D2* locus and heterozygous for *Gli-A2* (Fig. 3m). It was found that polypeptide β3, having a higher EM, has a lower MW (31.5 kDa) as compared to β3* (32.3 kDa), whereas polypeptides α1 and α1* have approximately the same MW (approximately 33.0 kDa). Heterozygous genotypes demonstrated dose effects of alleles in triploid endosperm. For example, genotypes heterozygous for *Gli-D2* locus and homozygous for allele *Gli-A2b* had either two doses of the *Gli-D2b* allele (Fig. 3l) or two doses of the *Gli-D2e* allele (Fig. 3h).

The *Gli-D2r* allele is unique, since it controls synthesis of gliadin (indicated by β3**) characterized by significantly higher EM as compared to β3 (Figs. 1, 4f). Comparison of two-dimensional electrophoretograms of varieties Pandas (*Gli-D2b*, Fig. 3a) and Costantino (*Gli-D2r*, Fig. 3b) showed that gliadin β3** MW is lower than β3 MW and is approximately 30.8 kDa.

One-dimensional PAGE demonstrates differences between *Gli-D2b* and *Gli-D2c* alleles in EM of β-gliadin in the β-zone on electrophoregram at the border with the γ-zone. Moreover, the polypeptide controlled by *Gli-D2b* allele (designated as β4) has a lower EM than β4* from *Gli-D2c* alleles (Figs. 1, 4d). Analysis of two-dimensional electrophoregrams for Pandas (*Gli-D2b*), Bezostaya 1 (*Gli-D2b*), and Siete Cerros (*Gli-D2c*) varieties (Figs. 3a, 3e, 3c, respectively) showed that gliadin β4 has higher MM (34.8 kDa) as compared with β4* (34.2 kDa).

*Gli-D2j* allele encodes β-gliadin (β4**), which has (in the same way as β4*) a higher EM as compared to gliadin β4 alleles at *Gli-D2b* (Figs. 1, 4b). It was figured out that the higher EM of gliadin β4** is accompanied by a lower (the same way as for β4*) MM as compared to β4 (Figs. 3d, 3e). In this case, the MM of gliadins β4* and β4** differs insignificantly (Figs. 3c, 3d), although β4* has higher EM than β4** (Figs. 1, 4c, 4d).

## DISCUSSION

Three groups of gliadins, synthesized in common weat seeds (α, γ and ω), differ among themselves by structure and amino acid composition [8]. Predominant protein, α-gliadin (up to 30% of total seed protein), is encoded by the *Gli-2* locus [10, 13]. In fact, α-gliadins are components of many food products [10, 17].

The group of α-gliadin-encoding genes is characterized by exceptionally high level of polymorphism [13]. The use of variants of two-dimensional electrophoresis permitted the identification of approximately 30 different polypeptides of α-gliadin per common weat variety [9]. However, Chinese Spring has up to 90 and more α-gliadin-encoding genes capable of expression [13]. For *Gli-2* loci (*Gli-A2*, *Gli-B2*, and *Gli-D2*), 28–35 alleles per locus are described [9]. Each allele encodes a group (block) of gliadin polypeptides

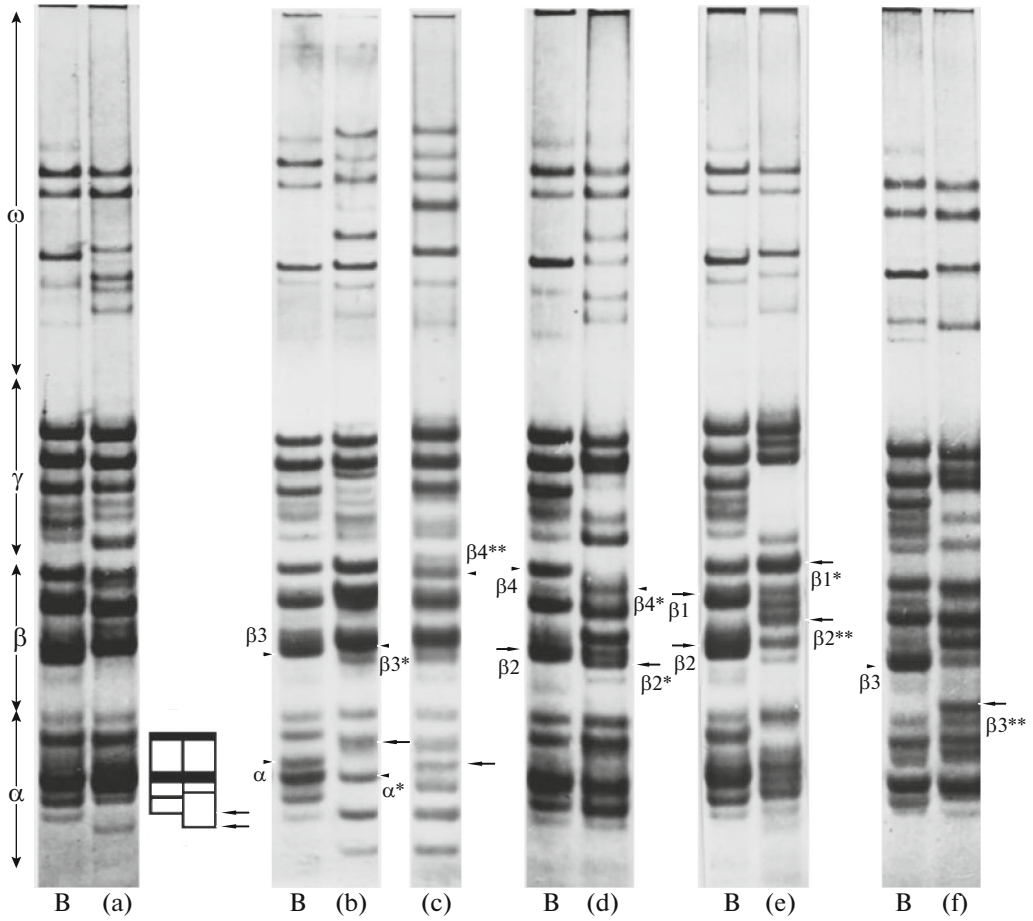


**Fig. 4.** PAGE electrophoregrams of gliadin from varieties and genotypes of common weat [4] containing alleles of *Gli-2* loci: (a) Pliska (*Gli-A2p*), (b) Mironovskaya 808 (*Gli-A2n*, *Gli-D2e*), (c) Promin (*Gli-A2f*, *Gli-D2j*), (d) Siete Cerros (*Gli-B2c*, *Gli-D2c*), (e) Lutescence 62 (*Gli-B2o*), (f) Poljarka (*Gli-D2r*), B—variety Bezostaya 1 (universal control). Long-tail arrows indicate gliadin polypeptides encoded by alleles at *Gli-A2* locus; short-tail arrows indicate *Gli-B2*; no-tail indicate *Gli-D2*. Schema demonstrates blocks encoded by *Gli-A2b* and *Gli-A2p* alleles.

(PAGE electrophoretic components) localized in α- and β-zones on PAGE electrophoregrams [2–4].

In a typical gliadin-type polypeptide, five domains are identified: the signaling sequence is followed by N-terminal domain (113–134 amino acids), it is composed of moderately conservative repeats of 5–7 amino acids in length, followed by two polyglutamine domains alternating with two unique domains. The usual length of polyglutamine domain is 7–28 amino acids [8, 11, 13]. In variety Cheyenne, an unusual α-gliadin gene containing polyglutamine domain of 107 amino acids in length was identified [10].

Genes encoding α-gliadins differ among themselves mostly in substitutions of nucleotides as well as small deletions/insertions [11–13]. Groups (families) of α-gliadin-encoding genes are described. They differ in the details of their structure [8, 11, 13].

Blocks encoded by the *Gli-A2* locus can be divided into two families that differ in PAGE block components [7]. Two-dimensional electrophoresis of gliadin isolated from F2 seeds from crosses of the varieties that have contrasting alleles at the *Gli-A2* locus has been performed. Inheritance of all polypeptides composing a "two-dimensional" block was confirmed [18].

Some blocks encoded by alleles at the first of two families (Bezostaya 1 type or Cheyenne type [7]) differ in one-dimensional PAGE only by EM of one or two α-gliadins [4]. This could indicate a close similarity between those blocks and corresponding alleles at the *Gli-A2* locus (Fig. 1). Two-dimensional blocks encoded by alleles of this family were much more complicated than "one-dimensional" blocks: each two-dimensional block had a unique combination of 5–10 α-gliadins that differed from each other by EM and MW (Fig. 2).

Two alleles—*Gli-A2f* and *Gli-A2n* from *Gli-A2* locus (Chinese Spring type [7])—differ only by EM of one α-gliadin [4]. Two-dimensional electrophoregrams of blocks encoded by these alleles are composed of several apparently identical polypeptides. Never-

theless, the difference between the blocks controlled by alleles *Gli-A2f* and *Gli-A2n* is more complex. It could not be explained by mutation-altering EM of one α-gliadin (Figs. 2d, 2e).

Two-dimensional electrophoregrams of blocks encoded by alleles of the second family of the *Gli-A2* locus have no gliadin polypeptide for which EM and MW would coincide with other gliadins from the first family. At the same time, blocks with an intermediate set of components were not detected [9]. Alleles of the *Gli-A2* locus from the first and second families also differ in the set of restriction fragments of genomic DNA (identified by the RFLP method) [19]. Obviously, common weat inherited these contrasting family of alleles in the *Gli-A2* locus from different genotypes of a polymorphic donor of genome A [9, 19].

Analysis of two-dimensional electrophoregrams of common weat varieties demonstrated that many alleles at the *Gli-B2* locus control synthesis of a pair of β-gliadins (designated β1 and β2) having MW of approximately 36.5 kDa. Evidently, this pair developed via gene duplication and subsequent divergence of the two copies. This causes development of two genes encoding β-gliadins in the *Gli-B2* locus. They have the same MW and different EM. It is well known that duplications in the *Gli-2* loci induced development of a large number of copies in a family of genes that have α-gliadin encoding sequences [20]. Duplicated genes can undergo significant divergence [20, 21].

The *Gli-B2o* allele is of special interest, since it has a positive effect on common weat adaptability [16]. This allele more often than others from the *Gli-B2* locus is inherited by progeny of crosses [9]. Analysis of segregating progeny [16] demonstrated that the *Gli-B2o* allele encoded β1* and β2* gliadins instead of β1 and β2 (Fig. 1). Two-dimensional electrophoresis was used to evaluate MW of β1* and β2* gliadins: approximately 41.0 and 30.0 kDa, respectively. Thus, MW of two β-gliadins is almost the same for those gliadins encoded by *Gli-B2b*, *Gli-B2p*, *Gli-B2h*, *Gli-B2q*, and β1 and β2 polypeptides encoded by other alleles (73 kDa), and product of *Gli-B2o* allele (71 kDa). It could be hypothesized that the *Gli-B2o* allele developed via unequal crossing over at the region of *Gli-B2o* locus, where duplicated genes encoding β1- and β2-gliadins were located. There are other possible events at the DNA level that could induce development of the *Gli-B2o* allele [21].

*Gli-D2c* and *Gli-D2j* alleles differ from the *Gli-D2b* allele by higher EM (Fig. 1) and lower MW of the gliadin polypeptide located at the end of the zone. It is interesting that, despite different EM, β4* gliadins (allele *Gli-D2c*) and β4** (*Gli-D2j*) had MW (approximately 34.0 kDa) difficult to distinguish with SDS-PAGE. Polypeptides α1 and α1*, encoded by alleles *Gli-D2b* and *Gli-D2e*, respectively, also had different EM and the same MW. The difference in EM did not coincide with MW variation and could be caused by difference in the number of charged amino acids in gliadin polypeptides.

As a rule, *Gli-2* locus polypeptides with a higher EM had a lower MW. For example, when comparing three variants of the most mobile β-gliadin encoded by the *Gli-D2* locus (β3*, β3, and β3** in the order of increasing EF, Fig. 1), it was found that a polypeptide with a higher EM has a lower MW. Curiously, the gliadins β3 and β3** are found only in blocks that are coded alleles of *Gli-D2b* and *Gli-D2r*, respectively, while β3* occurs in many alleles of this locus [4]. The difference in MW between polypeptides β3*, β3, and β3** is less than 1 kDa, so development of β3 and β3** was probably associated with a mutational loss of one repeat in the gliadin N-terminal domain or with a decrease in the length of a polyglutamine domain.

In most comparisons, the MW difference in two allelic variants of gliadin polypeptides was approximately 0.5 kDa, and faster gliadin had a lower MW. This difference was characteristic of most mobile α-gliadins. Their EM was different for blocks controlled by *Gli-A2p* and *Gli-A2b* alleles, one α-gliadin in spontaneous mutant, and another one in control variety Skorospelka Uluchshennaya. Gliadin β2* encoded by *Gli-B2c* allele had higher EM [4] and lower MW (36.0 kDa) as compared with gliadin β2 (36.5 kDa). Gliadins encoded by alleles *Gli-D2c* (β4*) and *Gli-D2j* (β4**) differed from gliadin β4 alleles of *Gli-D2b* by higher EM [4] and lower MW by 0.5 kDa. Approximately the same difference in MW was observed between gliadins β3 (allele *Gli-D2b*) and β3* (*Gli-D2e*).

It is well known that the length (MW) of α-type gliadin polypeptides depends on the number of repeats at the N-terminal domain and the length of the polyglutamine domains [8, 10–13, 17]. We detected a difference in MW (approximately 0.5 kDa) between allelic variants of gliadins. This difference in MW value was lower than MW for a repeat in the N-terminal domain of the polypeptide (up to 7 amino acids). Since glutamine MW is 0.128 kDa, the observed differences between allelic variants of α-type gliadins could be explained by shortening of the polyglutamine domain length by 3–4 amino acids or deletions/insertions of this size. Polypeptides of α-type gliadin, differing only by the length of the polyglutamine domain, were described previously [11, 13, 17].

On the contrary, the difference between γ-gliadin molecules encoded by alleles at *Gli-A1* and *Gli-D1* loci almost always exceeds 1 kDa. Therefore, it could be explained by varying number of repeats (6–8 amino acids [22]) in γ-gliadin repetitive domain [14]. Moreover, the analysis of cloned genes demonstrated that the length of γ-gliadin depends upon the number of repeats in the second polypeptide domain (repetitive domain) [22, 23]. Thus, while the differences in MW in α-type gliadin depends upon the length of polyglutamine domain, the difference in MW between alleles

of γ-gliadins could be explained by the number of repeats in the domain [9].

The number of alleles of the *Gli-2* locus is at least 1.5 times higher than the number of alleles in *Gli-1* loci [4, 9]. Our results permit us to speculate that the cause of higher polymorphism in *Gli-2* loci in comparison to *Gli-1* loci is associated with special features of those loci and the number of α- and γ-types encoded by them.

Firstly, the number of α-type polypeptides encoded by the *Gli-2* locus (Figs. 1–3) is higher than the number of γ-gliadins encoded by any allele of the *Gli-1* locus [9]. Changes in any gene in the *Gli-2* locus could be identified by PAGE and interpreted as identification of a new allele resulting from a mutation. Evidently, the higher the number of genes expressed in the locus, the higher the probability of development of a new allele inside the locus.

Secondly, DNA sequences encoding the polyglutamine domains of the gliadin peptide are variants of microsatellites [10, 13]. Microsatellites have high frequency of spontaneous mutation [24]. Indeed, PAGE of individual common weat seeds demonstrated that the frequency of spontaneous mutations inducing changes in EM of polypeptides is higher in *Gli-2* versus *Gli-1* [5].

Thus, the frequency of development of new alleles (and polymorphism extension) in *Gli-2* loci should be much higher than in *Gli-1* loci. A portion of changes in *Gli-2* loci is difficult to detect due to the overlap of electrophoretic components encoded by the above-mentioned loci [9]. Nevertheless, the number of *Gli-2* alleles in the catalog [4] is significantly higher than the number of alleles in *Gli-1*. We would like to stress that, for *Gli-1* loci, starch electrophoresis in acid buffer provides approximately the same polymorphism detection capabilities as PAGE, but the capabilities of differentiation of alleles at *Gli-2* loci are different [2, 3]. Apparently, small differences in α-gliadin MW associated with polyglutamine domains are unresolvable in starch gels.

## REFERENCES


1. Metakovsky, E.V. and Graybosch, R.A., Gliadin alleles in wheat: identification and application, in *Gliadin and Glutenin. The Unique Balance of Wheat Quality*, Wrigley, C., Bekes, F., and Bushuk, W., Eds., AACC Internat., 2006, pp. 85–114.
2. Sozinov, A.A. and Poperelya, F.A., Genetic classification of prolamins and its use for plant breeding, *Ann. Technol. Agric.*, 1980, vol. 29, pp. 229–245.
3. Sobko, T.A. and Poperelya, F.A., Frequencies of occurrence of alleles of gliadin-coding loci in winter common wheat, *Visn. S.-G. Nauki*, 1986, no. 5, pp. 84–87.
4. Metakovsky, E.V., Gliadin allele identification in common wheat. 2. Catalogue of gliadin alleles in common wheat, *J. Genet. Breed.*, 1991, vol. 45, no. 4, pp. 325–344.
5. Metakovsky, E.V., Davydov, S.D., Chernakov, V.M., and Upelniek, V.P., Gliadin allele identification in common wheat. 3. Frequency of occurrence and appearance of spontaneous mutations at the gliadin-coding loci, *J. Genet. Breed.*, 1993, vol. 47, no. 3, pp. 221–236.
6. Upelniek, V.P., Novoselskaya, A.Yu., Sutka, J., Galiba, G., and Metakovsky, E.V., Genetic variation at storage protein coding loci of common wheat (cv Chinese Spring) induced by nitrosoethylurea and by cultivation of immature embryos in vitro, *Theor. Appl. Genet.*, 1995, vol. 90, nos. 3–4, pp. 372–379.
7. Kasarda, D.D., Okita, T.W., Bernardin, J.E., Baecker, P.A., Nimmo, C.C., Lew, J.L., Dietler, M.D., and Green, F.C., Nucleic acid (cDNA) and amino acid sequence of α-type gliadins from wheat (*Triticum aestivum*), *Proc. Nat. Acad. Sci. U. S. A.*, 1984, vol. 81, no. 15, pp. 4712–4716.
8. Barak, S., Mudgil, D., and Khatkar, B.S., Biochemical and functional properties of wheat gliadins: a review, *Crit. Rev. Food Sci. Nutr.*, 2015, vol. 55, no. 3, pp. 357–368.
9. Metakovsky, E.V., *Wheat Grain Storage Proteins: Classical Genetics, Mutations, Phylogeny, Seed Breeding, and Grain Quality*, Lambert Acad. Publ., 2015, 320 p.
10. Anderson, O.D. and Greene, F.C., The α-gliadin gene family. 2. DNA and protein sequence variation, subfamily structure, and origins of pseudogenes, *Theor. Appl. Genet.*, 1997, vol. 95, no. 1, pp. 59–65.
11. Li, J., Wang, S.-L., Cao, D.-W., Subburaj, L.S., Li, X.H., Zeller, F.J., Hsam, S.L.K., and Yan, Y.-M., Cloning, expression, and evolutionary analysis of α-gliadin genes from *Triticum* and *Aegilops* genomes, *J. Appl. Genet.*, 2013, vol. 54, no. 2, pp. 157–167.
12. Li, Y., Xin, R., Zhang, D., and Li, S., Molecular characterization of α-gliadin genes from common wheat cultivar Zhengmai 004 and their role in quality and celiac disease, *Crop J.*, 2014, vol. 2, no. 1, pp. 10–21.
13. Noma, S., Kawaura, K., Hayakawa, K., Abe, C., Tsuge, N., and Ogihara, J., Comprehensive molecular characterization of the α/β-gliadin multigene family in hexaploid wheat, *Mol. Genet. Genom.*, 2016, vol. 291, no. 1, pp. 65–77.
14. Metakovsky, E.V., Melnik, V.A., Vaccino, P., and Rodriguez-Quijano, M., Comparison of alleles at *Gli-1* loci of common wheat by means of two-dimensional electrophoresis of gliadin and RFLP analysis, *Cytol. Genet.*, 2018, vol. 52, no. 1, pp. 16–27.
15. Sapirstein, H.D. and Bushuk, W., Computer-aided wheat cultivar identification and analysis of densitometric scanning profiles of gliadin electrophoregrams, *Seed Sci. Technol.*, 1986, vol. 14, no. 3, pp. 489–517.
16. Koval, S.F. and Metakovsky, E.V., Adaptive value of some quantitative and qualitative characters in the experimental hybrid population of *T. aestivum, Selskokhoz. Biol.*, 1985, no. 11, pp. 48–52.
17. Huang, Z., Long, H., Wei, Y.-M., Yan, Z.H., and Zheng, Y.L., Allelic variations of α-gliadin genes from species of *Aegilops* section *Sitopsis* and insights into evolution of α-gliadin multigene family among *Triticum* and *Aegilops, Genetics*, 2016, vol. 144, no. 2, pp. 213–222.

18. Metakovsky, E.V. and Sozinov, A.A., Genetics of gliadin proteins and the problems of interpreting results obtained with somaclonal variation in wheat, in *Biotechnology in Agriculture and Forestry (Wheat)*, Bajaj, Y.P.S., Ed., 1990, vol. 13, pp. 526–537.

19. Vaccino, P. and Metakovsky, E.V., Gliadin alleles in DNA RFLP patterns of common wheat: implication for analysis of organization and evolution of complex loci, *Theor. Appl. Genet.*, 1995, vol. 90, no. 2, pp. 173–181.

20. Gu, Y.Q., Crossman, C., Kong, X., Luo, M., You, F.M., Coleman-Derr, D., and Dubcovsky, J., Genomic organization of the complex α-gliadin gene loci in wheat, *Theor. Appl. Genet.*, 2004, vol. 109, no. 3, pp. 648–657.

21. Zmienko, A., Samelak, A., Kozlowski, P., and Figlerowicz, M., Copy number polymorphism in plant genomes, *Theor. Appl. Genet.*, 2015, vol. 127, no. 1, pp. 1–18.

22. Qi, P.F., Wei, Y.M., Ouellet, T., Chen, Q., Tan, X., and Zheng, Y.L., The γ-gliadin multigene family in common wheat (*Triticum aestivum*) and its closely related species, *BMC Genom.*, 2009, vol. 10, no. 1, pp. 168–181.

23. Wang, S., Shen, X., Ge, P., Saminathan, J.L., Li, S.X., Zeller, F.J., Hsam, K.L., and Yan, Y., Molecular characterization and dynamic expression patterns of two types of γ-gliadin genes from *Aegilops* and *Triticum* species, *Theor. Appl. Genet.*, 2012, vol. 125, no. 7, pp. 1371–1384.

24. Raquin, A.-L., Depaulis, F., Lambert, A., Galic, N., Brabant, P., and Goldringer, I., Experimental estimation of mutation rates in a wheat population with a gene genealogy approach, *Genetics*, 2008, vol. 179, no. 4, pp. 2195–2211.

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