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Genetic analysis of gliadin components in winter wheat using two-dimensional polyacrylamide gel electrophoresis

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Summary. Blocks of gliadin components found both in a number of varieties and in single F_2 grains of winter wheat intervarietal hybrids have been studied by two-dimensional electrophoresis combining electrophoresis in acidic aluminium-lactate buffer (pH 3.1) and SDS-electrophoresis. Gliadin components (spots) have been shown to be inherited as linked groups (blocks), co-dominantly and in accordance with a gene dosage in triploid endosperm. Blocks include components differing in their electrophoretic mobility and molecular weight. Some allelic variants of blocks differ only in presence of few additional components or in the electrophoretic mobility of components with similar molecular weights; other variants may contain no similar components. Apparently, in the course of evolution, mutations in individual genes of gliadin-coding loci and processes changing the number of expressing genes and the sizes of their structural part occurred.

Key words: *Triticum aestivum* – Gliadins – Two-dimensional electrophoresis – Hybridological analysis

Introduction

To study the component composition of a highly polymorphic storage protein, gliadin, polyacrylamide (Bushuk and Zillman 1978; Mecham et al. 1978) or starch (Ellis and Beminster 1977; Sozinov and Poperelya 1980) gel electrophoresis in acidic aluminium-lactate buffer and SDS-electrophoresis (Shewry et al. 1978) has been used. Obviously, the genetic analysis of components with similar electrophoretic mobility in one-dimensional gels presents considerable difficulties. To separate overlapped components several modifications of two-dimensional electrophoresis have been proposed (Brown and Flavell 1981; Mecham et al. 1978; Novoselskaya et al. 1983; Payne et al. 1982; Wrigley and Shepherd 1973). In our opinion, the combination of electrophoresis in aluminium-lactate buffer with SDS-electrophoresis in polyacrylamide gel (AL/SDS EP) has the largest number of advantages. Polyacrylamide gel electrophoresis in aluminium-lactate buffer was recommended as a standard procedure for studying the component composition of gliadin (Autran et al. 1979). The inheritance of gliadin components in a number of winter wheat varieties has already been studied by this method and the catalogue of linked inherited components (blocks) has been revealed (Metakovsky et al. 1984). The use of SDS-electrophoresis in the second dimension not only increases significantly the resolution but also makes it possible to determine the apparent molecular weight of individual gliadin components.

Earlier we used AL/SDS EP to study the inheritance of ω-gliadins (Novoselskaya et al. 1983). The task of the present work is the analysis of inheritance of gliadin components in complex α- and β-regions by means of two-dimensional electrophoresis. Moreover, the use of the AL/SDS EP procedure permitted two-dimensional forms of some common wheat blocks to be revealed and the molecular weight of their components to be compared.

Materials and methods

The following varieties of winter wheat *Triticum aestivum* were used: 'Bezostaya 1', 'Mikronovskaya Yubileinaya', 'Rusalka', 'Dneprovskaya 521', 'Zaporozhskaya ostistaya', 'Koncho', 'Kavkaz', 'Promin', 'Odesskaya 16' as well as F_2 grains from hybrids 'Bezostaya 1' × 'Mironovskaya Yubileinaya'.

Extraction of gliadins, preparation of samples and polyacrylamide gel electrophoresis in aluminium-lactate buffer were carried out by the standard technique according to Bushuk and Zillman (1978) with modifications described previously (Metakovsky et al. 1984; Novoselskaya et al. 1983).

In the case of two-dimensional electrophoresis, a gel strip representing a sample or a group of fractions of a sample under study was cut out of the one-dimensional slab and incubated first at room temperature for 1.5 h in distilled water to remove the aluminium-lactate buffer, then for 1.5 h in a buffer containing SDS and mercaptoethanol (O'Farrell 1975). It was then applied onto a prepared SDS-polyacrylamide gel slab according to O'Farrell (1975). Electrophoresis in the second dimension was carried ot for 15–17 h at 100 v. Bovine serum albumin (67,000) and ovalbumin (45,000) were used as the molecular weight standards. Gels were stained with 0.1% Coomassie R-250 in 10% TCA acid and rinsed in 10% TCA acid for 24 h. The regions of the gliadin component spectrum were designated α, β, γ and ω in accordance with the nomenclature originally used for one-dimensional starch gel (Woichik et al. 1961).

Results

Using the AL/SDS EP method, it is possible to separate gliadins in 1.5–2.0 times more individual components as compared to one-dimensional electrophoresis at pH 3.1. For instance, one-dimensional and two-dimensional electrophoreses reveal 24 and 41 components, respectively, in 'Bezostaya 1' (Fig. 1 a), 26 and 45 components in 'Kavkaz' (Fig. 1 b), 26 and 45 components in 'Rusalka' (Fig. 1 c). As it is seen from Fig. 1, the greatest increase of the resolution is achieved in the α and β gel regions.

In 123 F_2 single grains from the 'Bezostaya 1' × 'Micronovskaya Yubileinaya' hybrid 9 main (disregarding the doses of parental genes in the triploid endosperm) variants of component (spot) distribution in γ-α gel region have been detected (Fig. 2). The counting of grains with different sets of components has yielded the following results (Table 1). As it is seen from the table, the obtained distribution of grains among the classes agrees with the theoretically expected one for two independent pairs of characters with codominant expression. Each of the varieties studied has two groups of jointly inherited gliadin components (Fig. 2 b, d). In 'Bezostaya 1', one group includes two components, the other – nine (Fig. 2 a, b). In 'Mikronovskaya Yubileinaya' one group consists of two spots, the other – of 10 (Fig. 2 c, d). Grains containing all gliadin components of both parental varieties apparently represent double heterozygotes (Fig. 2 e, f). The gliadin spectra presented in Fig. 2 g, h, i, j, k are found in samples heterozygous for one and homozygous for the other gliadin-coding locus. All possible variants of double homozygotes are shown in Fig. 2 a, c, l, m.

According to the analysis of the same cross by one-dimensional electrophoresis the components in the α and β gel regions differing in these two parental varieties make up two pairs of blocks (Metakovsky et al. 1984). 'Bezostaya 1' has blocks 6A1 and 6D1, 'Mironovskaya Yubileinaya' – 6A4 and 6D2: each of the blocks are composed of five components. Two-dimensional separation has shown that blocks 6A1 and 6A4 are minimally composed of 9 and 10 components, respectively, which differ in mobility and molecular weight (Fig. 2 b, d). "Two-dimensional" blocks 6D1 and 6D2, as well as one-dimensional ones, have five components, and both methods of separation indicate the identity of three of five components in these two blocks. These three components have been ascribed to blocks 6D1 and 6D2 on the basis of an analysis of other hybrid combinations (Metakovsky et al. 1984).

Dosage effects of allelic variants of blocks are easily revealed by two-dimensional electrophoresis. For instance, the two spectra with blocks 6D1, 6D2 and 6A1 differ in staining intensities of the differing components of allelic variants of blocks 6D1 and 6D2 (Fig. 2 g, h). The 6D1 components in Fig. 2 g and the 6D2 components in Fig. 2 h are more intensive and therefore contain more protein. Similar differences in the relative expression of allelic variants of blocks were also observed for the pair of blocks 6A1 and 6A4 (compare Fig. 2 e and f). No other ratios in the staining intensity of components of allelic blocks (for instance, equal expression of both variants) have been found. The staining intensity of components obviously depends on

Table 1. Distribution of single F_2 grains from the cross 'Bezostaya 1' × 'Micronovskaya Yubileinaya' for phenotypical classes based on the analysis of the α-, β- and γ-gliadins by AL/SDS EP

Pattern of gliadin components in Fig. 2		a	c	e, f	g, h	i	j	k	l	m
Phenotypical classes (blocks of gliadins controlled by chromosome 1A and 1D)	1A	1	4	1/4	1	4	1/4	1/4	1	4
	1D	1	2	1/2	1/2	1/2	1	2	2	1
Grain number observed		11	7	33	17	11	9	17	8	10
Expected grain number		7.7	7.7	30.8	15.4	15.4	15.4	15.4	7.7	7.7

123 F_2 grains have been studied in total, $\chi^2 = 6.59$ at d.f. $= 6$, $0.50 > P > 0.25$

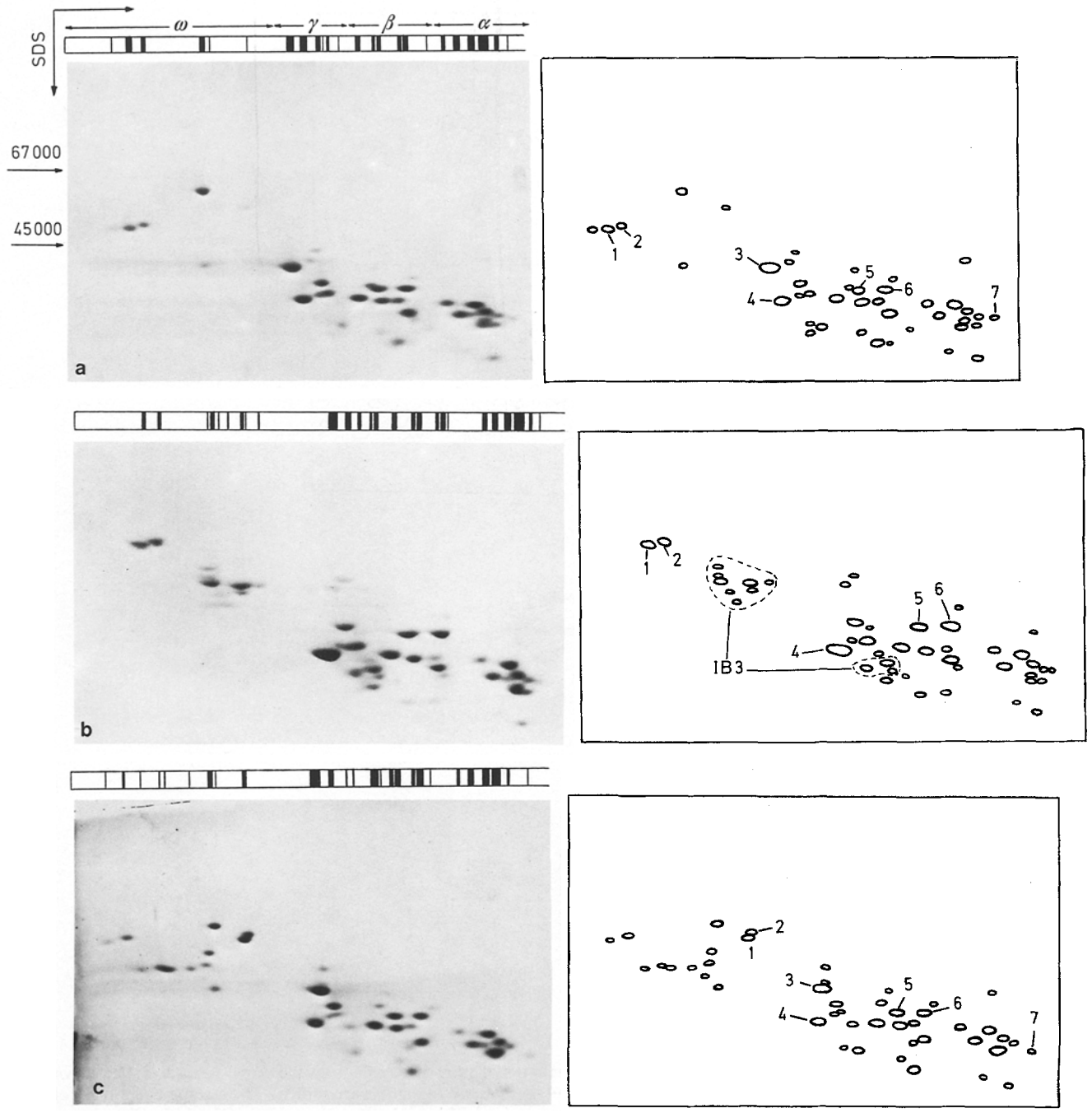

Fig. 1. Two-dimensional AL/SDS EP separation of gliadins of different wheat varieties: **a** 'Bezostaya' 1; **b** 'Kavkaz'; **c** 'Rusalka'. See text for data over the components marked by numbers

the number of corresponding homologous chromosomes in the triploid endosperm. Therefore, the genetic analysis makes it possible to reveal in the two-dimensional gliadin composition groups of jointly inherited components (spots) analogous to blocks of components (bands) detected in one-dimensional electrophoresis.

The analysis of some hybrid combinations and comparison with the results of one-dimensional separation permit a two-dimensional "form" of some common variants of blocks to be obtained (Table 2). It can be seen that some allelic blocks contain similar components while others do not. Some pairs of allelic blocks may differ only by the presence or absence of additional components (blocks 1B1, and 1B7, block 6A1 in 'Bezostaya 1' and 'Koncho', not shown), others – by the mobility and molecular weight of some components

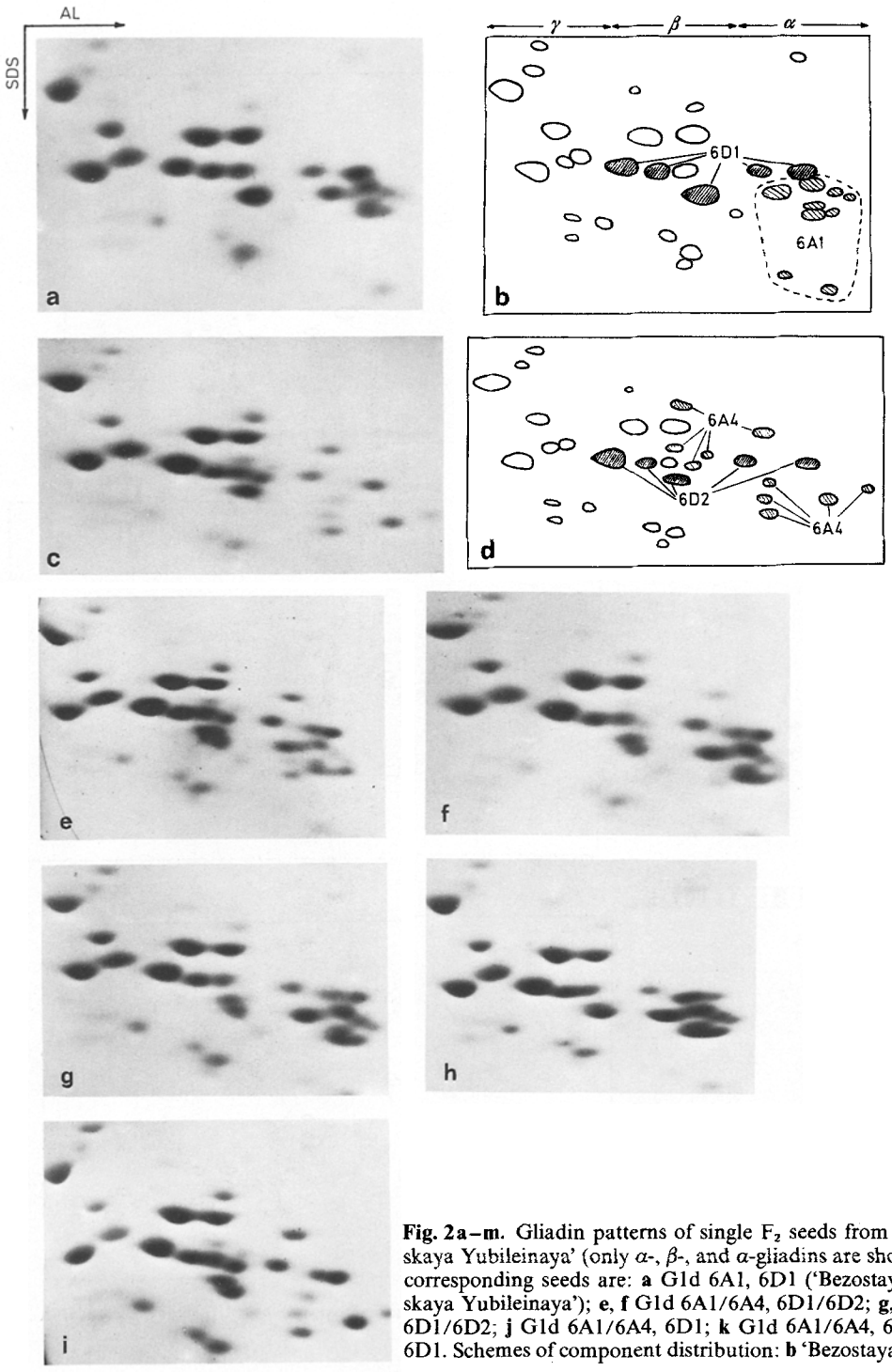

Fig. 2a–m. Gliadin patterns of single F_2 seeds from the cross 'Bezostaya 1' × 'Mironovskaya Yubileinaya' (only α-, β-, and α-gliadins are shown). The assumed genotypes of the corresponding seeds are: **a** G1d 6A1, 6D1 ('Bezostaya 1'); **c** G1d 6A4, 6D2 ('Mironovskaya Yubileinaya'); **e, f** G1d 6A1/6A4, 6D1/6D2; **g, h** G1d 6A1, 6D1/6D2; **i** G1d 6A4, 6D1/6D2; **j** G1d 6A1/6A4, 6D1; **k** G1d 6A1/6A4, 6D2; **l** G1d 6A1, 6D2; **m** G1d 6A4, 6D1. Schemes of component distribution: **b** 'Bezostaya 1'; **d** 'Mironovskaya Yubileinaya'

(blocks 6D1, 6D2, Fig. 2a, b). For one more example let us compare mobility of components N7 (Fig. 1a, c) in blocks 6A1 and 6A8. All chromosome 1D-controlled blocks include a similar component with the molecular weight of 34,000 (component N4, Fig. 1a–c). Many blocks controlled by chromosome 1B have a component with an apparent molecular weight of 41,000, the electrophoretic mobility of this protein in aluminium-lactate buffer, however, being essentially different in different blocks (component N3, Fig. 1a, c). One can see that a block may include components greatly differing (up to two times) in their molecular weights.

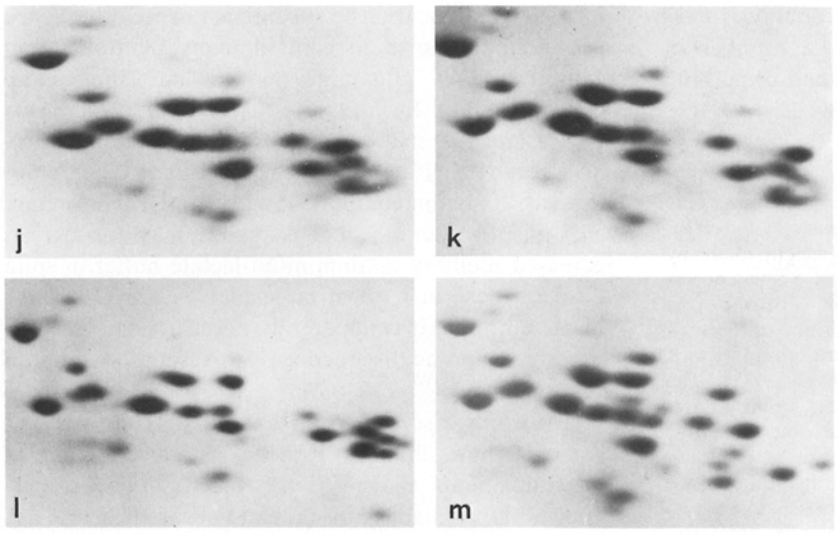

Fig. 2

Table 2. Apparent molecular weight and relative intensity of the components of some blocks in winter wheat

Block	Molecular weight[a] and intensity[b] of components
1A1	46++, 47++, 32+++++
1A4	38++++
1B1	60+++, 56+, 41+++++, 30++
1B2	57+++, 53+++, 54+++, 41+++++, 30+
1B3	49++, 47+, 45+++, 43+, 42+, 45+++, 44++, 45+, 33++, 33+++, 33++
1D1	50+, 50+++, 51++, 34+++++, 30++
1D4	52+, 53++, 44++, 45++, 44++, 34+++++, 30++
1D5	52++, 53++, 44++, 45++, 46+, 34+++++, 30++
6A1 (in Bezostaya 1)	33+++, 28+, 34+++, 32++, 31++, 27++, 33++, 31++, 33+
6A1 (in Koncho)	33+++, 28+, 32++, 31++, 27+, 33++, 31++, 31+
6A4	35+, 39++, 34+++, 35+, 36+++, 32+, 31++, 33+, 32+++, 32++
6A8	33+++, 28+, 32++++, 34+++, 27++, 33++, 31+
6B1	45+, 36+++, 36+++, 37++++, 37+++++
6B2	42+, 40+++, 30++++
6B4	41++, 34++, 37+++++, 37++++
6D1	35++++, 34++++, 32++++, 34+++, 34++++
6D2	35++++, 34+++, 33++++, 34+++, 34++++
6D6	35++++, 34+++, 32++++, 34+++, 34++++

[a] in kilodaltons
[b] +weak minor; ++minor; +++medium; ++++intensive; +++++very intensive. The components in one block are ranged in the order of increasing electrophoretical mobility

Of particular interest is block 1B3, controlled by a fragment of rye genetic material translocated onto chromosome 1B of hexaploid wheat. This block consists of two groups of proteins which are located in the γ and ω gel regions, similar to the wheat gliadins controlled by chromosome 1B. Each group consists of several components of similar molecular weight and electrophoretic mobility. Eight and three components included into the 1B3 block are found in ω and γ regions, respectively (Fig. 1 b, Table 2).

Discussion

This is the first report in which the inheritance of particular components has been examined using a two-dimensional separation of the gliadin of single F_2 seeds, and the results fully confirm previous findings. "One-dimensional" forms of blocks described earlier in starch (Sozinov and Poperelya 1980) and polyacrylamide gels (Metakovsky et al. 1984) and "two-dimensional" ones differ only in the number of their components. Since

the procedure of two-dimensional electrophoresis does not permit a rapid analysis of a large number of samples, the best results are achieved when using both methods and comparing results obtained.

We did not aim at defining the precise molecular weight of gliadin components as it is considered that the molecular weight of prolamines determined by SDS-electrophoresis may be overestimated (Hamauzu et al. 1974; Shewry et al. 1982). However, our estimations of the molecular weight made by AL/SDS EP for α-, β-, and γ-gliadins, are in a good accordance with values of the molecular weight of the corresponding components obtained by equilibrium ultracentrifugation or on the basis of amino acid composition (Ewart 1977; Patey and Waldron 1976; Sexson et al. 1978). The apparent molecular weight of the ω-gliadins found in our experiments was slightly lower than the one determined by Charbonnier (1974) with the help of gel-filtration or by Booth and Ewart (1969) by means of ultracentrifugation. So we assumed that, at least, the equal mobility of some two components in the second dimension indicates the similarity of their molecular weight.

The comparison of two-dimensional forms of allelic variants of blocks yields additional information on the possible evolutionary pathways of gliadin-coding loci. It is assumed that single genes of a gliadin-coding locus have arisen by duplication followed by divergence of sequences as a result of the mutation process (Kasarda 1980). Some data obtained by us are in good agreement with this concept. For instance, one can see that all components controlled by chromosome 6D have similar molecular weights and differ mostly in their electrophoretic mobility in aluminium-lactate buffer (Fig. 2, Table 2). Analogous similarities exist between two intensive components included into the 6B1 and 6B4 blocks (components NN 5 and 6, Fig. 1 a–c). A number of blocks controlled by chromosome 1B include an intensive component with an apparent molecular weight of 41,000 and varying electrophoretic mobility. The differences in the electrophoretic mobility, with no marked changes in the molecular weight of polypeptides, may be due to point mutations in the initially identical genes. There is some evidence that single changes in the amino acid composition may influence markedly the electrophoretic mobility of gliadin components (Ewart 1977).

It is of interest that a block often has pairs of components with similar molecular weights and staining intensity, the component of greater molecular weight having a bit higher electrophoretic mobility. Such pairs are, for example, components 1 and 2 (Fig. 1 a–c), and others. Pairs of components can be assumed to be formed of two initially identical (duplicated) genes due to a single mutational event. One may speculate that increased mobility of the heavier component is associated with an insertion into one of two copies of some genetic material coding for a polypeptide fragment with a comparatively higher content of basic amino acids. The specific amino acid sequence of gliadin is known to contain many neutral amino acids, especially glutamine and proline (Bietz et al. 1977; Booth and Ewart 1969; Charbonnier 1974; Ewart 1977; Hamauzu et al. 1974; Patey and Waldron 1976). As a result, gliadins are less mobile than other grain proteins. A gliadin component with an inserted foreign, "non-gliadin", amino acid sequence may possess an increased mobility in aluminium-lactate buffer in spite of an increase in its own molecular weight. To check this assumption, it is apparently necessary to study the primary amino acid sequence of corresponding gliadin components.

The comparison of α-gliadins in 'Bezostaya 1' and 'Koncho' shows that the 'Koncho' variety lacks one of the components controlled by chromosome 6A. Differences in α-gliadins of these two varieties are not practically revealed if only one-dimensional separation is used. Complete loss (or appearance) of individual components, the rest of a block remaining identical, may indicate that some genes of gliadin-coding loci may be "switched off" (or "switched on") or may be eliminated from the chromosome by deletions or rearrangements of the genetic material.

Thus, the comparison of blocks of gliadin components detected by the AL/SDS EP method indicates that at least in some gliadin-coding loci individual genes could really have arisen by duplications from some precursor gene and then diverged to a different degree in course of the natural mutation process. This has already been proposed (Kasarda 1980). Furthermore, an essential role in the course of the evolution of gliadin-coding loci must have been played by some events which resulted in changes of the size of protein-coding part of genes and in the number of expressing genes.

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