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Blocks of gliadin components in winter wheat detected by one-dimensional polyacrylamide gel electrophoresis

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Summary. Inheritance of gliadin components in winter wheat has been studied by one-dimensional polyacrylamide gel electrophoresis. Single F_2 grains from 36 intervarietal hybrid combinations have been analysed. The genetic analysis has revealed blocks, including 1–6 gliadin components, which are inherited as individual mendelian traits. About 80 variants of blocks have been detected. On the basis of the allelism test they are grouped into 6 series in accordance with the number of known gliadin-coding loci located on chromosomes of the homoeologous groups 1 and 6. Each series includes 8–18 blocks controlled by different alleles of one gliadin-coding locus. Blocks of components have been confirmed to be inherited codominantly in accordance to the gene dose in the triploid endosperm. The highest similarity between members of one series is observed in groups of blocks controlled by chromosomes 1D and 6D. On the contrary, many blocks controlled by chromosomes 1A and 1B have no bands in common. The presented catalogue of blocks of components may be used to make up gliadin genetic formulae and to compare electrophoregrams obtained by different authors. Blocks of gliadin components are suitable genetic markers for use in revealing and studying heterogeneity of wheat varieties, in tracing their origin, in identifying recombinations, translocations and substitutions of the genetic material and in solving many other problems of the origin, evolution and selection of hexaploid wheat.

Key words: *Triticum aestivum* – Gliadins – Electrophoresis – Hybridological analysis – Genetic nomenclature

Introduction

The synthesis of gliadin, a polymorphic reserve protein, is known to be controlled in hexaploid wheat almost exclusively by 6 chromosomes of two homoeologous groups, 1 and 6 (Wrigley and Shepherd 1973; Rybalka 1975; Zehatschek et al. 1981; Brown and Flavell 1981; Garcia-Olmedo et al. 1982). The number of gliadin components revealed by one- and two-dimensional electrophoresis in different varieties is 15–30 (Shewry et al. 1978; Lookhart et al. 1982; Wrigley et al. 1982) and 20–50 (Wrigley and Shepherd 1973; Mecham et al. 1978; Brown and Flavell 1981; Novoselskaya et al. 1983), respectively. Each chromosome must, obviously, carry genes determining the synthesis of several gliadin components. Indeed, various authors have noted that gliadin components (bands) are inherited as linked groups or blocks (Doekes 1973; Sozinov et al. 1975; Baker and Bushuk 1978; Mecham et al. 1978; Damidaux et al. 1980; Branlard 1982b; Novoselskaya et al. 1983). Analysis of gliadin banding patterns in starch gel has resulted in the discovery of multiple allelism of gliadin-coding loci of the group 1 chromosomes. Two alleles for each gliadin-coding locus of the group 6 chromosomes have also been detected (Sozinov and Poperelya 1980). Recombinations in components of allelic variants of blocks are either not observed at all or occur too seldom to be observed (Sozinov et al. 1975; Baker and Bushuk 1978; Mecham et al. 1978; Damidaux et al. 1980; Sozinov and Poperelya 1980). Various gliadin components have a similar amino acid composition and can be divided into several groups with similar but not identical amino acid sequences (Bietz et al. 1970; Bietz et al. 1977; Autran et al. 1979b). A gliadin-coding locus is assumed to consist of several tightly linked genes which arise from duplication and subsequent divergence (Kasarda 1980). Blocks of gliadin components may serve as suitable genetic markers in evolution and population studies as well as in breeding (Sozinov and Poperelya 1980).

To describe the component composition of gliadins, the International Association for Cereal Chemistry has adopted the genetic nomenclature suggested by Sozinov and Poperelya (1980). The essence of the genetic nomenclature is that the electrophoretic gliadin spectrum of a given variety or specimen may be presented as a set of blocks of components determined by gliadin-coding loci. An optimal standard

technique of polyacrylamide gel electrophoresis of gliadins in aluminium-lactate buffer has recently been proposed (Bushuk and Zillman 1978; Autran et al. 1979a). However, the application of the genetic nomenclature worked out with the use of starch gel is impossible for gliadin spectra obtained in polyacrylamide gel because of differences in the distribution of components in the two types of gel, especially in the medium mobility region (Novoselskaya et al. 1983).

The task of our work was to analyse the inheritance of gliadin components and to reveal blocks of components using the standard procedure of polyacrylamide gel electrophoresis (Bushuk and Zillman 1978) with minimal modifications.

Materials and methods

Single F_2 grains of the following hybrid combinations and corresponding parental varieties of winter wheat *Triticum aestivum* have been studied: 32 combinations in which one of the parents is 'Bezostaya 1' and the other – 'Aurora', 'Kavkaz', 'Donskaya bezostaya', 'Dukat', 'Voskhod', 'Dneprovskaya 521', 'Kishinevskaya 102', 'Krasnodonka', 'Lesostepka', 'Mironovskaya 808', 'Mironovskaya Yubileinaya', 'Odesskaya semidwarf', 'Odesskaya 26', 'Odesskaya 16', 'Peresvet', 'Promin', 'Tarasovskaya', 'Ukrainka', Towstik's lines 4/147 and 6/24, 'Ackermanns Cara', 'Cluj 650', 'Dankowska Iasna', 'Gernot', 'Kaprock', 'Kiszombori 1', 'Kremena', 'Levent', 'Newcaster', 'Rusalka', 'Szegedi 6', 'Zg 2639/73'; combinations 'B16'× 'Odesskaya semidwarf', 'B16'×'Zirka', 'Severokubanka'× 'Promin', 'Concho'×'Kaprock'.

To perform gliadin electrophoresis, the standard procedure (Bushuk and Zillman 1978) with modifications similar to ones previously developed (Tkachuk and Metlish 1980) was used. Flour from single grains (30–40 mg) was incubated with 0.1 ml of 70% ethanol (v/v) for 20–40 min at 40 °C. After centrifugation (10 min, 3,000 rpm) 0.1 ml of aluminium-lactate buffer containing 80% of sucrose and pyronin dye was added to the supernatant (Novoselskaya et al. 1983). The electrophoresis was carried out in vertical slabs (1.8 mm × 15 cm × 15 cm) of 8% polyacrylamide gel at 550 v for 3 h at a temperature not exceeding 25 °C (Novoselskaya et al. 1983). The gel slabs were fixed in 10% trichloro-acetic acid and stained with 0.04% Coomassie R-250 in 10% trichloro-acetic acid overnight. Then they were photographed on glass in transmitted light. For illustration, individual lines were cut out from the slabs and arranged in an order required. The regions of the gliadin component spectrum in photos and patterns were designated for convenience as α, β, γ, ω in accordance with the nomenclature originally used for starch gel (Woychik et al. 1961).

Results

1 Inheritance of gliadin components in 'Bezostaya 1' × 'Odesskaya semidwarf' crossing (monohybrid crossing)

The distribution of gliadin components in 'Bezostaya 1' and 'Odesskaya semidwarf' varieties is presented in Fig. 1 (lines 1 and 4). The two varieties differ in the number and distribution of bands of medium electrophoretic mobility (β and γ gliadins). Among 164 single

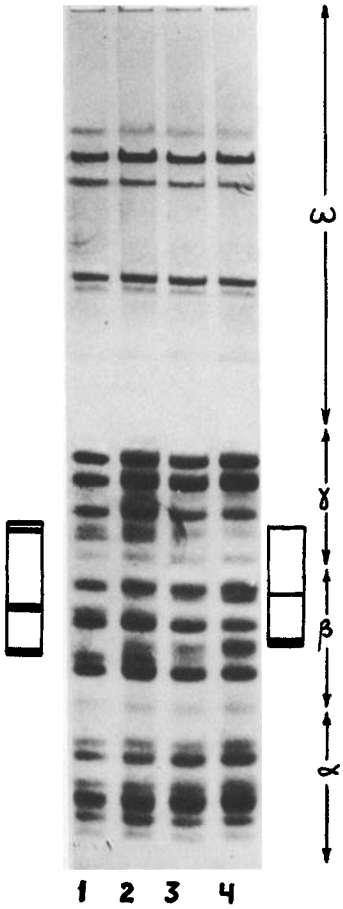

Fig. 1. Electrophoretic gliadin spectra in the varieties 'Bezostaya 1' (*line 1*), 'Odesskaya semidwarf' (*line 4*) and their hybrids (*lines 1–4*). The scheme shows blocks of jointly inherited components (see the text about the *arrows*)

F_2 grains of the 'Bezostaya 1' × 'Odesskaya semidwarf' hybrid, four variants of the distribution of gliadin components corresponding to four phenotypic classes were observed. Two electrophoretic patterns included all components of the both parents but differed in the degree of expression of the parental components (Fig. 1, lines 2 and 3); the other two were identical to the parental ones. The number of grains in phenotypic classes 1, 2+3 and 4 (38, 89 and 37, respectively) was in good agreement with the theoretically expected distribution in the case of monohybrid and codiminant inheritance of two groups (blocks) of gliadin components ($\chi^2 = 1.21$) (Fig. 1). Variants 2 and 3 obviously represent two possible heterozygotes with two doses of gliadin components from 'Bezostaya 1' or from 'Odesskaya semidwarf', respectively. The number of grains with variants 2 and 3 was equal ($\chi^2 = 0.00$). No samples in which one of the pair of blocks has an incomplete set of components have been detected.

Thus, the genetic analysis shows that the synthesis of differing gliadin components in 'Bezostaya 1' and 'Odesskaya semidwarf' is controlled by allelic variants of one gliadin-coding locus. All bands composing the given block are inherited as a single codominant mendelian trait.

2 Inheritance of gliadin components in 'Bezostaya 1' × 'Mironovskaya Yubileinaya' crossing (trihybrid crossing)

As it is seen from Fig. 2, the parental varieties differ in the regions α, β, and ω. Among 238 F_2 grains from the 'Bezostaya 1'×'Mironovskaya Yubileinaya' hybrid 27 basic (with no regard for gene dosage effects in the triploid endosperm) gliadin banding patterns or phenotypic classes have been revealed. The obtained distribution of grains in different phenotypic classes satisfied the assumption of independent and codominant inheritance of three pairs of alleles ($\chi^2 = 19.95$) (Fig. 2). In Fig. 3 eight rare combinations of components are presented which correspond to the phenotypic classes of triple homozygotes. Fig. 2 (line 2) also shows a spectrum of components in the most abundant class of triple heterozygotes (the number of grains – 27 out of 238).

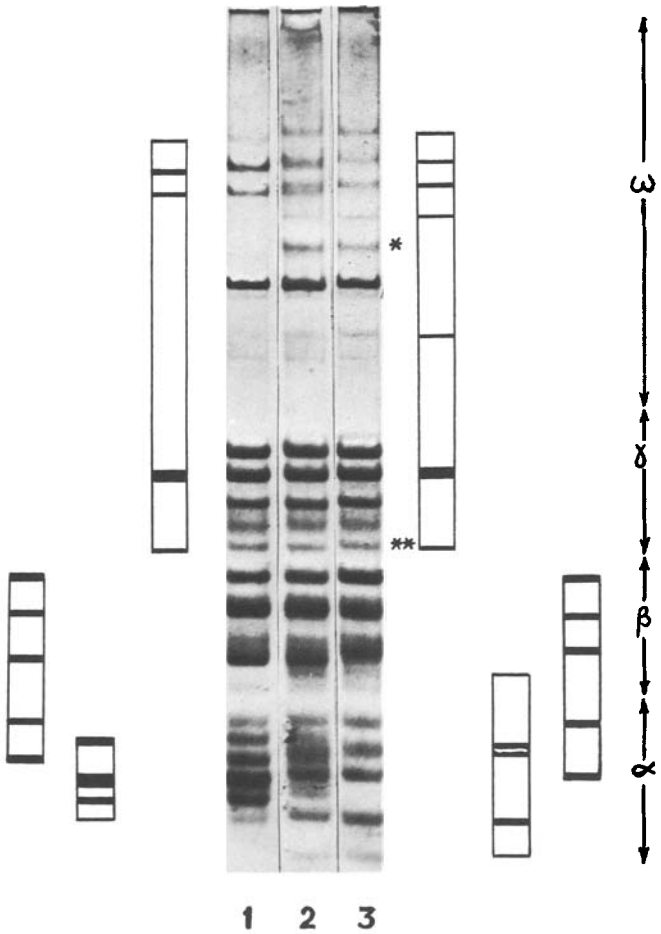

Fig. 2. Electrophoretic gliadin spectra in the varieties 'Bezostaya 1' (*line 1*), 'Mironovskaya Yubileinaya' (*line 3*) and in the most abundant phenotypic class of their hybrids (*line 2*). The scheme shows blocks of components (see the text about the components indicated by *asterisks*)

The inheritance of one component in the ω-region (indicated by an asterisk in Fig. 2) has remained obscure in this crossing. This component exhibited dosage effects, was inherited in accordance with the segregation 3 : 1 (presence : absence), and was independent of each of the three loci studied in this crossing.

3 Inheritance of gliadin components in 'Bezostaya 1' × 'Levent' crossing (tetrahybrid crossing)

The parental varieties differed in the distribution of gliadin components in all gel regions (Fig. 4). The analysis of 184 single F_2 grains in the 'Bezostaya 1'× 'Levent' cross has revealed four pairs of blocks of gliadin components displaying clear dosage effects (Fig. 5). The ratio of grains in phenotypic classes 1, 2+3, 4 for each pair of blocks (Fig. 5a–d) satisfied the theoretically expected segregation 1 : 2 : 1 ($\chi^2 = 0.24$, 0.05, 0.52, 1.11, respectively). Both types of heterozygotes (lines 2 and 3, Fig. 5) had similar frequencies in each pair. Consequently, each pair of blocks is controlled by different alleles of one gliadin-coding locus. Moreover, the number of grains in nine phenotypic classes for any two pairs of blocks is in good agreement with the theoretically expected distribution in the case of two non-linked codominant traits (Table 1). Thus, the synthesis of gliadin components differing in 'Bezostaya 1' and 'Levent' is controlled by four independent loci.

The comparison of the results of the analysis of F_2 grains from intervarietal combinations shows that in crosses 'Bezostaya 1'×'Mironovskaya Yubileinaya' and 'Bezostaya 1'×'Levent' three different alleles for each of the two independent gliadin-coding loci are available. Each allelic state of a given locus was

Table 1. χ^2 values for distribution of grains into 9 phenotypic classes for each two pairs of blocks detected in the 'Bezostaya 1'×'Levent' cross

	a[a]	b[a]	c[a]
b[a]	3.37		
c[a]	6.45	7.72	
d[a]	2.69	6.15	5.95

[a] a, b, c, d – corresponding blocks presented in Fig. 5

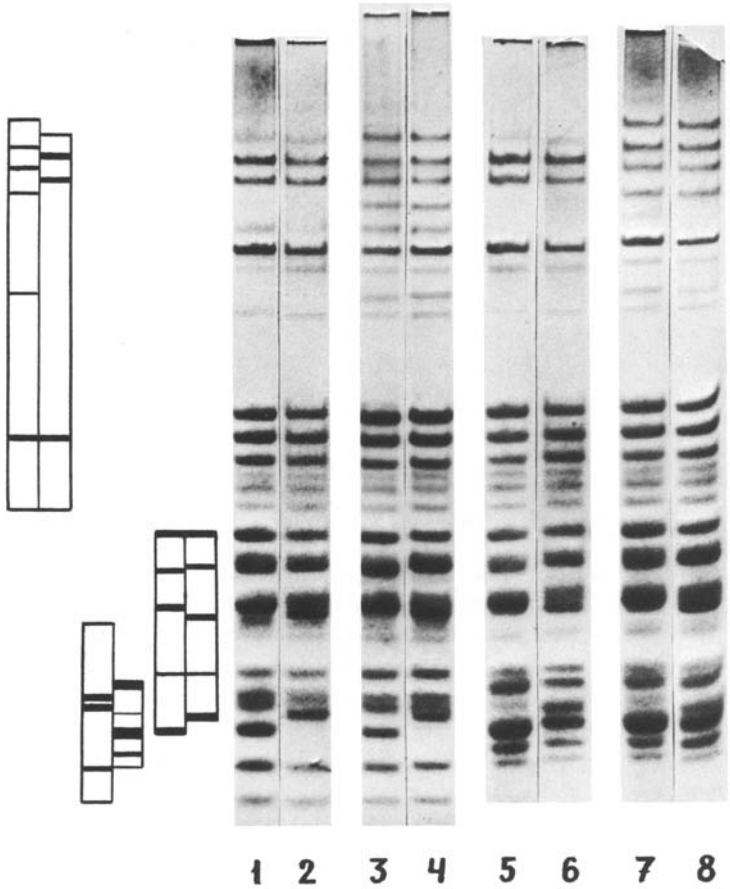

Fig. 3. Rare patterns of gliadin distribution in F_2 hybrids between 'Bezostaya 1' and 'Mironovskaya Yubileinaya' corresponding to the classes of triple homozygotes. On the *left,* three pairs of blocks of components are shown schematically.

characterized by a corresponding group of electrophoretic gliadin components (Figs. 2 and 4). The comparative analysis of a number of crosses not only demonstrates the allelism of different variants of blocks but also helps to determine more precisely their component composition. For instance, the fast component in the γ region (indicated by two asterisks in Fig. 2) can be assigned to a corresponding block only after analysis of the 'Bezostaya 1'×'Levent' cross since in the 'Bezostaya 1'×'Mironovskaya Yubileinaya' cross this component is identical in both parents.

4 Catalogue of blocks of gliadin components

Single F_2 grains from 36 crosses involving 39 winter wheat varieties and lines have been analysed. The sample size in 5 crosses was over 100 grains, and in 20 crosses 60–80 F_2 grains were analysed. At the initial stages of the work six independently inherited blocks of gliadin components in 'Bezostaya 1' wheat were identified. These blocks included almost all components of this variety. In most other varieties blocks were identified on the basis of allelism with respect to the blocks of bands in 'Bezostaya 1'. If a variety under study had the same blocks as in 'Bezostaya 1' and/or blocks similar to those observed previously in other varieties we analysed 30–50 F_2 grains (11 crosses). In all cases allelism of blocks was established on the basis of determining groups of jointly inherited components (by analysing electrophoregrams), counting the number of parental and heterozygous grains, and checking the resultant segregation by the Chi-squared test. Relatively small samples do not permit a confident estimation of a possible percentage of recombinations between components of allelic blocks. Within our samples pairs of allelic blocks were inherited as stable non-recombinative groups of bands.

The results of the analysis make it possible to distinguish 6 basic independent groups, or series, of blocks of gliadin components (Fig. 6). The similarity of Fig. 6 with the catalogue of blocks of gliadin components worked out earlier with the use of starch gel (Sozinov and Poperelya 1980; Poperelya, unpublished) is beyond doubt. The determination of gliadin-coding loci, the loci to which the series of blocks presented in Fig. 6 pertain, was carried out on the basis of comparison with the previously published (Sozinov and Poperelya 1980) catalogue, and independently on the

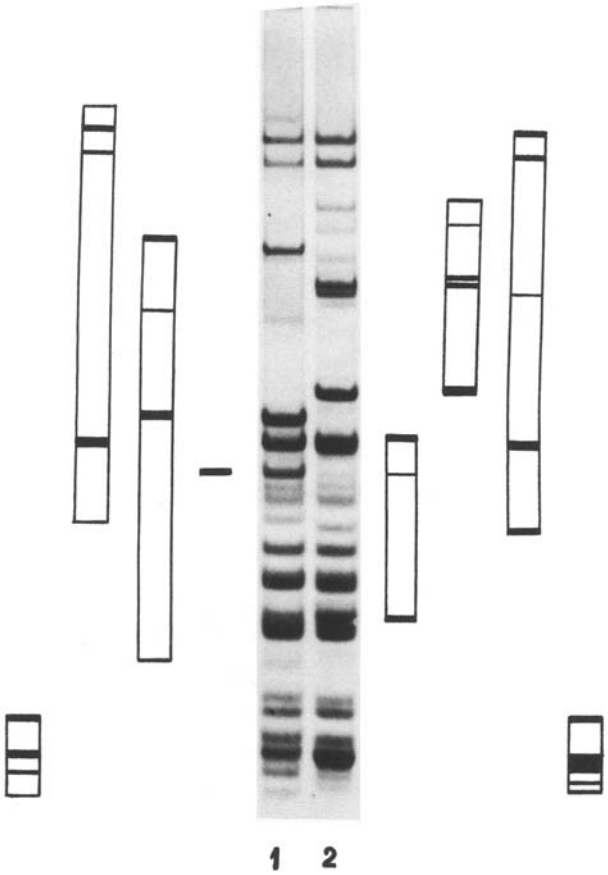

Fig. 4. Electrophoretic gliadin spectra in the varieties 'Bezostaya 1' (*line 1*) and 'Levent' (*line 2*). The scheme shows blocks of jointly inherited components.

basis of comparison of the component distribution in these series of blocks with the gliadin electrophoretic spectrum in the 'Chinese Spring' variety. Analysis of changes in the gliadin banding pattern in nullitetrasomic strains has made it possible to refer the groups of components ("blocks") in 'Chinese Spring' to the known gliadin-coding chromosomes (unpublished). The subsequent comparative analysis of electrophoretic mobility and intensity of the components has demonstrated, in particular, that an identical group of bands is controlled by chromosome 6D in 'Chinese Spring' and presents a block identified by genetic analysis in a number of winter wheat varieties. This block (6D6), as well as the whole series it is included into, is apparently controlled by different alleles of chromosome 6D (Fig. 6). There was also similarity between a pair of components controlled by chromosome 1A in 'Chinese Spring' and two bands included into one of the blocks in the variety 'Levent' (block 1A11, Fig. 6). Three components controlled by chromosome 6A in 'Chinese Spring' correspond in their mobility to specific bands of one of the blocks discovered in 'Mikronovskaya Yubileinaya' (block 6A4) and in some other varieties (Fig. 6, blocks 6A3–7). The same specific components that disappear from the gliadin spectrum in 'Chinese Spring' in the absence of chromosome 1D are not observed in null alleles of 1D locus (unpublished). Analysis of the data presented in Fig. 6 shows that, on the whole, the "blocks" of components of the 'Chinese Spring' variety are typical of only definite series of allelic variants of blocks. The data presented agree with the results obtained earlier on starch gel using the hybrid material from crossing 'Chinese Spring' to some winter varieties (Rybalka 1975).

The catalogue of allelic variants of blocks is intended to make up formulae of the gliadin component composition in different varieties and specimens in accordance with the genetic nomenclature (Sozinov and Poperelya 1980). For instance, the component composition of gliadin the the varieties studied is described by the following formulae (Figs. 1, 2 and 4). In 'Bezostaya 1' chromosomes 1A, 1B, 1D, 6A, 6B, 6D control blocks 4, 1, 1, 1, 1, 1, respectively. The specimen of 'Odesskaya semidwarf' variety employed in the cross studied has a gliadin formula 4.1.1.1.2.1, 'Mironovskaya Yubileinaya' – 4.1.5.4.1.2, 'Levent' – 11.12.3.2.1.1. Gliadin formulae of some other varieties studied by us are shown in Table 2. Having a sufficiently extensive catalogue, a presumable formula of gliadin of any variety can be inferred after analysing

Table 2. Gliadin formulae for for some winter wheat varieties

	1A	1B	1D	6A	6B	6D
1. 'Aurora'	3	3	1	1	1	6
2. 'Bezostaya 1'	4	1	1	1	1	1
3. 'Dneprovskaya 521'	1	2	1	9	1	7
4. 'Kavkaz'	4	3	2	1	1	6
5. 'Krasnodonka'	4	10	3	10	1	2
6. 'Lesostepka'	8	17	5	10	8	2
7. 'Mironovskaya 808'	3	1	5	4	5	2
8. 'Mironovskaya Yubileinaya'	4	1	5	4	1	2
9. 'Odesskaya 16 (line)	2	1	5	3	2	6
10. 'Odesskaya 26'	5	1	4	3	1	2
11. Odesskaya semidwarf (line)	4	1	1	1	2	1
12. 'Promin'	4	1	4	3	4	4
13. Zaporozhskaya ostistaya (line)	4	1	5	1	1	2
14. 'Zirka'	4	2	1	3	4	4
15. 'Ackermanns Cara'	2	5	1	13	4	6
16. 'Cluj 650'	2	14	9	12	8	6
17. 'Dankowska Iasna'	12	13	2	2	9	6
18. 'Kaprock'	7	7	9	6	7	6
19. 'Kremena'	11	8	1	2	1	3
20. 'Levent'	11	12	9	2	1	1
21. Rusalka (line)	4	7	4	8	4	1
22. 'Szegedi 6'	1	9	9	12	6	6
23. 'Zg 2639/73'	16	16	9	2	4	6

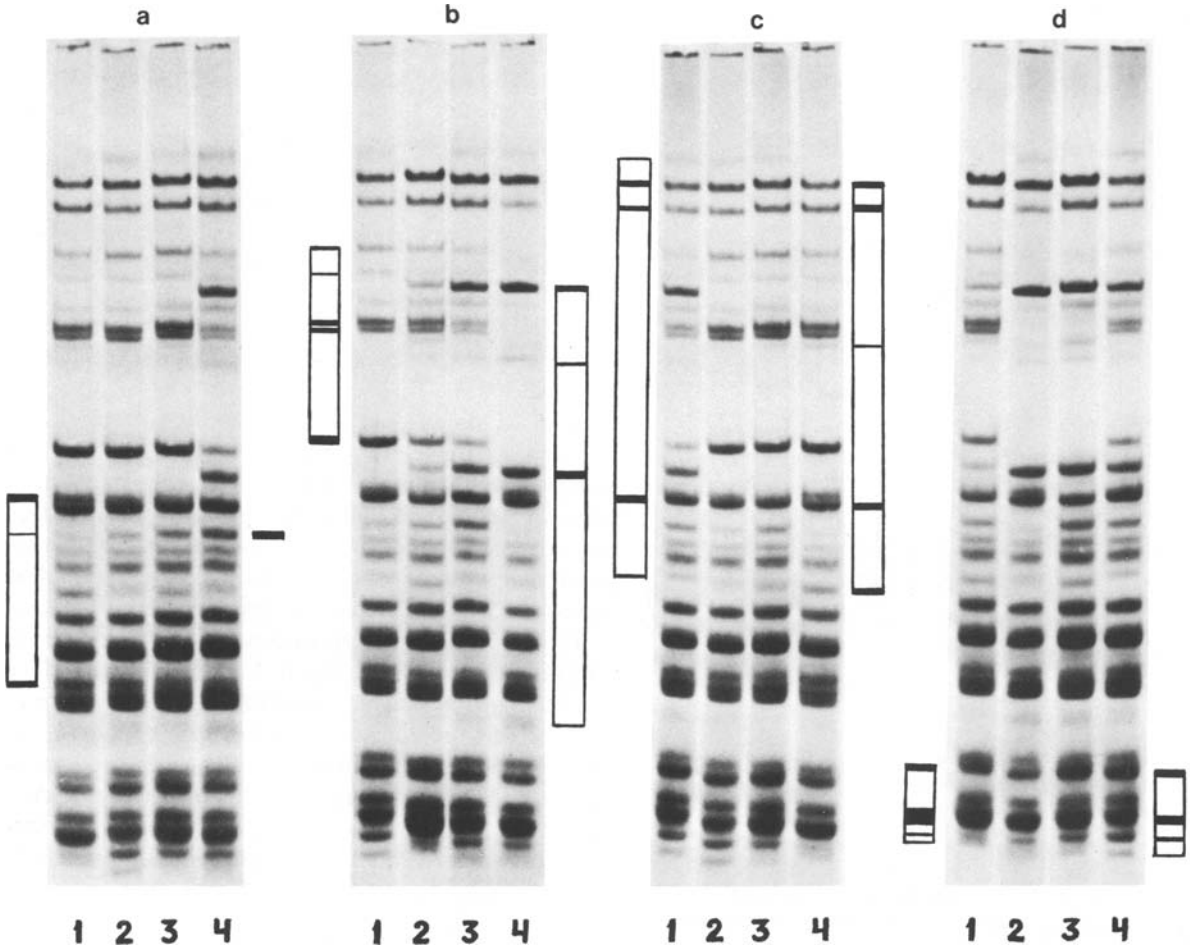

Fig. 5 a – d. Dose effects of four pairs of blocks of components in hybrids between 'Bezostaya 1' and 'Levent'. *1, 4* – homozygotes; *2, 3* – heterozygotes for the blocks illustrated. The genotypes of the specimens presented in Fig. 5 a – d for all four pairs of blocks of gliadin components are as follows (*from left to right*): het, L, het, B; het, het, L, L; B, het, het, het; L, B, het, het; L, L, L, L; het, L, het, B; het, L, het, het; B, het, het, het; het, het, B, B; het, L, het, B; het, L, het, het; L, L, L, L; het, het, L, L; L, B, het, het; B, B, L, het; het, het, B, B (L – a block from 'Levent', B – a block from 'Bezostaya 1', het – both allelic variants of blocks are present)

only the distribution of gliadin components on gel without hybridological analysis. In complicated cases (superposition of components, small differences in the relative mobility of components as compared to those mentioned in the catalogue) it is necessary to compare thoroughly, on neighbouring lines of the same slab, an analysed specimen with a suitable variety studied earlier by hybridological analysis. It should be noted that the peculiar intensive component N 50 of the variety 'Marquis', which has recently been purified to be used as a marker in electrophoresis (Howes and Kosmolak 1982), is clearly a component of the 1A1 block present in this variety.

5 Distribution of gliadin components in different gel regions and the possibility of their genetic analysis

The method of electrophoresis proposed by Bushuk and Zillmann (1978) to study gliadins and used by us (with minimal modifications) to prepare a catalogue of blocks of components yields quite satisfactory and reproducible results. Thus, this method makes it possible not only to detect a doublet of components in the middle gel region in the variety 'Marquis', which is assumed (Lookhart et al. 1982) to be a criterion of good electrophoresis quality, but also to reveal small differences in the mobility of the slow member of this doublet which are observed in different blocks of the 1B series.

The distribution of gliadin components along the gel in one-dimensional polyacrylamide and starch gel electrophoresis is uneven. In the ω region most bands are inherited in accordance with control by a single dominant gene. Some bands in this region consist of two co-migrating components and their presence-absence conforms to the segregation 15 : 1. Such components with a similar mobility can be separated and identified, in some cases with the help of prolonged

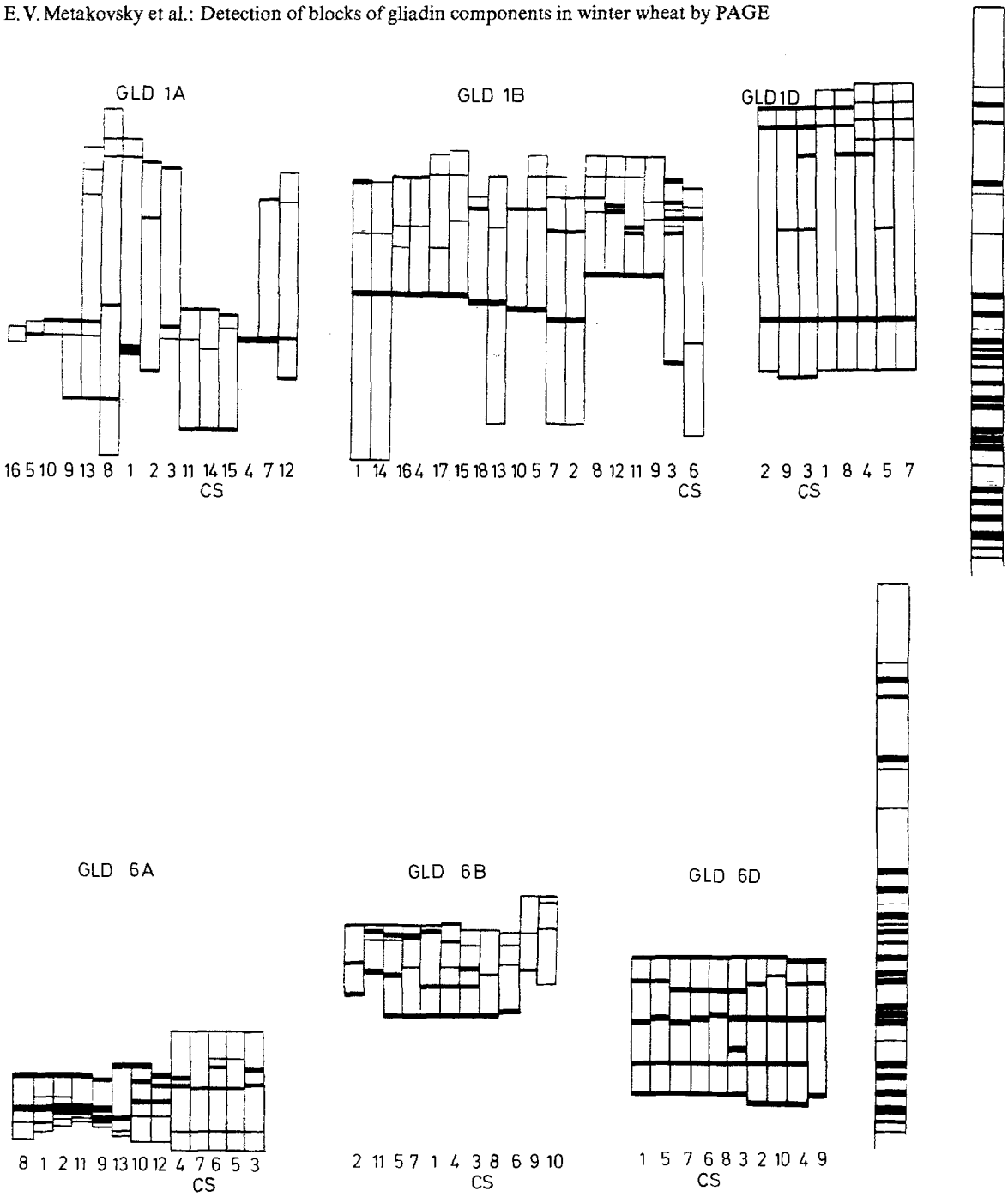

Fig. 6. The catalogue of blocks of gliadin components. On the *right* – the pattern of gliadin bands in 'Bezostaya 1'. CS – "blocks" of gliadin components in 'Chinese Spring' detected by the analysis of electrophoretic spectra in different nullitetrasomics. Some numbers of blocks are omitted to bring this catalogue into correspondence with the catalogue of blocks detected in starch gel

electrophoresis or small changes in pH aluminium-lactate buffer. Minor components are sufficiently stable and their inheritance was easily traced by analysing F_2 grains. We have failed to assign to any series of allelic variants of blocks some minor components observed in many varieties (including 'Bezostaya 1') in the middle of the ω region. A special study on starch gel has shown that the synthesis of these components is apparently controlled by an additional locus on chromosome 1A (Sobko; Poperelya, unpublished).

Bands of the γ region, if they are really complex, consist of an intensive component and minor components of similar mobility which are revealed only by

two-dimensional electrophoresis combining electrophoresis in aluminium-lactate buffer and electrophoresis in the presence of sodium dodecyl sulphate (Metakovsky, unpublished). Inheritance of the main components of this region, which may be controlled by chromosomes 1A, 1B, 1D and 6B, is established relatively easily by analysis of hybrid material.

Many components of the α and β regions are complex, and components controlled by chromosomes 1A, 1B, 6A, 6B and 6D were found in the β region. In the β region inheritance of either most intensive or single components can be analysed by one-dimensional electrophoresis (Fig. 1). Genetic analysis of gliadins in the α region is simply due to the fact that, as shown by one-dimensional (Fig. 6) and two-dimensional (in preparation) electrophoresis, all components in this region in the varieties studied are controlled by only two chromosomes, 6A and 6D (see also Kasarda 1980).

It should be noted that in several varieties some bands have been assigned to none of the blocks. Such bands may include several components of approximately equal mobility controlled by different loci. All doubtful cases of complex components or unclearly identified minor components were not taken into account when preparing a catalogue of blocks. As a result, some of the blocks presented in Fig. 6 are probably simplified.

Discussion

The analysis of inheritance of electrophoretic gliadin components in 39 varieties has permitted a catalogue of nearly 80 blocks to be prepared. In the course of the work the main features of inheritance of gliadin components revealed previously (Doekes 1973; Rybalka 1975; Sozinov et al. 1975; Baker and Bushuk 1978; Damidaux et al. 1980; Branlard 1982 a, and others) with the use of starch, polyacrylamide and SDS-polyacrylamide gels have been confirmed: blocks of jointly inherited components, codominance and dosage effects of blocks, low recombination frequencies of components of allelic blocks. We did not tackle the latter point especially in this work, but 1 presumptive recombinant for 1B chromosome locus has been found in the 'Bezostaya 1'×'Levent' cross (unpublished). Multiple allelism demonstrated previously for gliadin-coding loci of chromosomes 1A, 1B and 1D (Sozinov and Poperelya 1980) has also appeared significant for the homoeologous group 6 chromosomes. In the course of the F_2 grain analysis, neither appearance nor reproducible intensification of any band in a hybrid as compared to parental variants was recorded by us, in contrast to Branlard (1982 a). We observed only some intervarietal variation in the intensity of the same band or spot in two-dimensional electrophoresis (Novoselskaya et al. 1983). The staining intensity of single (non-superimposed) bands in hybrids was within the range of intensity of these components in parents, as it was previously reported (Sozinov et al. 1975; Baker and Bushuk 1978; Mecham et al. 1978).

We recognize that the catalogue of allelic variants of blocks presented here will undergo certain changes with time. Firstly, analysis of non-studied varieties will reveal new blocks. Secondly, some presented blocks may acquire minor components which will probably be located in complex gel regions. Thirdly, some blocks (Fig. 6) may represent families of blocks distinguishable by hitherto undetected minor components and by very faint differences in band mobility. Gliadin components of different varieties having an equal electrophoretic mobility in aluminium-lactate buffer may differ in another electrophoretic system or upon isoelectric focusing. Nevertheless, the catalogue encompasses practically all distinct bands in the analysed varieties and may serve both for practical use and as a basis for more detailed studies in future.

The catalogue of gliadin allelic blocks, including 12–18 blocks in each of 6 independent groups or series, suggests an exclusively high polymorphism of gliadin. Not only are multiple alleles observed for each gliadin-coding locus, but blocks themselves controlled by one locus significantly differ and may have no bands in common. In this connection, a clear distinction of series controlled by the D genome from other groups should be pointed out. A series of blocks controlled by the D genome from other groups should be pointed out. A series of blocks controlled by a gliadin-coding locus of chromosome 1D is the smallest and the blocks are very similar (Fig. 6). They include 2–4 components with a minimal electrophoretic mobility and a pair of components in the γ region. One of the components in the γ region is identical in all blocks of this series. This component, together with other bands controlled by chromosome 1D, was absent, firstly, from corresponding nullitetrasomics of the 'Chinese Spring' variety and, secondly, from some mutant lines (Sozinov and Kopus 1983).

Blocks of components controlled by chromosome 6D are also very similar and almost always have 5 distinct components. They have only small differences in the mobility of some components which are probably caused by single mutations in corresponding genes.

Blocks of components controlled by chromosomes of A and B genomes are much more diverse. Subgroups or families of blocks including one or several components characterized by equal electrophoretic mobility may be distinguished among them.

Chromosome 6A controlled blocks may be divided into 3–4 subgroups depending on the mobility of the fastest components. The family of blocks 6A3–7 has three similar bands and the blocks differ by the mobility of the remaining 1–3 components. It may be assumed that this subgroup of blocks has a common precursor and the observed polymorphism is a result of mutation accumulation. Thus, different gliadin components controlled by one gliadin-coding locus and included into one block are subject to the action of natural mutation process in a different degree or are of different selective value.

Almost all blocks controlled by chromosome 1B have an intensive band in the region of the slowest γ-gliadins. In accordance with the electrophoretic mobility of this band, several subgroups of 1B blocks may be distinguished. Their members are, probably, more closely related than members of different subgroups. Several subgroups of blocks may also be distinguished in the series of blocks controlled by chromosome 6B. The series of chromosome 1A-controlled blocks containing components in β, γ and ω gliadins is very diverse.

It is believed that hexaploid wheat *Triticum aestivum* appeared about 10,000 years ago due to hybridization of the tetraploid form AABB with the D genome donor *Ae. squarrosa* (Konzak 1977). It is natural to suppose that the rate of changes in the blocks of linked components (changes in the number, mobility and intensity of components caused by mutations, duplications, deletions, unequal crossingover etc. in corresponding genes) is equal in the series of blocks controlled by the A, B and D genomes. In this case, an essentially higher diversity of blocks controlled by the A and B genomes as compared to series controlled by the D genome may be explained by higher selective values of certain components of 1D and 6D blocks maintained in the relatively conservative form. Another possible explanation is that the D genome was introduced into the tetraploid forms AABB several times. Gliadin blocks controlled by the A and B genomes must be different in these tetraploid forms and might have given rise to subgroups or families of blocks observed nor. The repeated AABBDD hexaploid formation might have been promoted by the specific effect of the D genome on photosynthesis, water balance, properties of grain proteins and other important parameters of the plant (Cole et al. 1981; Planchon and Fesquet 1982). The presumptive differences of the gliadin component composition in the tetraploid forms AABB may be also due to heterogeneity of the diploid donors of the A and B genomes (see also Cole et al. 1981). Judging by gliadin blocks controlled by chromosomes 6D and 1D, the D genome donor was much more homogeneous.

The results obtained agree with the hypothesis on the complex structure of gliadin-coding loci. Each of these loci controls the synthesis of a series of components with different electrophoretic mobilities in aluminium-lactate buffer and different molecular weights (Novoselskaya et al. 1983). Genes controlling the synthesis of these components are apparently closely linked on the chromosome. However, in the gliadin-coding locus there may be recombinations, mutations and other events changing the mobility, number and intensity of the components of this block. Judging by the great diversity of allelic blocks, including ones controlled by the genome D which appeared relatively recently in the hexaploid wheat genome, these changes were not very rare and were not eliminated by natural selection and/or by breeding.

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References

Autran JC, Bushuk W, Wrigley CW, Zillmann RR (1979a) Wheat cultivar identification by gliadin electrophoregrams. 4. Comparison of international methods. Cereal Food World 24:471–475

Autran JC, Lew EJL, Nimmo CC, Kasarda DD (1979b) N-terminal amino acid sequencing of prolamines from wheat and related species. Nature 282:527–529

Baker RJ, Bushuk W (1978) Inheritance of differences of gliadin electrophoregrams in the progeny of 'Neepawa' and 'Pitic 62' wheats. Can J Plant Sci 58:325–329

Bietz JA, Huebner FR, Rothfus JA (1970) Chromatographic comparisons of peptic digests of individual gliadin proteins. Cereal Chem 47:393–404

Bietz JA, Huebner FR, Sanderson JE, Wall JS (1977) Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. Cereal Chem 54:1070–1083

Branlard G (1982a) Study of genetic determination of 20 gliadin bands. Theor Appl Genet 64:155–162

Branlard G (1982b): Correlation between gliadin bands. Theor Appl Genet 64:163–168

Brown JWS, Flavell RB (1981) Fractionation of wheat gliadin and glutenin subunits by two dimensional electrophoresis and the role of group 6 and group 2 chromosomes in gliadin synthesis. Theor Appl Genet 59:349–359

Bushuk W, Zillmann RR (1978) Wheat cultivar identification by gliadin electrophoregrams. 1. Apparatus, method and nomenclature. Can J Plant Sci 58:505–515

Cole EW, Fullington JG, Kasarda DD (1981) Grain protein variability among species of *Triticum* and *Aegilops:* quantitative SDS-PAGE studies. Theor Appl Genet 60:17–30

Damidaux R, Autran JC, Grignac P, Feillet P (1980) Déterminisme génetique des constituants gliadines de *Triticum durum* Desf. associés a la qualité culinaire intrinsèque des variétés. CR Acad Sci, Ser D 192:585–588

Doekes GJ (1973) Inheritance of gliadin composition in bread wheat, *Triticum aestivum* L. Euphytica 22:28–34

Garcia-Olmedo F, Carbonero P, Jones BL (1982) Chromosomal locations of genes that control wheat endosperm proteins. In: Pomeranz Y (ed) Adv Cereal Sci Technol, vol 5. Am Assoc Cereal Chemists Inc, St. Paul Min, pp 2–47

Howes NK, Kosmolak FG (1982) Purification and characterization of a specific gliadin component from the wheat cultivar 'Marquis'. Cereal Chem 59:485–488

Kasarda DD (1980) Structure and properties of α-gliadins. Ann Technol Agric 29:151–173

Konzak CF (1977) Genetic control of the content, amino acid composition, and processing properties of proteins in wheat. In: Caspari EW (ed) Adv genet, vol 19. Academic Press, London New York, pp 407–550

Lookhart GL, Jones BL, Hall SB, Finney KF (1982) An improved method for standardizing polyacrylamide gel electrophoresis of wheat gliadin proteins. Cereal Chem 59: 178–181

Mecham DK, Kasarda DD, Qualset CO (1978) Genetic aspects of wheat gliadin proteins. Biochem Genet 16: 831–853

Novoselskaya AYu, Metakovsky EV, Sozinov AA (1983) The study of gliadin polymorphism of some wheat varieties by means of one- and two-dimensional electrophoresis. Cytol Genet (USSR) 17 (5):45–49

Planchon C, Fesquet J (1982) Effect of the D genome and of selection on photosynthesis in wheat. Theor Appl Genet 61:359–365

Rybalka AI (1975) Hybridization and monosomic analyses of gliadins. Dis VSGI, Odessa

Shewry PR, Faulks AJ, Pratt HM, Miflin BJ (1978) The varietal identification of single seeds of wheat by sodium dodecyl sulphate polyacrylamide gel electrophoresis of gliadin. J Sci Food Agric 29:847–849

Sozinov AA, Kopus MM (1983) The mutation of gliadin-coding locus of chromosome 1D. Cytol Genet (USSR) 17:19–24

Sozinov AA, Poperelya FA (1980) Genetic classification of prolamines and its use for plant breeding. Ann Technol Agric 29:229–245

Sozinov AA, Poperelya FA, Stakanova AI (1975) Hybridological analysis as a method for study of the genetical regularities in gliadin biosynthesis. Nauchno-Tekh Byull Uses Sel-Genet Inst: 10–15

Tkachuk R, Metlish VJ (1980) Wheat cultivar identification by high voltage gel electrophoresis. Ann Technol Agric 29:207–212

Wrigley CW, Shepherd KW (1973) Electrofocusing of grain proteins from wheat genotypes. Ann NY Acad Sci 209: 154–162

Wrigley CW, Autran JC, Bushuk W (1982) Identification of cereal varieties by gel electrophoresis of the grain proteins. In: Pomeranz Y (ed) Adv Cereal Sci Technol, vol 5. Am Assoc Cereal Chem Inc, St. Paul Min, pp 211–259

Woychik JH, Boundy JA, Dimler RJ (1961) Starch gel electrophoresis of wheat gluten proteins with concentrated urea. Arch Biochem Biophys 94:477–482

Zehatschek W, Günzel G, Fischbeck G (1981) Electrophoretic analysis of gliadins in wheat, *Triticum aestivum* L., for determining their inheritance and chromosomal localization. Z Pflanzenzücht 87:45–57