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Genetic control of endosperm proteins in wheat

2. Variation in high molecular weight glutenin and gliadin subunits of *Triticum aestivum*

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Summary. Endosperm protein subunits of 109 primitive and modern lines of hexaploid wheat, *Triticum aestivum* L. em. Thell., were fractionated by one-dimensional, high resolution, sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE). A wide range of both qualitative and quantitative variation was observed in the fractions of the high molecular weight (HMW) glutenin and gliadin subunits of the different lines. The qualitative variation was expressed in the number of subunits per fraction and in their molecular weight, as determined by the differential rate of migration. The quantitative variation was expressed in the differential staining intensity of several subunits. The widest variation was detected in the HMW glutenin and gliadin subunits controlled by chromosome 1B while a much smaller variation was observed in those subunits controlled by chromosome 1A and further smaller variation in the subunits controlled by 1D. Only a small number of subunits in both fractions was found to be controlled by chromosome 1A indicating that diploidization of endosperm protein genes in common wheat has been non-random. The genetic and evolutionary implications of these findings are discussed.

Key words: Common wheat, *Triticum aestivum* – Electrophoresis – Endosperm proteins – Glutenins – Gliadins

Introduction

The biochemistry and genetics of endosperm proteins in common wheat have been studied very intensively in recent years (for review, see Kasarda et al. 1976; Konzak 1977; Wall 1979). A considerable progress was achieved in these studies by the application of the technique of sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE). This technique enables a detailed study as to the number, size distribution and the genetic control of specific proteins at the subunit level.

The high molecular weight (HMW) glutenin subunits, which are well resolved in this system, appear as the heaviest fraction of the endosperm proteins ranging between 140 kilo dalton (KD) and 80 KD. Different lines of common wheat possess between three and six subunits of HMW glutenins (Lawrence and Shepherd 1980; Payne et al. 1981 b). Analysis of these proteins in various aneuploids and intervarietal substitution lines of the cultivar 'Chinese Spring' has revealed that all of these subunits are controlled by the long arm of chromosomes of homoeologous group 1 (Bietz et al. 1975; Brown et al. 1979, 1981; Lawrence and Shepherd 1980; Galili and Feldman 1983). The glutenin subunits which are controlled by the long arm of chromosome 1B (1BL) tend to occupy the central part of the HMW glutenin fraction in the gel, while subunits controlled by chomosomes 1D (1DL) and 1A (1AL) tend to occupy the upper and lower parts of this fraction.

In contrast to the HMW glutenins, the application of SDS PAGE to the HMW gliadins has been very limited. These subunits, which are probably identical to the ω gliadins (Bietz et al. 1977), occupy the central part of the SDS gel, ranging between 68 KD and 50 KD (Galili and Feldman 1983). Brown et al. (1979), Lawrence and Shepherd (1980) and Galili and Feldman (1983) have shown that in the cultivar 'Chinese Spring', these subunits are controlled by genes located on the short arm of chromosomes of homoeologous group 1.

In this report, 109 different lines of *T. aestivum*, representing a wide spectrum of common wheat germplasm, most of which have not yet been studied, were analysed as to the qualitative and quantitative variation

of the HMW glutenin and gliadin subunits. The high resolution one-dimensional SDS PAGE, previously described, (Galili and Feldman 1983) was employed in this study.

Materials and methods

Plant material

One hundred and nine lines representing a wide range of genotypes of *T. aestivum* L. em. Thell. were used in this study. These lines are divided into the following groups: a) Forty-six outdated and/or modern commercial varieties of var. 'aestivum' including Israeli commercial material as well as lines originating in other countries. The SDS PAGE pattern of ten out of these lines has been reported in a previous work (Payne et al. 1981 b) while the rest of the lines have not yet been studied; b) Fifty-three lines of var. 'aestivum' are being grown as land races in various fields in Judea and Samaria where traditional agriculture is still being practiced. This material was collected and kindly supplied by M. Kislev, Bar-Ilan University, Ramat-Gan; c) One to three lines from each of the following botanical varieties of *T. aestivum*, namely vars. 'macha', 'vavilovii', 'spelta', 'compactum' and 'sphaerococcum'.

Grains of all of these lines are maintained in the stock of our laboratory seed collection.

Extraction and fractionation of proteins

Extraction of endosperm protein subunits was performed from embryo-less mature kernels by Al-lactate followed by sample buffer solution (consisting of 10% glycerol, 3% SDS, 5% 2-mercaptoethanol, 66 mM Tris-HCl pH 6.8). Fractionation was carried out in SDS PAGE of 7–12% acrylamide gradient which is a modification of Laemmli (1970). A detailed description of the procedure was previously described (Galili and Feldman 1983).

Quantitative analysis of the gels

Coomassie brilliant blue R-250 stained gels were photographed and a 1 : 1 transparent sheet was prepared. Each lane from this sheet was scanned by a Gilford Spectrophotometer scanning apparatus at 500 nm. With the extraction conditions used a linear correlation was found between the volume of protein extract loaded on each lane and the peak area of each band of the HMW glutenins and gliadins. The relative area of each peak was expressed by its weight in mg.

Estimation of MW

The MW of the various endosperm protein subunits was estimated by comparison with protein markers of known MW (Pharmacia) which were fractionated in a parallel lane. The protein markers were phosphorilase-B (94 KD), bovine serum albumin (67 KD), ovalbumin (43 KD) and carbonic anhydrase (30 KD).

Results and discussion

The range of variation of HMW glutenin and gliadin subunits was determined in 109 different lines of *T. aestivum* by the use of a high resolution one-dimensional SDS PAGE. Representative examples of typical SDS PAGE patterns of these proteins from outdated and modern cultivars, Israeli land races of var. 'aestivum' and lines of the other five botanical varieties of hexaploid wheat are shown in Figs. 1, 2 and 3, respecitvely. The HMW glutenins (corresponding to

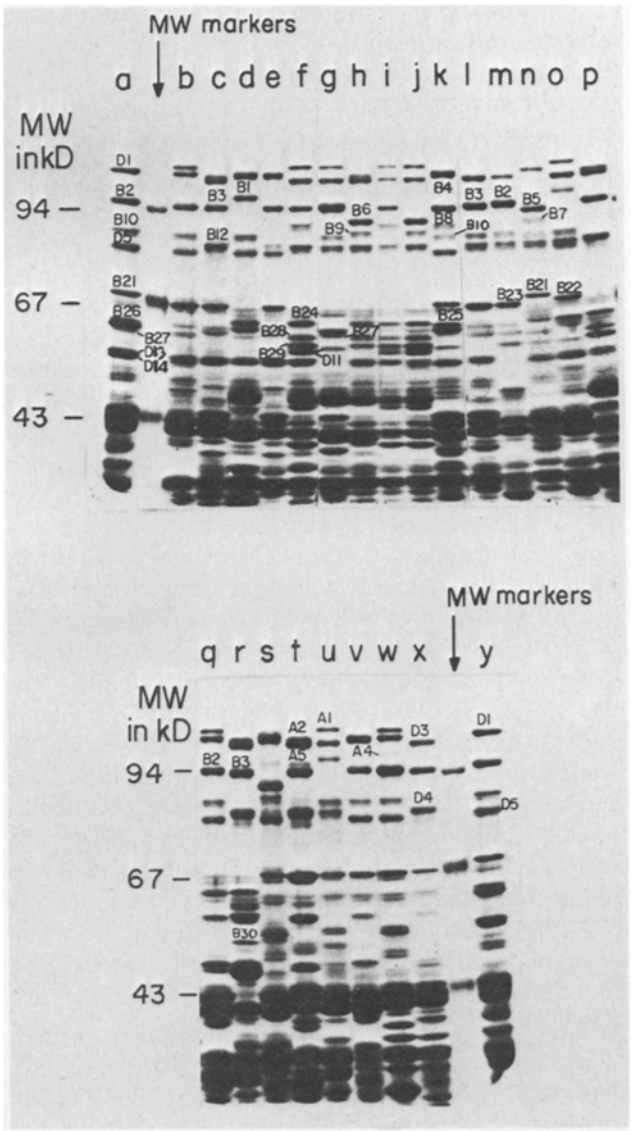

Fig. 1. SDS PAGE migration pattern of total endosperm proteins extracted from a representative sample of outdated and modern commercial varieties of *T. aestivum* var. 'aestivum'. The lines included are the following: *a* 'Chinese Spring'; *b* 'Merav'; *c* 'Florence Aurore'; *d* 'Ribeiro'; *e* 'Lackish'; *f* 'Miriam'; *g* 'Jaral'; *h* 'Tanori'; *i* 'Inia 66'; *j* 'Sonora 66'; *k* 'TAA19'; *l* 'Hazera 112'; *m* 'Hazera 776'; *n* 'Mabrouk'; *o* 'Selkirk'; *p* 'WA 6389'; *q* 'Red Egyptian'; *r* 'Thatcher'; *s* 'Timstein'; *t* 'Cheyenne'; *u* 'Hope' *v* 'Centurk'; *w* 'TAA36'; *x* 'TAA35'; *y* 'Chinese Spring'. Proteins of known *MW* were fractionated in parallel lanes with *MW* indicated on the left

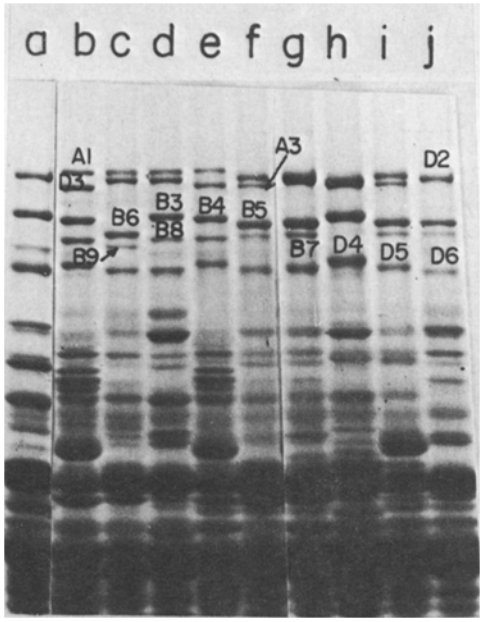

Fig. 2. SDS PAGE migration pattern of total endosperm proteins extracted from several Israeli land races of *T. aestivum* var. 'aestivum'. The collection numbers of the various lines are as follows: *a* 'Chinese Spring' (given as a reference); *b* 'TAA106'; *c* 'TAA107'; *d* 'TAA108'; *e* 'TAA51'; *f* 'TAA54'; *g* 'TAA70'; *h* 'TAA84'; *i* 'TAA91'; *j* 'TAA93'

subunits D1, B2, B10 and D5 of 'Chinese Spring') occupy the upper part of the gel while the HMW gliadins (corresponding to subunits B21, B26, B27, D13 and D14 of 'Chinese Spring'), occupy the middle part of the gel (Figs. 1–3, lane a). A wide range of variation was observed in both fractions, particularly in the commercial lines, in which nearly each of the tested lines exhibited a characteristically unique pattern. This was mainly due to the variation in the number and size of the different subunits in each line. In addition, quantitative variation was reflected by the intensity of band staining which is strongly correlated with the protein content.

Qualitative variations

HMW glutenins. A total of 23 differentially migrating bands was detected in the fraction of the HMW glutenins in all the lines tested, ranging in MW from 114 to 78 KD (Figs. 1–4). The number of these subunits in each of the tested lines varied from three to seven, four or five were the most frequent. However, while the commercial lines (Fig. 1) exhibited the full range of variability (three to seven subunits per line) the variation in the land races (Fig. 2) was more restricted (four to six subunits per line).

The range of variation in the HMW glutenins, as reported here, is wider, in all respects, than previously described by Lawrence and Shepherd (1980) and Payne et al. (1981b). This may be attributed to the wider spectrum of wheat genotypes used and/or to the higher resolution of the one-dimensional SDS PAGE employed (Galili and Feldman 1983).

HMW gliadins. A total of 14 differentially migrating protein bands was detected in the region of HMW gliadins, ranging in MW between 68 to 52 KD (Figs. 1–3 and 6). The number of these bands in each of the tested lines varied from two to seven, while three to five bands per line appeared in most of the lines. To the best of our knowledge, variation of HMW gliadins in SDS PAGE has not been previously reported. These proteins probably belong to the group of ω gliadins (Bietz et al. 1977).

Classification into chromosomal groups

The various subunits were divided into three chromosomal groups (Fig. 4) according to their chromosomal control, namely, subunits controlled by chromosomes

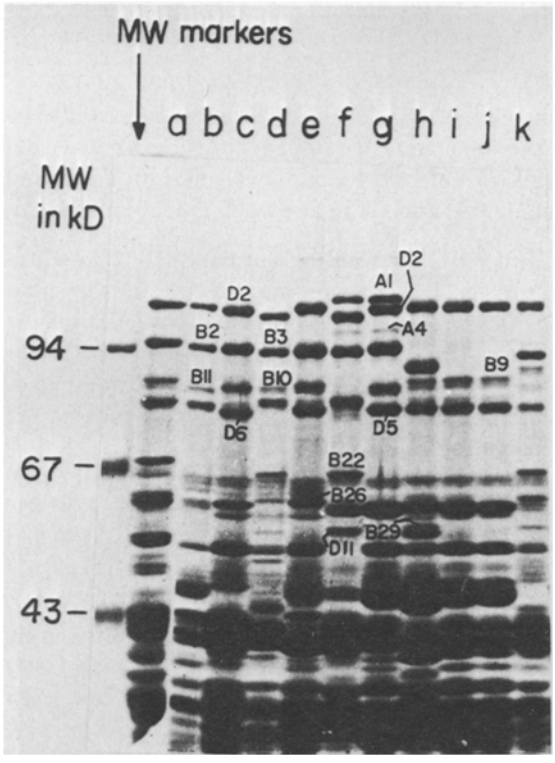

Fig. 3. SDS PAGE migration pattern of total endosperm protein subunits extracted from the various botanical varieties of *T. aestivum.* The varieties and their collection numbers are as follows: *a* var. 'aestivum' cv. 'Chinese Spring', TAA01; *b, c* var. 'vavilovii', TAV02 and TAV01; *d* var. 'macha', TAM01; *e – g* var. 'spelta', TAS04, TAS03 and TAS01; *h – j* var. 'sphaerococcum', TAP03, TAP02 and TAP01; *k* var. 'compactum', TAC01. Proteins of known *MW* were fractionated in a parallel lane with *MW* indicated on the left

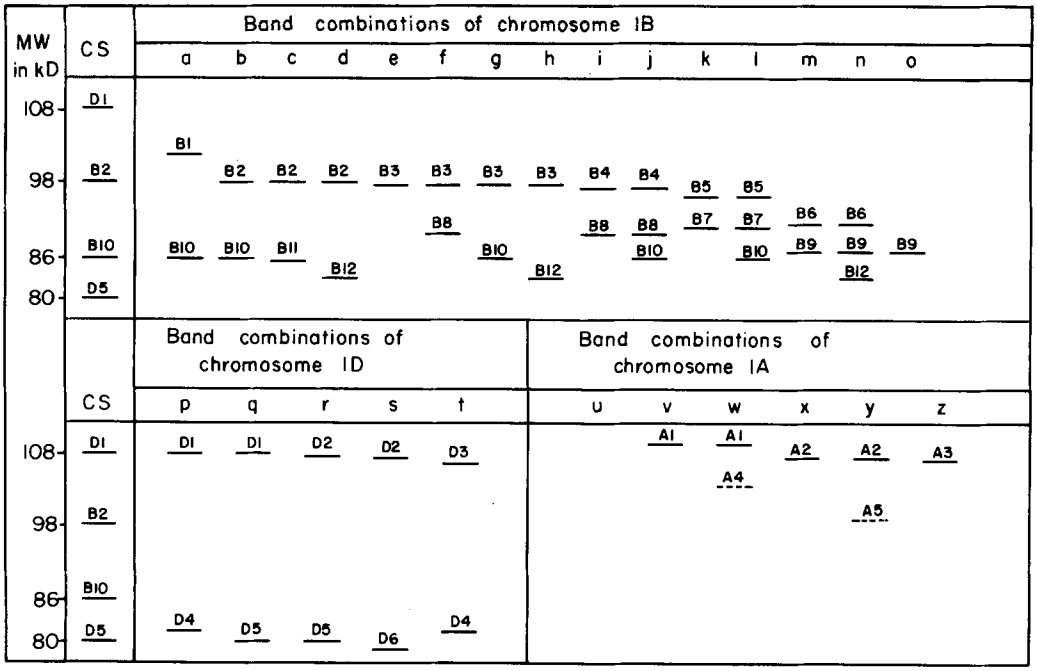

Fig. 4. Schematic diagram of the different band combinations which were detected in hexaploid wheat for HMW glutenin subunits controlled by chromosomes *1B* (*a* – *o*), *1D* (*p* – *t*) and *1A* (*u* – *z*). The four HMW glutenin bands of the cultivar 'Chinese Spring' (*CS*) and their *MW* are indicated at the left of the diagram

1B, 1D and 1A. Data on the chromosomal control of these subunits were compiled from Lawrence and Shepherd (1980); Payne et al. (1980, 1981 b); Galili and Feldman (1983) and Galili and Feldman, unpublished.

HMW glutenins. Twelve different bands were found to be controlled by chromosome 1B in all the lines studied. Their MW ranged between 102–92 KD for the slowly migrating subunits (B1–B6) and 91–84 KD for the rapidly migrating ones (B7–B12). The number of bands per line varied from one to three, two being the most frequent (Figs. 1–4).

Six different bands were found to be controlled by chromosome 1D. Their MW varied between 108–106 KD for the slowly migrating subunits (D1–D3) and 84–78 KD for the rapidly migrating ones (D4–D6). There were, invariably, two bands per line (Figs. 1–4).

Five different bands were found to be controlled by chromosome 1A. Two of these bands (A4 and A5) appeared relatively faint and in some cases could not be detected in the reproduced photographs. The MW of the subunits controlled by chromosome 1A ranged between 114–105.5 KD for the major subunits (A1–A3) and 103–100 for the minor subunits (A4–A5). The number of bands per line varied from zero to two (Figs. 1–4).

HMW gliadins. Out of the 14 HMW gliadin bands ten were found to be controlled by chromosome 1B, four by chromosome 1D and none by chromosome 1A (Fig. 6). The assignment of bands to chromosomal groups in this fraction was based on results obtained from the analysis of intervarietal substitution lines (Galili and Feldman, unpublished). Bands B22 and B25 were assigned to chromosome 1B indirectly, on the basis of their rate of migration, which was within the MW range of subunits controlled by this chromosome. The ten different bands controlled by chromosome 1B ranged in their MW between 68 and 52 KD while the four bands controlled by chromosome 1D ranged between 57 and 53.5 KD.

In both the HMW glutenins and gliadins, chromosomal group 1B possesses not only the largest number of bands but also the most variable ones in molecular weight. This finding confirms previous results regarding the HMW glutenins (Lawrence and Shepherd 1980; Payne et al. 1981 b). The wider variation of the proteins controlled by chromosome 1B is in accord with the fact that the B genome of polyploid wheats, which has been described as a "modified genome" (Zohary and Feldman 1962), is highly variable cytologically and genetically. This variation is presumably due to interspecific and intergeneric hybridizations which took place on the polyploid level.

Chromosomal group 1D contains more bands than chromosomal group 1A, yet, in group 1D there is less variation in the number of bands per line than in the other two groups.

Classification into subgroups

The protein bands in both fractions of each chromosomal group were further divided into several distinct

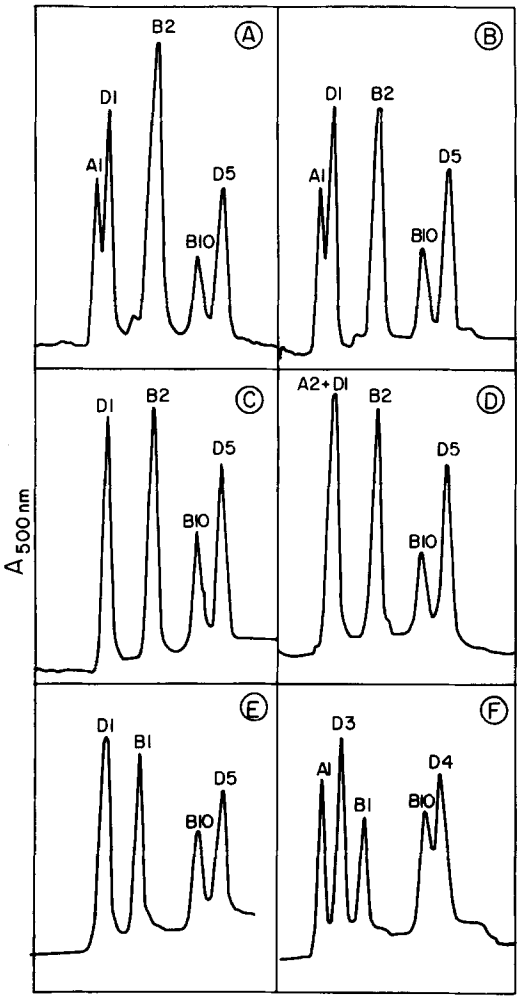

Fig. 5. The densitometer tracings of HMW glutenin subunits from several representative cultivars of hexaploid wheat. Note the variation in bands B1 and B2. The cultivars are: *A* 'TAA36'; *B* 'Red Egyptian'; *C* 'Chinese Spring'; *D* 'Centurk'; *E* 'Ribeiro'; *F* 'Hope'

subgroups on the basis of their occurrence in the various lines; subunits belonging to a particular subgroup never appear together in the same line. Only in a hybrid do such bands appear together in a quantitative endospermic (3n) ratio of 2:1, depending on the direction of the cross. Therefore, protein bands of one subgroup may represent the products of different alleles of one genetic locus. This sub-division conforms to the electrophoretic mobility of the bands in one-dimensional SDS PAGE.

HMW glutenins. On the basis of the criteria described above, chromosomal group 1B contains three different subgroups namely, Glt-B1, Glt-B2 and Glt-B3 (Table 1). Subgroup Glt-B1 contains six slowly migrating subunits (bands B1 to B6); subgroup Glt-B2 contains three rapidly migrating subunits (bands B7 to B9) while subgroup Glt-B3 contains the three most rapidly migrating subunits of this chromosomal group (bands B10 to B12). Chromosomal group 1D contains two subgroups, namely, Glt-D1 which contains the three slowly migrating subunits (D1 to D3) and Glt-D2 which contains the three rapidly migrating subunits of this chromosomal group (bands D4 to D6). Chromosomal group 1A contains two subgroups, Glt-A1 and Glt-A2, the first of which contains the three slowly migrating subunits (bands A1 to A3) while the latter contains the two rapidly migrating subunits of this chromosomal group (bands A4 and A5). Similar sub-

Table 1. The assignment of the HMW glutenin and gliadin bands into chromosomal groups and subgroups

Chromosomal groups		Chromosomal subgroups	Band designation	MW (KD)
A	*Glutenins*			
	Chromosome 1B	Glt-B1	B1	102
			B2	98
			B3	97
			B4	95.5
			B5	94
			B6	92
		Glt-B2	B7	91
			B8	90
			B9	88
		Glt-B3	B10	86
			B11	85.5
			B12	84
	Cromosome 1D	Glt-D1	D1	108
			D2	107.5
			D3	106
		Glt-D2	D4	84
			D5	80
			D6	78
	Chromosome 1A	Glt-A1	A1	114
			A2	107
			A3	105.5
		Glt-A2	A4	103
			A5	100
B	*Gliadins*			
	Chromosome 1B	Gld-B1	B21	68
			B22	67
			B23	66
			B24	63
		Gld-B2	B25	62
		Gld-B3	B26	61
		Gld-B4	B27	59.5
			B28	59
		Gld-B5	B29	57.5
		Gld-B6	B30	52
	Chromosome 1D	Gld-D1	D11	57
			D12	56
		Gld-D2	D13	54.5
		Gld-D3	D14	53.5

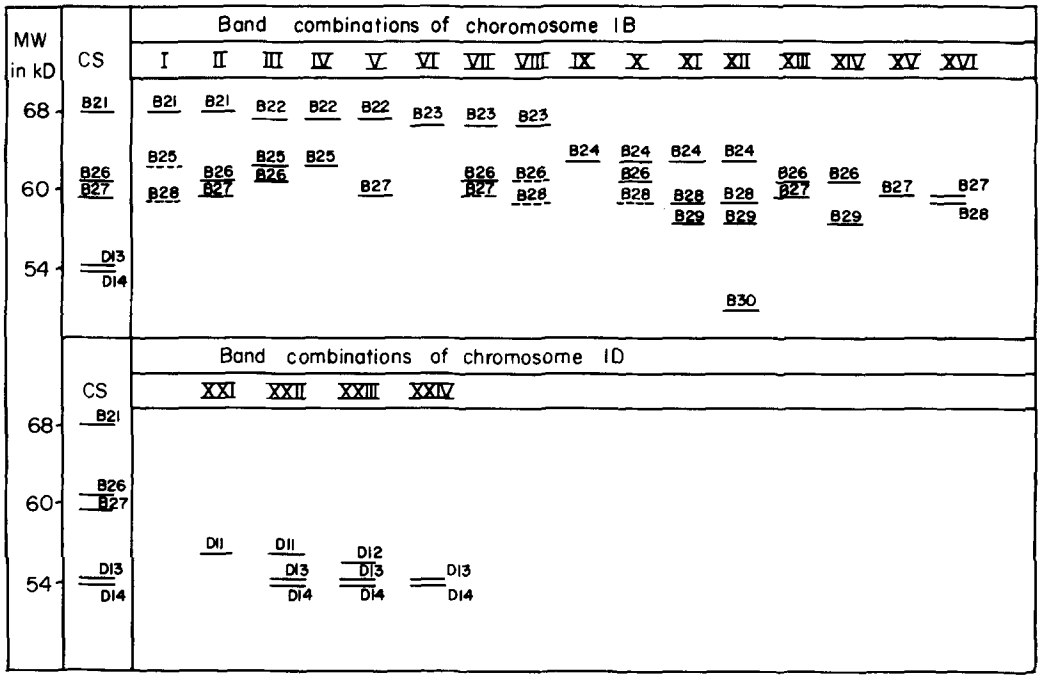

Fig. 6. Schematic diagram of the various band combinations which were detected in hexaploid wheat for HMW gliadin subunits controlled by chromosomes *1B* (*I – XVI*) and *1D* (*XXI – XXIV*). The HMW gliadin subunits controlled by these chromosomes in the cultivar 'Chinese Spring' (*CS*) as well as their *MW* are indicated on the left of the diagram

grouping of the HMW glutenin bands was also suggested by Payne et al. (1981b) on the basis of their electrophoretic mobility. They described two subgroups in each of the chromosomal groups 1B and 1D and only one subgroup in chromosomal group 1A. However, the appearance in several lines of three HMW glutenin bands controlled by chromosome 1B and of two bands controlled by chromosome 1A (Figs. 1–4) imply a larger number of subgroups in chromosomal groups 1B and 1A than that suggested by Payne et al. (1981b). Three bands controlled by chromosome 1B were also detected in a combined iso-electric focusing and SDS two-dimensional PAGE (Holt et al. 1981).

HMW gliadins. Six different subgroups were detected in chromosomal group 1B, namely Gld-B1 (bands B21 to B24), Gld-B2 (band 25), Gld-B3 (band 26), Gld-B4 (bands B27 and B28), Gld-B5 (band B29) and Gld-B6 (band 30) (Table 1). Similarly, the four bands of chromosomal group 1D were divided into the following three subgroups: Gld-D1 (bands D11 and D12), Gld-D2 (band D13) and Gld-D3 (band D14) (Table 1).

Protein bands belonging to different subgroups of one chromosomal group may represent either products of different genes, or alternatively, products of a single gene that underwent different degrees of post-translational modifications. The latter possibility seems unlikely since preliminary evidence from peptide mapping and amino acid analysis of several HMW glutenin bands revealed structural differences between protein subunits of different subgroups of the same chromosome (Galili and Feldman, unpublished). It therefore seems more plausible that these bands represent products of different genes and that bands of each chromosomal subgroup represent allelic products of one genetic locus. Actually, Payne et al. (1980) presented evidence indicating that the variation in several HMW glutenin subunits amongst varieties is due to allelic genes. Accordingly, chromosome 1B probably carries up to three different loci that code for HMW glutenin subunits (Glt-B1, Glt-B2 and Glt-B3) and up to six loci for HMW gliadin subunits (Gld-B1 to Gld-B6); chromosome 1D carries two loci that code for HMW glutenin subunits (Glt-D1 and Glt-D2) and up to three different loci which code for HMW gliadins (Gld-D1 to Gld-D3); chromosome 1A carries up to two loci coding for HMW glutenins (Glt-A1 and Glt-A2) and zero loci coding for HMW gliadin subunits. Altogether, in contrast to previous reports, our data are consistent with seven HMW glutenin genes and nine HMW gliadin genes in hexaploid wheat.

About 150 different collections of the diploid species which are closely related to the ancestors of polyploid wheat, namely, *T. monococcum*, *T. tauschii* and species of section *Sitopsis* were analyzed in our laboratory (Galili and Feldman, unpublished). Most of

them possess two bands of HMW gutenins and several bands of HMW gliadins however several collections of wild forms of *T. monococcum, T. speltoides* and *T. sharonensis* possess three HMW glutenin bands. In most hexaploid lines chromosome 1B also codes for two HMW glutenin bands but in several lines it codes for three such bands. Thus, the third gene of chromosome 1B may have been derived from a diploid line possessing three HMW glutenin bands. Alternatively, it may have been evolved on the polyploid level either by duplication or via introgression from another species.

On the other hand, the SDS PAGE pattern of both HMW glutenin and gliadin fractions in hexaploid wheat does not represent the total number of subunits existing in its diploid progenitors. One-fifth of the examined lines lack HMW glutenin subunits controlled by chromosome 1A while the rest possess, in equal proportions, either one or two bands. Moreover, in the fraction of HMW gliadins, none of the hexaploid lines possess any detectable subunit controlled by chromosome 1A. Similarly, four of the examined lines possess only one HMW glutenin subunit controlled by chromosome 1B which belong either to subgroup Glt-B1 or to Glt-B2 (Fig. 4). Regarding the HMW gliadins, in spite of the fact that there are six different subgroups controlled by chromosome 1B, most of the lines examined possess two to four subunits of this fraction. In contrast to chromosomes 1A and 1B, all the hexaploid lines examined possess, invariably, two subunits of HMW glutenins controlled by chromosome 1D. There is some variation, however, in the number of HMW gliadin bands controlled by this chromosome. The above data indicate that a considerable process of diploidization of endosperm protein genes has occurred on the polyploid level. It is also obvious that this process has been non-random, affecting the genes of both fractions controlled by chromosome 1A much more than those of 1B while those of 1B were more affected than the genes of 1D. A similar non-random process of diploidization of gliadins and of total endosperm proteins was observed in the cultivar 'Chinese Spring' using various high resolution, one and two-dimensional, gel electrophoresis systems (Wrigley and Shepherd 1973; Brown et al. 1979; Galili and Feldman 1983).

The high degree of diploidization of genes controlling endosperm proteins in hexaploid wheat is in sharp contrast to the relatively small extent of diploidization which was noticed in genes controlling enzymes. Hart (1979) described in hexaploid wheat only one out of sixteen enzymes studied in which diploidization was detected. This may be accounted for by assuming that heterozygosity between homoeologous loci may provide greater flexibility regarding enzymatic activity while such a genetic make-up provides no special advantage for storage proteins. Moreover, in the latter case, activity of all the homoeoalleles may result in overproduction and lack of efficiency.

The various chromosomal subgroups (genes) contain different numbers of bands. Assuming that each differentially migrating band in a given subgroup represents the product of a distinct allele then the number of such alleles varies from one to six in the various genes. The slowly migrating subgroup of the HMW glutenins of chromosome 1B, namely, Glt-B1, not only comprises the largest number of bands but also exhibits the widest range in MW.

Occurrence of endosperm proteins in band combinations

As was previously noticed (Lawrence and Shepherd 1980; Payne et al. 1981 b), subunits of different subgroups (genes) which belong to the same chromosomal group and appear together in a particular line form a specific band combination. The participation of the subunits in each band combination was found to be non-random in both HMW glutenin and gliadin fractions.

HMW glutenins. Fifteen different band combinations were detected in chromosomal group 1B, as compared with five and six band combinations found in chromosomal groups 1D and 1A, respectively (Fig. 4). As was pointed out by Lawrence and Shepherd (1980), in many band combinations of chromosomal groups 1B and 1D, heavier subunits of the slowly migrating subgroups tend to associate with lighter subunits of the rapidly migrating subgroups. This tendency is exemplified by band combinations b and m of chromosomal group 1B and band combinations q and t of chromosomal group 1D (Fig. 4). The nature of this non-random association is not known.

The participation frequency of the different subunits forming the various band combinations is not equal. This is particularly evident in the bands of chromosomal group 1B in which, for example, subunit B1 appears in only one combination while B3 appears in four different ones (Fig. 4).

The various band combinations (Fig. 4) occur in different frequencies in the various lines. While the combinations b and h of chromosome 1B and q and t of chromosome 1D are the most frequent in the commercial lines, the combination 1 of chromosome 1B and q of chromosome 1D are the most frequent in the land races.

HMW gliadins. The ten subunits of chromosomal group 1B form 16 different band combinations while the four subunits of chromosomal group 1D form four different such combinations (Fig. 6). The frequency of

participation of the different subunits in the various band combinations of chromosomal groups 1B and 1D is different, e.g., while band B21 appears only in two band combinations B26 appears in seven combinations. Moreover, the assembly of the subunits in each band combination seems to be non-random.

Band combinations of the different chromosomal groups in both fractions tend to associate at random with each other in the commercial lines. In the land races, however, these associations were significantly non-random, at the 0.05% level, as tested by χ^2. This is presumably due to the fact that the land races represent repetitions of a small number of genotypes.

The number of band combinations found in the HMW glutenins and gliadins of hexaploid wheat (data of Lawrence and Shepherd 1980; Payne et al. 1981 b; as well as those presented in this paper) is much lower than the expected number of such combinations on the basis of random association. The smaller number of band combinations indicates that the different genes in each fraction are organized in closely linked clusters. Such close linkage was demonstrated by Lawrence and Shepherd (1981) and by Payne et al. (1981 a) for several HMW glutenin subunits. However, Lawrence and Shepherd (1980) have shown that some missing phenotypes expected for subunits derived from chromosome 1D in hexaploid wheat do exist in *T. tauschii*, the diploid donor of the D genome. This may result from mutations or recombinations on the diploid level which does not or rarely occur in the hexaploid level. One hexaploid cultivar (Fig. 1, lane n and Fig. 4 phenotype p) was exceptional in possessing a pattern of HMW glutenins controlled by chromosome 1D which may be a result of crossing-over between the two band combinations q and t. This line possesses subunits D1 and D4 while most of the other lines possess either subunits D1 and D5 or D3 and D4.

In the commercial lines, band combinations of HMW glutenins or gliadins controlled by the three chromosomes tend to associate at random with each other. The same is true for the association of band combinations of HMW glutenins with those of gliadins controlled by the same chromosome (Lawrence and Shepherd 1981). These two groups of genes which are located on the long and short arms of chromosomes of homoeologous group 1 (Brown et al. 1979; Lawrence and Shepherd 1980; Galili and Feldman 1983) were shown to recombine in high frequency (Galili and Feldman, unpublished).

Quantitative variation of endosperm proteins

HMW glutenins. The densitometer tracings of six representatives of the lines studied are given in Fig. 5. The calculated relative peak areas of the different HMW glutenin bands from these lines are presented in Table 2. The results are presented as follows:

Table 2. Comparison of the relative peak areas of the various HMW glutenin subunits derived from the lines presented in Fig. 5. The relative peak areas were normalized to dry kernel weight

Patterns according to Fig. 5	The relative peak area weight of the specified HMW glutenin subunits (in mg)							
	A1	D1	D3	B1	B2	B10	D4	D5
A	21	30			68	19		28
B	20	33			37	19		29
C		34			39	20		30
E		35		24		17		21
F	20		31	16		23	30	

Quantitative variation of the same band in the different lines

The staining intensity of each of the bands controlled by chromosomes 1D and 1A and of those belonging to the subgroups Glt-B2 and Glt-B3 of chromosome 1B was relatively similar in the various lines studied (Table 2 and Fig. 5). On the other hand, two of the slowly-migrating bands belonging to subgroup Glt-B1 of chromosome 1B, namely B1 and B2, each exhibited variation in their staining intensity in the different lines studied. This is evident by comparing the peak area of band B2 in tracings A and B or the peak area of band B1 in tracings E and F in Fig. 5 and Table 2. These differences in the staining intensity of bands B1 and B2 amounted to 67% and 57%, respectively.

Quantitative variation within subgroups

Generally, the staining intensity of the different bands belonging to the same subgroup is relatively similar. Only the bands of subgroup Glt-B1 differ markedly from each other in their staining intensity. This variation amounts, in certain cases, to more than four-fold (compare the peak area of band B2 in tracing A with that of band B1 in tracing F of Fig. 5 and Table 2).

Quantitative variation between subgroups of the same chromosomal group

In addition to the similarity of staining intensity in most bands within each subgroup, the staining intensity of bands belonging to the two subgroups of chromosome 1D, namely, Glt-D1 and Glt-D2, is, more or less, similar. In other words, the amount of protein in each band of chromosome 1D is similar to that of the other bands. In chromosome 1A, however, bands belonging to the two different subgroups (Glt-A1 and Glt-A2), differ considerably in their staining intensity, i.e., the bands of the slowly-migrating subgroup Glt-A1 are

heavily stained while those of the rapidly-migrating subgroup, Glt-A2, appear in all cases to be very faint. The staining intensity of band A2 of subgroup Glt-A1 could not be measured since it was not resolved from band D1 or D2, in the densitometer tracings (Fig. 5D). Similarly, also in chromosome 1B, the staining intensity of most bands composing the slowly-migrating subgroup Glt-B1 is much higher than that of the bands of the more rapidly-migrating subgroups, Glt-B2 and Glt-B3 (Table 2 and Fig. 5).

Quantitative variation between subgroups of different chromosomal groups

The relative staining intensity of bands belonging to the subgroup Glt-A1 of chromosome 1A is equal to that of bands belonging to the rapidly-migrating subgroups of chromosome 1B (Glt-B2 and Glt-B3) and is about two-thirds of that of bands composing either of the subgroups of chromosome 1D. The relative staining intensity of bands composing subgroups Glt-B1 varies from twice as much down to one-half of bands controlled by chromosome 1D.

HMW gliadins. No specific order could be detected in this fraction. However, subunits of chromosomal group 1B showed the widest range of quantitative variation. Some of the subunits controlled by this chromosome were very faint in some lines while heavily stained in others (compare, for example, bands B26 and B27 in Fig. 1, lanes a and c).

The genetic and molecular nature of variation of endosperm proteins in wheat is yet unknown. Evidence from amino terminal sequence of gliadins (Bietz et al. 1977; Autran et al. 1979; Shewry et al. 1980) support the hypothesis that the different genes in each fraction were derived from one ancestral locus. Duplication or amplification of the ancestral gene should have occurred very early in the evolution of wheat since most of the diploid species of this group already possess two or more subunits in both fractions of HMW glutenins and gliadins (Galili and Feldman, unpublished).

Possible applicable contribution

The wide extent of variability of endosperm protein genes in wheat as determined from the SDS PAGE presented in this report clearly points to the applicable advantage in using this specific PAGE for the identification of different lines and homogeneity within the lines in breeding programs. In addition, these results may contribute to our knowledge on the correlation between electrophoretic patterns of HMW glutenins and gliadins with several traits such as grain protein content, amino acid composition, bread making quality and others.

It is important to note that a much wider variation of HMW glutenins exists in wild populations of wheat such as *T. tauschii* (Lawrence and Shepherd 1980) and *T. turgidum* var. 'dicoccoides' (Galili and Feldman, unpublished) and that these variations may be used in improving desirable characteristics of wheat.

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