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Genetic control of endosperm proteins in wheat

3. Allocation to chromosomes and differential expression of high molecular weight glutenin and gliadin genes in intervarietal substitution lines of common wheat *

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Summary. Total endosperm proteins extracted from both several common wheat cultivars and some intervarietal substitution lines derived from them were fractionated according to their molecular weight in a high resolution one-dimensional gel electrophoresis. The four donor cultivars and the recipient one – 'Chinese Spring', possessed differentially migrating protein bands in the fractions of high molecular weight (HMW) glutenins and gliadins. Several of these bands were identified for the first time in this study. By utilizing intervarietal substitution lines the control of the HMW glutenins and gliadins by chromosomes of homoeologous group 1 was either reaffirmed or, for the new bands, established. Several HMW gliadin subunits showed a considerable variation in their staining intensity in the intervarietal substitution lines indicating that their expression was dependent on the genetic background.

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

Introduction

The availability of several series of intervarietal substitution lines in common wheat, in which individual chromosome pairs of a recipient parent are substituted for by the homologous chromosome pair derived from a donor line, enabled studies on the chromosomal location and phenotypic expression of many endosperm protein genes (for review see Wall 1979; Payne et al. 1982).

Previous studies of endosperm proteins extracted from intervarietal substitution lines, using one-dimensional sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE), were restricted mainly to the fraction of HMW glutenins due to the relatively poor resolution of the gliadins (Lawrence and Shepherd 1980; Payne et al. 1980). A more detailed analysis of gliadins from these lines was achieved by fractionating total endosperm proteins in two-dimensional PAGE (Brown et al. 1981). Recently, we have reported on a high resolution one-dimensional SDS PAGE (Galili and Feldman 1983 a, b) which reveals a large size variation in both HMW glutenin and gliadin subunits in common wheat. This technique was employed in this work in order to determine the chromosomal control of HMW glutenins and, particularly, of HMW gliadins in different common wheat cultivars, by using their intervarietal substitution lines.

Materials and methods

Plant material

Four series of intervarietal substitution lines, their donor varieties, 'Hope', 'Timstein', 'Cheyenne' and 'Thatcher', as well as the recipient variety 'Chinese Spring', were studied. All the substitution lines were backcrossed at least seven times to the recipient variety, except for the substitution lines of 'Thatcher', namely, 'CS' ('Thatcher') which were backcrossed to 'Chinese Spring' only four times. The substitution line 'CS' ('Cheyenne-2B') was not available for analysis.

Seeds of the varieties 'Chinese Spring', 'Hope', 'Timstein' and 'Thatcher', as well as of their intervarietal substitution lines, were kindly provided by E. R. Sears. Seeds of the variety 'Cheyenne' and the substitution lines of 'CS' ('Cheyenne') were kindly provided by R. Morris.

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Methods

Extraction of total endosperm proteins, fractionation by SDS PAGE, staining and destaining of the gels, the use of MW protein markers and quantitative analysis of specific bands from densitometer tracings were as previously described (Galili and Feldman 1983a, b).

Results

Endosperm proteins extracted from the recipient line 'Chinese Spring', the four donor cultivars and their intervarietal substitution lines were analysed by SDS PAGE. The PAGE patterns of all four donor cultivars differed from that of 'Chinese Spring' (Figs. 1–4, lanes f, g). The designation of the various subunits in the SDS PAGE patterns is in accordance with our previous report (Galili and Feldman 1983b). The PAGE pattern of most substitution lines did not differ markedly from that of 'Chinese Spring', except for the intervarietal substitution lines for chromosomes of homoeologous group 1 which are shown in Figs. 1–4, lanes b–d. The chromosomal control of the various subunits was inferred from the presence or absence of particular bands in the gel patterns of the various intervarietal substitution lines. These results are summarized for homoeologous group 1 in Table 1.

In addition to confirming previously identified bands, several HMW glutenins (the faint bands A4 and A5) and gliadins (B24, B28, B29, B30, B31, D11 and D15) were identified for the first time in the present study and their chromosomal control established (Table 1).

All donor cultivars, but not 'Chinese Spring', possessed a slowly migrating HMW glutenin band controlled by chromosome 1A. In order to clarify the nature of the absene of this subunit in 'Chinese Spring', the reciprocal cross 'Chinese Spring' × 'CS' ('Hope-1A') was performed. All F_1 grains possessed this HMW glutenin band (A1) of 'Hope' with staining intensity reduced by about 1/3 or 2/3 of its original staining intensity as appeared in 'Hope' (Fig. 5). This differential reduction in staining intensity was dependent on the direction of the cross, as could be expected from the nature of the endosperm tissue. Analysis of F_2 grains of this cross revealed a 3 : 1 ratio between grains possessing or lacking band A1. These findings indicate that the absence of subunit A1 in 'Chinese Spring' is not due to genetic suppression.

The intervarietal substitution line 'CS' ('Timstein-1D') lacked the rapidly migrating HMW glutenin band D5 controlled by chromosome 1D of 'Timstein' (Fig. 2b). This observation has been discussed elsewhere (Galili and Feldman 1984).

Table 1. Chromosomal control of HMW glutenins and gliadins as inferred from the SDS PAGE migration pattern of certain intervarietal substitution lines[a]

Chromosome	Line	Subunits pattern		Reference
		HMW glutenins	Gliadins	
1A	'Chinese Spring'	None	None	Figs. 1–4; e, g
	'Hope'	A1, A4 (faint)*	None	Fig. 1, d
	'Timstein'	A2[a]	None	Fig. 2, d
	'Cheyenne'	A2, A5 (faint)*	None	Fig. 3, d
	'Thatcher'	A2[a], A5 (faint)*	None	Fig. 4, d
1B	'Chinese Spring'	B2, B10	B21, B26, B27	Figs. 1–4; e, g
	'Hope'	B1, B10	B23, B26[a] (faint)	Fig. 1, c
	'Timstein'	B6, B9, B12[a] (faint)	B23, B28 (faint)*	Fig. 2, c
	'Cheyenne'	B3, B12	B23, B28 (faint)*	Fig. 3, c
	'Thatcher'	B3[a], B12[a]	B24*, B28*, B29*, B30*, B31*	Fig. 4, c
1D	'Chinese Spring'	D1, D5	D13, D14	Figs. 1–4; e, g
	'Hope'	D3, D4	D13, D14	Fig. 1, b
	'Timstein'	D1, D5	D13, D14	Fig. 2, b[b]
	'Cheyenne'	D3, D4	D11*, D15*	Fig. 3, b
	'Thatcher'	D3, D4	D11*, D15*	Fig. 4, b[c]

[a] Identification and chromosomal control of subunits marked by an asterisk (*) were determined in this work; those underlined were previously identified in different cultivars; unmarked subunits were previously identified in the same cultivar

[b] The absence of band D5 of 'Timstein' in intervarietal substitution line 'CS' ('Timstein-1D') is discussed in detail in Galili and Feldman (1984)

[c] The assumed substitution line 'CS' ('Thatcher-1D') was found to be contaminated

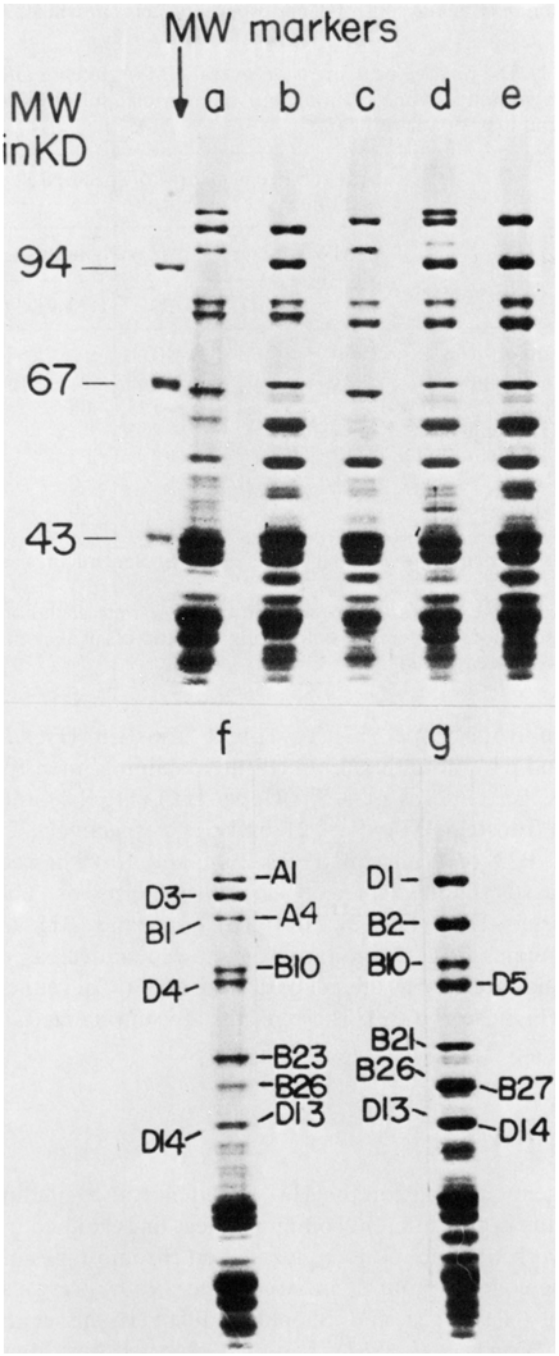

Fig. 1. The SDS PAGE migration pattern of total endosperm proteins extracted from the donor cultivar 'Hope', the recipient cultivar 'Chinese Spring' and three of their intervarietal substitution lines: *a, f* 'Hope'; *b* 'CS' ('Hope-1D'); *c* 'CS' ('Hope-1B'); *d* 'CS' ('Hope-1A'); *e, g* 'Chinese Spring'. Protein markers of known MW were fractionated in a parallel lane with MWs indicated on the *left*

The substitution line 'CS' ('Thatcher-1D') exhibited the gel pattern of 'Chinese Spring', although the donor line 'Thatcher' did not possess the HMW gliadin subunits D13 and D14 of 'Chinese Spring'. This substitution line was therefore assumed to be a contamination. Interestingly, this substitution line, as does 'CS' ('Timstein-1D'), also lacked the rapidly migrating HMW glutenin subunit controlled by chromosome 1D (Fig. 4 b).

Quantitative analysis

The staining intensity of the various subunits in each line was measured from densitometer tracings of each lane of the gels. Measurements were done by normalizing the area under each band to the area under

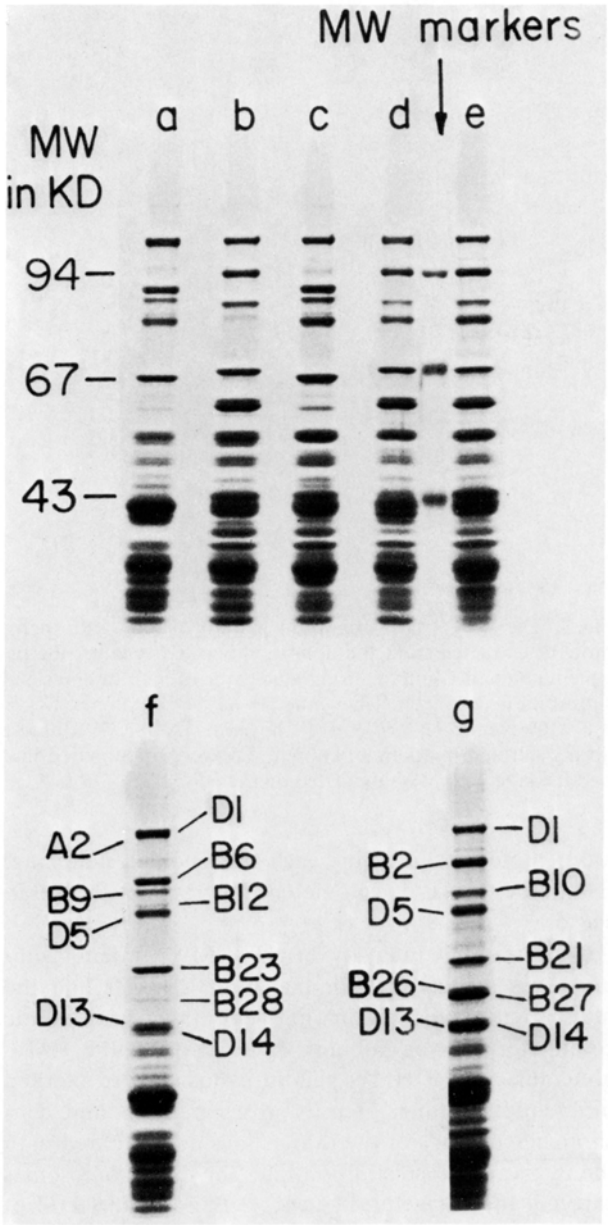

Fig. 2. The SDS PAGE migration pattern of total endosperm proteins extracted from the donor cultivar 'Timstein', the recipient cultivar 'Chinese Spring' and three of their intervarietal substitution lines: *a, f* 'Timstein'; *b* 'CS' ('Timstein-1D'); *c* 'CS' ('Timstein-1B'); *d* 'CS' ('Timstein-1A'); *e, g* 'Chinese Spring'. Protein markers of known MW were fractionated in a parallel lane with MWs indicated on the *left*

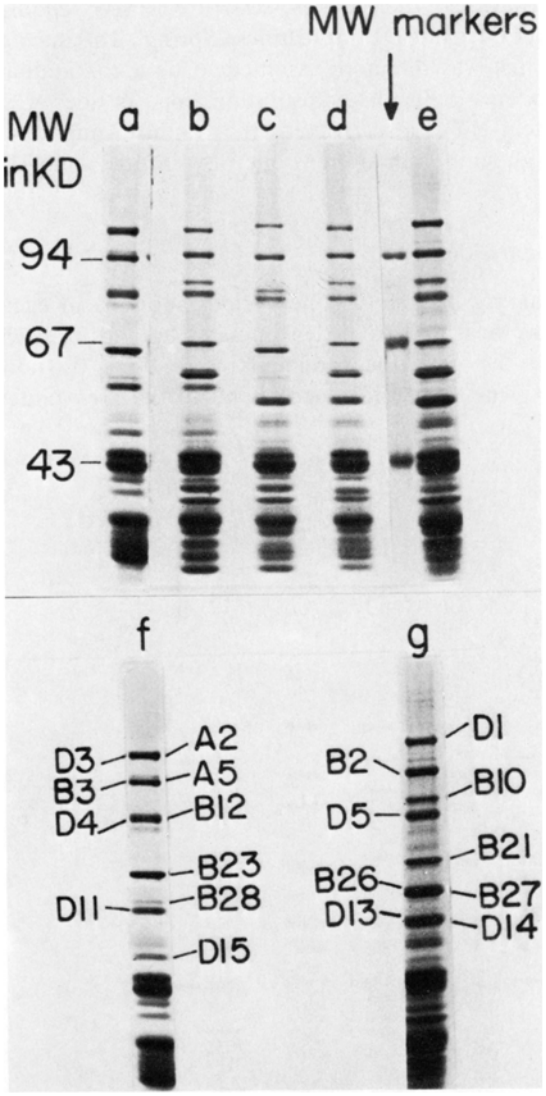

Fig. 3. The SDS PAGE migration pattern of total endosperm proteins extracted from the donor cultivar 'Cheyenne', the recipient cultivar 'Chinese Spring' and three of their intervarietal substitution lines: *a, f* 'Cheyenne'; *b* 'CS' ('Cheyenne-1D'); *c* 'CS' ('Cheyenne-1B'); *d* 'CS' ('Cheyenne-1A'); *e, g* 'Chinese Spring'. Protein markers of known MW were fractionated in a parallel lane with MWs indicated on the *left*

total protein bands in each densitometer tracing. Examples of six densitometer tracings are shown in Fig. 6.

The staining intensity of the HMW glutenin subunits was similar in both the donor cultivar and the intervarietal substitution line for the chromosome coding for a given subunit. In contrast to the HMW glutenins, several HMW gliadin bands showed marked differential staining intensity in gel patterns and densitometer tracings of the donor lines as compared with the intervarietal substitution lines for the chromosomes carrying their structural genes: 1) Bands D13+D14 of the cultivars 'Hope' (Fig. 1 a, f) and 'Timstein' (Figs. 2 a, f and 6 b) more than doubled their staining intensity in the gel patterns of 'CS' ('Hope–1D') (Fig. 1 b) and 'CS' ('Timstein-1D') (Figs. 2 b and 6 c), respectively; 2) Band B23 of 'Timstein' (Figs. 2 a, f and 6 a) showed increased staining intensity in gel patterns of 'CS' ('Timstein-1B') (Figs. 2 c and 6 b); 3) Band B23 of 'Cheyenne' (Figs. 3 a, f and 6 d) showed a decreased staining intensity in the gel pattern of 'CS' ('Cheyenne-1B') (Figs. 3 c and 6 e). These results are summarized in Table 2.

Table 2. The relative peak areas of several HMW glutenin and gliadin subunits extracted from four intervarietal substitution lines and their donor cultivars[a]

Line	The relative peak area of specified subunits[b]				
	HMW glutenins			HMW gliadins	
	B3	B6	D3	B23	D13+D14
'Cheyenne'	5.00			10.60	
'CS' ('Cheyenne-1B')	4.27			5.69	
'Timstein'		4.99		5.11	3.69
'CS' ('Timstein-1B')		5.19		7.98	
'CS' ('Timstein-1D')					8.65
'Hope'			4.00		3.50
'CS' ('Hope-1D')			4.40		9.50

[a] Measurements are based on the densitometer tracings of Fig. 6

[b] The relative peak areas specified in the table were each normalized to total proteins by calculating the ratio of (peak area/total peak area) × 100

Discussion

The genes coding for the HMW glutenin and gliadin subunits in the different common wheat lines studied in this work, were found to be located on chromosomes of homoeologous group 1 in accordance with previous reports (Lawrence and Shepherd 1980; Payne et al. 1980; Brown et al. 1981). It is first reported here that the faint HMW glutenin subunits A1 and A5 are controlled by chromosome 1A; the HMW gliadin subunits B24, B28, B29, B30 and B31 by chromosome 1B and the HMW gliadin subunits D11 and D15 by chromosome 1D.

Our analysis concerning the HMW gliadins obtained by the one-dimensional SDS PAGE differs in some cases from those of Brown et al. (1981). For instance, band D11 of the cultivar 'Cheyenne' was found to replace the double band D13+D14 of 'Chinese Spring' in the gel pattern of 'CS' ('Cheyenne-1D') (Fig. 3 a, b, e); no similar differences between these two lines have been previously observed.

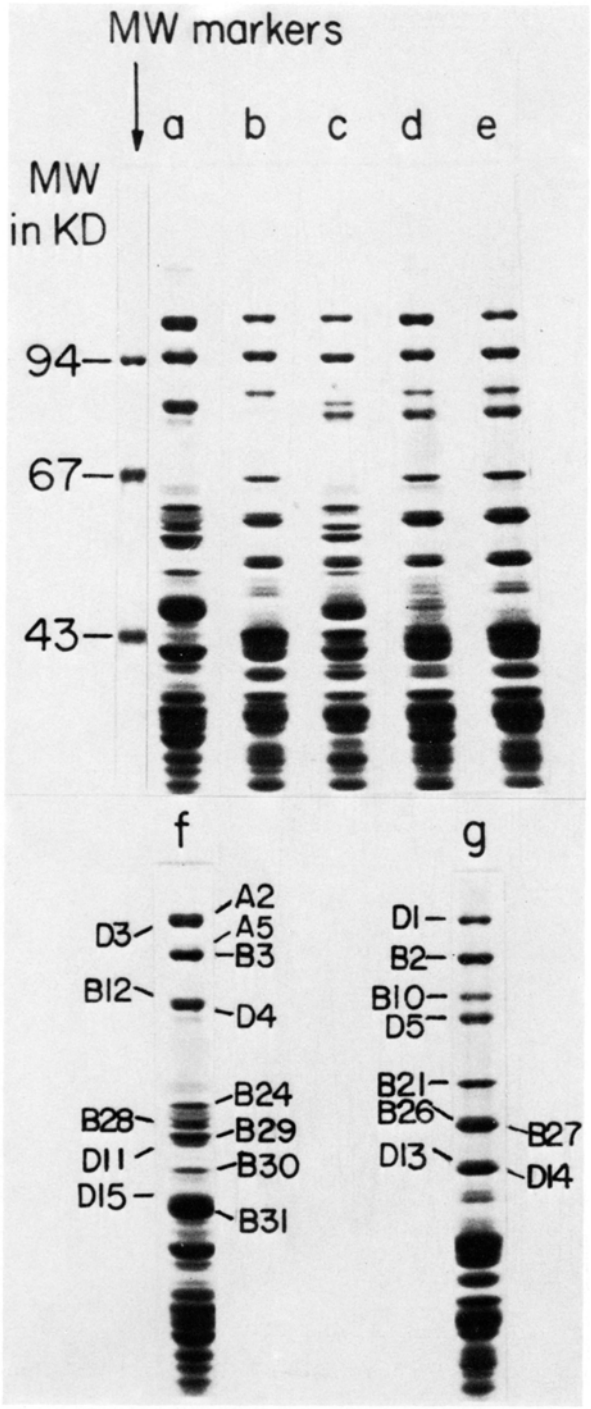

Fig. 4. The SDS PAGE migration pattern of total endosperm proteins extracted from the donor cultivar 'Thatcher', the recipient cultivar 'Chinese Spring' and three of their intervarietal substitution lines: *a, f* 'Thatcher'; *b* 'CS' ('Thatcher-1D'); *c* 'CS' ('Thatcher-1B'); *d* 'CS' ('Thatcher-1A'); *e, g* 'Chinese Spring'. Protein markers of known MW were fractionated in a parallel lane with MWs indicated on the *left*

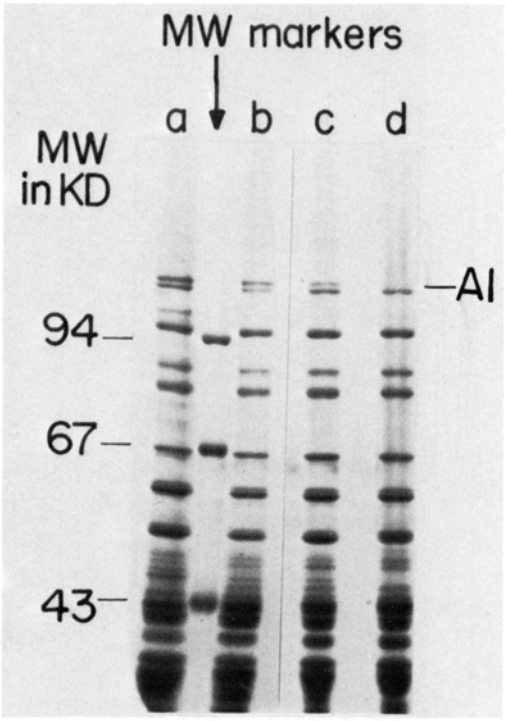

Fig. 5. The SDS PAGE pattern of total endosperm proteins extracted from: *a* 'CS' ('Hope-1A'); *b* F_1 grain of the cross 'CS' ('Hope-1A') × 'Chinese Spring'; *c* F_1 grain of the reciprocal cross 'Chinese Spring' × 'CS' ('Hope-1A'); *d* 'Chinese Spring'. Protein markers of known MW were fractionated in a parallel lane with MWs indicated on the *left*

Diploidization of endosperm protein genes in hexaploid wheat

Several of the lines studied in this report possess a reduced number of HMW glutenin bands controlled by genome A as compared with those of genomes B and D. Moreover, none of the lines studied possess any HMW gliadin subunit controlled by genome A but only subunits of genomes B and D. A similar massive and non-random diploidization was shown also to occur in the HMW glutenins of tetraploid wheat, *T. turgidum* (Galili and Feldman 1983c) and in other groups of endosperm proteins (Garcia-Olmedo et al. 1978) but not in genes coding for enzymes (Hart 1979). This shows the non-random nature of gene diploidization in polyploid wheats: genes coding for endosperm proteins were much more affected than genes coding for enzymes and endosperm protein genes of genome A were much more affected than those of genomes B and D.

The fact that endosperm protein genes, in contrast to most enzyme genes, are very active in producing a big mass of proteins, may provide a partial explanation for the massive, non-random diploidization of endo-

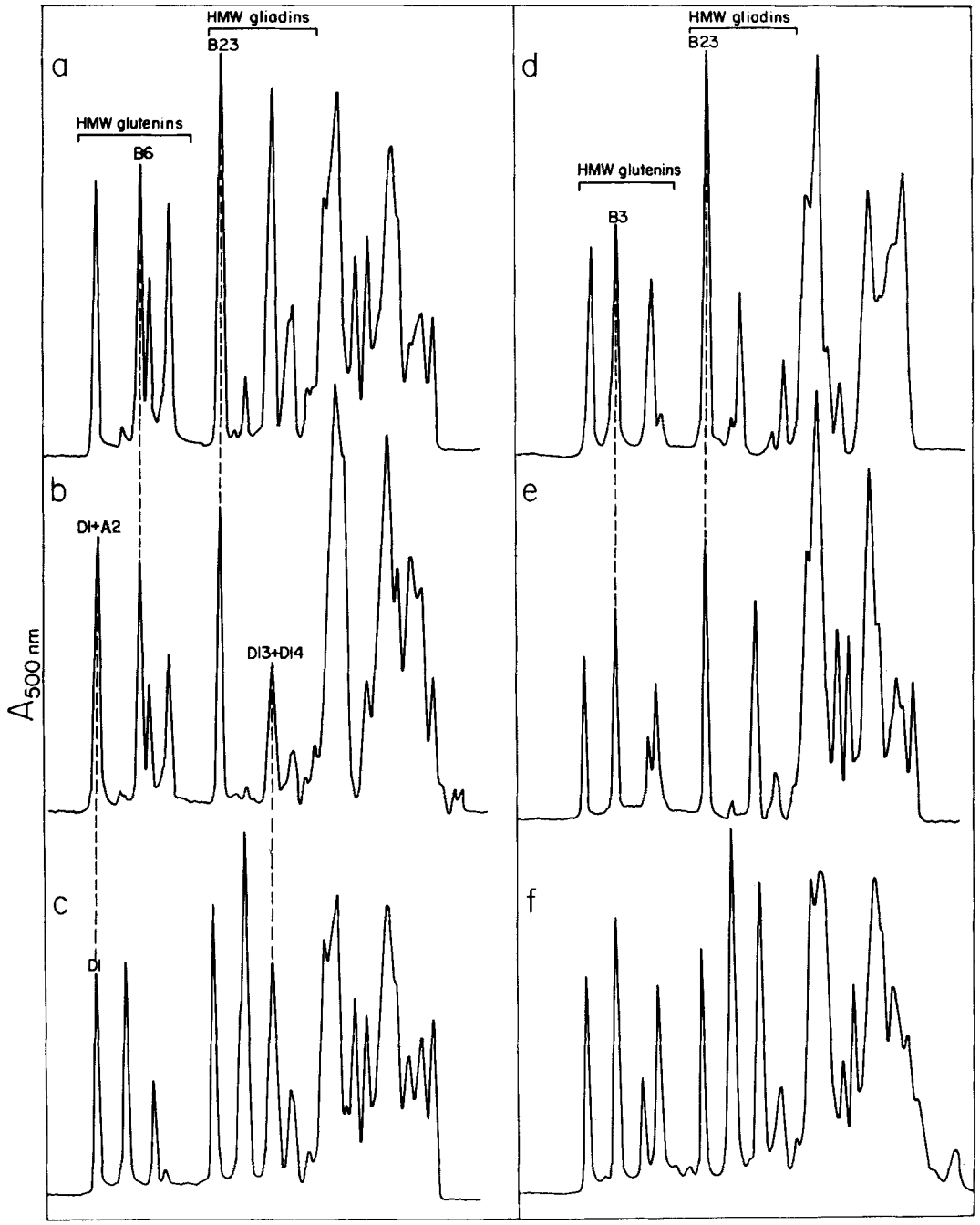

Fig. 6. Densitometer tracings of several gel patterns shown in previous figures: *a* 'CS' ('Timstein-1B'); *b* 'Timstein'; *c* 'CS' ('Timstein-1D'); *d* 'Cheyenne'; *e* 'CS' ('Cheyenne-1B'); *f* 'Chinese Spring'

sperm protein genes: multiplication of these genes in polyploid wheats may result in overproduction and lack of efficiency and thus must be balanced by a rapid silencing of the redundant genes. Another group of active genes which might suffer from overproduction and redundancy is the rRNA genes located in the nucleolar organizers. Interestingly, in the course of wheat evolution, this group of genes was also diploidized non-randomly in a similar manner to endosperm protein genes, i.e., those of genome A became inactive (Nevo 1979). It is noteworthy that both the rRNA genes and the gliadin genes are located on the short arm of chromosomes of homoeologous groups 1 and 6. The question as to whether there is any correlation between the similar chromosomal location of these two groups of genes and the similarity in their

active transcription and non-random diploidization remains to be solved. The possible role of the cytoplasm in the rapid diploidization of genome A genes, should be also considered since the cytoplasm of common wheat was derived from the diploid donor of genome B.

The analysis of F_1 and F_2 grains of the cross 'Chinese Spring' × 'CS' ('Hope-1A') showed that the lack of the HMW glutenin band A1 in 'Chinese Spring' was not due to genetic suppression but rather to a mutation or inactivation of this locus. Indeed, recent evidence has shown that DNA sequences of HMW glutenins exist in chromosome 1A of 'Chinese Spring' (Forde et al. 1983; Thompson et al. 1983).

Differential expression of endosperm protein genes

The similar staining intensity of the various HMW glutenin subunits under different genomic backgrounds indicates that their rate of expression is affected mainly by the structural genes themselves. In contrast, the differential staining intensity of several HMW gliadin subunits under various genomic background shows that their expression is affected by other factors located elsewhere in the genome. Such factors may regulate the gliadin genes directly, or indirectly through their effects on the availability of specific amino acids in the grain pool which might alter the final expression of these genes. Recently, Levy, Galili and Feldman (unpublished) found that the relative expression of HMW gliadin genes is markedly affected by N fertilizers while that of HMW glutenin genes is not.

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