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Gene-dosage compensation of endosperm proteins in hexaploid wheat *Triticum aestivum*

(posttranscriptional control/gel electrophoresis/glutenin/gliadin)

GAD GALILI, AVRAHAM A. LEVY, AND MOSHE FELDMAN

Department of Plant Genetics, The Weizmann Institute of Science, Rehovot 76 100, Israel

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ABSTRACT **Several aneuploid lines and one intervarietal substitution line of the hexaploid wheat *Triticum aestivum* ($2n = 6x = 42$; genomes AABBDD) cv. Chinese Spring were used to study the effects of different doses of chromosomes 1B, 1D, or 1A on the amount of the high molecular weight ("HMW") glutenins and gliadins of endosperm. These homeologous chromosomes carry HMW glutenin and gliadin gene clusters on their long and short arms, respectively. Increasing the dosage of chromosome 1B of Chinese Spring in plants having in their $3n$ endosperm zero or the normal three doses of the homeologue 1D, as well as in plants carrying in their endosperm one dose of 1B of the cultivar Timstein, had a dual effect: on one hand, a nonlinear increase in the amount of each subunit encoded by the chromosome whose dosage was elevated and, on the other hand, a compensating nonspecific decrease in the amount of other HMW glutenin and gliadin subunits encoded either by the homeoalleles on 1D or by the homoalleles on 1B of Timstein, respectively. Deletion of chromosome arm 1BL, which carries only a few HMW glutenin genes, had no significant effect on the amount of HMW glutenins encoded by 1DL and HMW gliadins encoded by 1DS and 1BS. However, deletion of 1BS or 1DS, each carrying many gliadin genes, caused a significant but nonspecific increase in the HMW glutenins and gliadins encoded by the remaining arms of 1B and 1D. The possible mechanism and evolutionary implications of gene-dosage compensation in polyploid wheat are discussed.**

Many structural genes of eukaryotes show a nearly linear correlation between their dosage level (i.e., copy number) and the amount of protein they produce (1). This phenomenon led Carlson (2) to propose that, in general, transcriptional control is the main rate-limiting step in the expression of eukaryotic structural genes. However, linear gene-dosage response is not always of physiological or evolutionary advantage. Several groups of genes, especially repetitive ones, may occur in superoptimal dose. For these genes, linear gene-dosage response might have resulted in overproduction and inefficiency. As expected, a nonlinear gene-dosage response—namely, gene-dosage compensation—was identified for such genes; noteworthy are the sex-linked genes of *Drosophila*, in which transcriptionally controlled dosage compensation in females has been demonstrated (3–5). Gene-dosage compensation also exists in the multigenic families of rRNA genes of several organisms (6, 7). For yeast histone genes, the levels of mRNA are not correlated with the elevated number of active genes; increased turnover of the histone transcripts indicates that gene-dosage compensation is achieved at the posttranscriptional step (8). Gene-dosage compensation has also been found for the alcohol dehydrogenase system in maize (9, 10). Schwartz (9) proposed the existence of a "super-repression mechanism" of gene expression, by which the expression of several genes may be dependent on a dosage ratio between the structural gene and a putative inducer or repressor.

The high molecular weight ("HMW") glutenins and gliadins of common wheat are encoded by multigenic families located on the long and short arms, respectively, of chromosomes of group 1 (11–13). These genes provide a most suitable system for studying gene-dosage compensation. The protein subunit encoded by each of these genes can be easily separated and identified and its relative quantity estimated. Moreover, being an allohexaploid organism ($2n = 6x = 42$; genomes AABBDD), common wheat contains these gene clusters in triplicate doses. Hence, since these genes are expressed in the $3n$ endosperm, a wider range of gene dosage can be produced for every locus and different dosage relationships between alleles can be obtained in hybrids, depending on the direction of the cross. A unique advantage of common wheat is the availability of various aneuploid lines with different dosage ratios between homologous, homeologous, and nonrelated chromosome arms (14). This enabled us to study the effect of different doses of HMW glutenin genes, HMW gliadin genes, or both on their expression or on that of other genes, either homoallelic, homeoallelic, or nonrelated. First, the effect of different doses of chromosome 1B of the common-wheat cultivar Chinese Spring on the amount of several HMW glutenin and gliadin subunits encoded by its genes was studied. This was done in the presence of zero or the normal three endosperm doses of the homeologous chromosomes 1A or 1D; the former carries inactive HMW glutenin and HMW gliadin genes, whereas the latter carries active ones. In addition, we examined the effect of increased dosage of 1B of Chinese Spring on the expression of HMW glutenin and gliadin genes of 1D of Chinese Spring as well as on that of 1B of the cultivar Timstein. We also determined the effect of deficiencies for the chromosomal arms 1AL, 1BL, 1BS, or 1DS on the expression of the HMW glutenin and gliadin genes.

MATERIALS AND METHODS

Plant Material. Seeds of the common-wheat cultivar Chinese Spring and of the following aneuploid lines of this cultivar were used: monosomic 1B (Mono 1B), tetrasomic 1B (Tetra 1B), ditelosomic 1AS (DT1AS), ditelosomic 1BS (DT1BS), ditelosomic 1BL (DT1BL), ditelosomic 1DL (DT1DL), and the nullisomic–tetrasomic lines nullisomic 1A tetrasomic 1B (N1AT1B) and nullisomic 1D tetrasomic 1B (N1DT1B). The monosomic and tetrasomic lines carry their $3n$ endosperm chromosome 1B in one (or two in rare cases) and six doses, respectively; the ditelosomic lines carry in their $3n$ endosperm the designated arm in the normal three doses and are deficient for the other arm; the nullisomic–tetrasomic lines are deficient for one pair of homologous

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Abbreviations: HMW glutenin and HMW gliadin, high molecular weight glutenin and gliadin.

chromosomes while carrying in the endosperm six doses of one of its homeologues. In addition, we used the intervarietal substitution line Chinese Spring ('Timstein 1B'), in which the pair of 1B chromosomes of Chinese Spring is replaced by the homologous pair of 1B chromosomes of the cultivar Timstein. All aneuploid lines were produced and kindly provided by E. R. Sears. All plants were grown under greenhouse conditions.

Extraction of Total Endosperm Proteins. Individual embryoless half-kernels were ground in a pestle and mortar and homogenized at a concentration of 20 mg/ml in a solution consisting of 10% (vol/vol) glycerol, 3% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 0.1% bromophenol blue, and 66.6 mM Tris·HCl (pH 6.8). The homogenate was allowed to stay at room temperature for 2 hr, incubated at 100°C for 2 min, and then centrifuged (Eppendorf) for 2 min; 10 μl of each supernatant was loaded on a separate lane of the gel.

Electrophoretic Procedure and Quantitative Analysis of Protein Bands. Preparation of $NaDodSO_4$/polyacrylamide slab gels by a modification of the procedure of Laemmli (15), fractionation conditions, staining and destaining of the gels, and quantitative analysis of protein bands from densitometer tracings were as previously described (12, 16). Under the conditions used, a linear correlation was found between the amount of protein loaded on each lane and the peak area for each band. The area of each peak (band) was measured by cutting the peak from the tracing and weighing it, and the relative area of each peak was expressed as percentage of the total peak weight.

Statistical Analysis. At least 10 seeds taken from two different plants were individually analyzed for each aneuploid line (genotype). Since no significant differences were found between plants, gels were considered as statistical blocks. Differences in the peak areas of the various bands in the different aneuploid lines were tested at a 5% level of significance, by Duncan's multiple-range test.

RESULTS

Grain-Protein Percentage. Protein percentage was determined by the semimicro-Kjeldhal procedure. No significant differences in grain-protein percentage were observed among all aneuploid lines tested, in spite of large differences in the dose of HMW glutenin and HMW gliadin genes. All aneuploid lines had about 18.5% protein and most of them differed significantly ($P < 0.05$) from Chinese Spring, which had 15.2%.

Gene-Dosage Effects of Chromosome 1B on HMW Glutenins and Gliadins Encoded by Its Own Genes and by Those of the Homeologue 1D. Fig. 1 shows examples of the $NaDodSO_4$/PAGE migration patterns of total endosperm proteins extracted from lines having in their 3*n* endosperm tissues one (Mono 1B), three (euploid), or six (Tetra 1B) doses of chromosome 1B and lines having six doses of 1B but zero dose of its homeologous chromosome 1A (N1AT1B) or 1D (N1DT1B). Densitometer tracings of these gel patterns were done, and the results of the quantitative analyses of the peak areas corresponding to individual HMW glutenin and gliadin bands are shown in Fig. 2. Both the HMW glutenin and the HMW gliadin subunits controlled by chromosome 1B of Chinese Spring—namely, the glutenin subunits B2 and B10 and the gliadin subunits B21, B26, and B27—exhibited a higher staining intensity at the elevated dosage of this chromosome. However, this positive response was not linear, especially for the gliadin subunits, as the increments of staining intensity tended to be smaller with each successive increase in the dosage of chromosome 1B from zero to one to three to six (Fig. 2). The increased staining intensity of subunits controlled by chromosome 1B was accompanied in both protein fractions by a simultaneous decrease in the staining intensity of homeologous subunits controlled by chromosome 1D of Chinese Spring—namely, the glutenin subunit D1 and the gliadin subunits D13 and D14 (Figs. 1 and 2). Subunit D5 could not be studied, since its migration position partially overlapped that of a faint band representing a subunit controlled by 1B; the latter is clearly observable in N1DT1B (Fig. 1, lane e). The reduction in intensity was at a slightly higher rate for the gliadin subunits D13 and D14 than for the glutenin subunit D1. Deletion of the homeologous chromosome 1A, which in Chinese Spring does not carry active HMW glutenin and HMW gliadin genes, at six doses of chromosome 1B, had no effect on any of the subunits studied (Figs. 1 and 2). Deletion of the homeologous chromosome 1D, which carries active HMW glutenin and gliadin genes, at six doses of chromosome 1B, had also no significant effect on the staining intensity of the HMW glutenin subunits and only a very small positive effect on the staining intensity of the gliadin subunits controlled by chromosome 1B (Figs. 1 and 2); significant increase ($P < 0.05$) was found only for bands B26 plus B27.

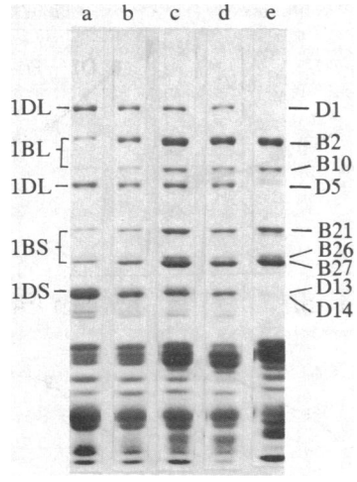

FIG. 1. $NaDodSO_4$/PAGE migration patterns of total endosperm proteins extracted from the cultivar Chinese Spring (lane b) and from four aneuploid lines derived from this cultivar: Mono 1B (lane a); Tetra 1B (lane c); N1AT1B (lane d); N1DT1B (lane e). The controlling chromosome arms and the subunit designations are given at left and at right, respectively. Bands D1–D5 represent the HMW glutenins; bands B21–D14 represent the HMW gliadins.

Gene-Dosage Effects of Chromosome 1B of Chinese Spring on HMW Glutenins and Gliadins Encoded by Genes of 1B of Timstein. In light of the above results, it was of interest to study the dosage effect of chromosome 1B of Chinese Spring on the amount of proteins coded by a homoallelic gene cluster, in this case carried on the homologous chromosome 1B of Timstein. Heterozygotes for chromosomes 1B from Chinese Spring and Timstein were produced by the crosses Chinese Spring Mono 1B × Chinese Spring ('Timstein 1B') and Chinese Spring Tetra 1B × CS ('Timstein 1B'). Mono 1B, used as female in the first cross, produces gametes having either 20 or 21 chromosomes, and the intervarietal substitution line Chinese Spring ('Timstein 1B'), used as male, produces gametes having 21 chromosomes; thus, two different types of progeny seeds, containing either 41 or 42 chromosomes, as observed in root-tip cells, could be selected from the first cross. These seeds contained in their 3*n* endosperm tissue one dose of chromosome 1B of Timstein derived from the male parent as well as zero or two doses, respectively, of chromosome 1B derived from Chinese Spring (the female parent). Seeds of the second cross with 43 chromosomes [22 of the Tetra 1B gametes plus 21 of the Chinese Spring ('Timstein 1B') gametes] had in their 3*n*

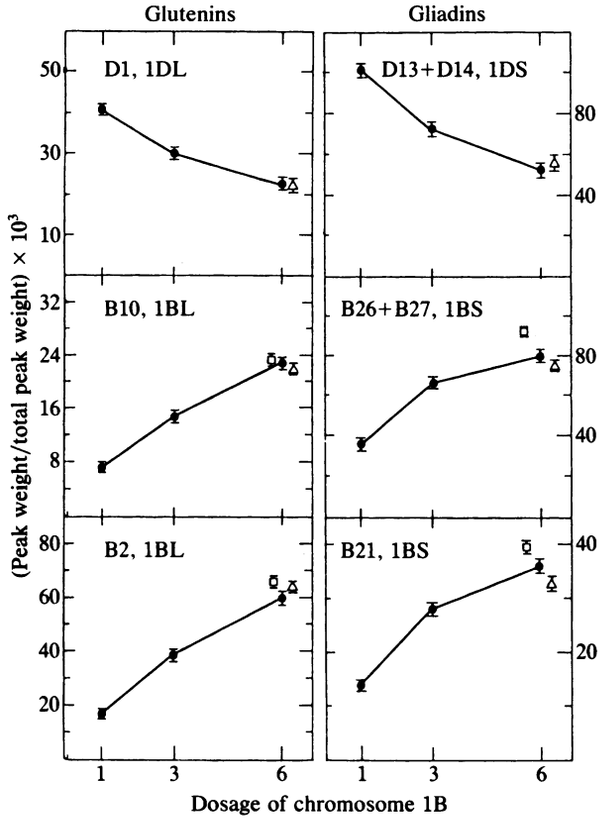

FIG. 2. Quantity of HMW glutenin (*Left*) and HMW gliadin (*Right*) subunits of the cultivar Chinese Spring as a function of dosage of chromosome 1B. Measurements are given on the basis of total protein (i.e., total peak weight). The subunit studied and its controlling chromosome are given for each curve. ●, Lines having the normal dosage of chromosomes 1D and 1A and one, three, or six doses of chromosome 1B in the 3*n* endosperm tissue (Mono 1B, Chinese Spring, and Tetra 1B, respectively). □, A line lacking chromosome 1D and having six doses of chromosome 1B (N1DT1B). △, A line lacking chromosome 1A and having six doses of chromosome 1B (N1AT1B). Bars represent SEM.

endosperm five doses of chromosome 1B: one of Timstein derived from the male parent and four of Chinese Spring derived from Tetra 1B (the female parent). Thus, the various progeny seeds from both crosses had in common one dose of chromosome 1B of Timstein but differed in having zero, two, or four doses of chromosome 1B derived from Chinese Spring.

The NaDodSO$_4$/PAGE patterns of endosperm proteins extracted from the three different types of progeny are shown in Fig. 3. Densitometer tracings of the gel patterns were done, and the results of the quantitative analyses of the peak areas of the HMW glutenins and gliadins are given in Fig. 4. The responses of the subunits controlled by chromosomes 1B of Timstein and 1D of Chinese Spring to increasing doses of chromosome 1B of Chinese Spring were very similar to those observed in the previous experiment (compare Figs. 2 and 4). Both HMW glutenin subunit B6 and HMW gliadin subunit B23, controlled by chromosome 1B of Timstein, whose dose was not changed, had a reduced staining intensity at elevated dose of 1B of Chinese Spring, similar to that of subunits D1 and D13 plus D14, controlled by chromosome 1D of Chinese Spring, as observed in the previous experiment. Thus, gene-dosage compensation showed no specificity regarding homologous or homeologous chromosomes.

Effect of Deficiency for a Chromosome Arm Carrying HMW Glutenin or Gliadin Genes on the Amount of These Proteins. Effects of deficiency for certain HMW glutenin or gliadin

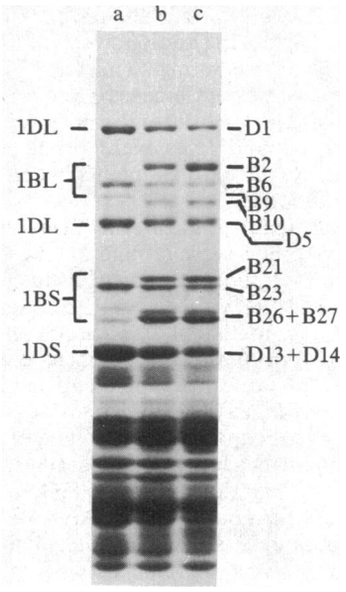

FIG. 3. NaDodSO$_4$/PAGE migration pattern of total endosperm proteins extracted from F_1 hybrids of the crosses Mono 1B × Chinese Spring ('Timstein 1B') (lanes a and b) and Tetra 1B × Chinese Spring ('Timstein 1B') (lane c). Thus, lanes a–c represent lines possessing in the 3*n* endosperm tissue one dose of chromosome 1B of Timstein and zero, two, and four doses of 1B of Chinese Spring, respectively. The HMW glutenin bands B6 and B9 are controlled by chromosome arm 1BL of Timstein, whereas B2 and B10 are controlled by 1BL of Chinese Spring. The HMW gliadin band B23 is controlled by chromosome arm 1BS of Timstein, and B21, B26, and B27 are controlled by 1BS of Chinese Spring.

genes on the amount of subunits of each of these fractions were studied in ditelosomic lines of Chinese Spring. These lines are deficient for one pair of chromosome arms, either the long arm carrying the HMW glutenin genes or the short arm carrying the HMW gliadin ones. We studied the effect of this deficiency on the expression of homeologous genes of the same protein fraction as well as of nonrelated genes of the second fraction. The NaDodSO$_4$/PAGE patterns of the ditelosomic lines studied are shown in Fig. 5. Deletion of the chromosome arms 1AL, carrying no active HMW glutenin and HMW gliadin genes, and of 1BL, carrying active HMW glutenin genes, had no significant effect on the amount of other subunits, whereas deletion of 1BS or 1DS, each carrying active gliadin genes, caused a significant increase in the amount of subunits of both protein fractions encoded by the other genes (Table 1). Also, in this case gene-dosage compensation was not specific: deficiency for the gliadin genes of 1BS or 1DS caused similar changes in the staining intensity of subunits encoded by the homeologous gliadin genes as well as of those encoded by the nonrelated glutenin genes.

DISCUSSION

Similarly to many other eukaryotic genes, the storage protein genes of wheat endosperm responded positively (i.e., by increased expression) to elevation in their dosage. This is in accord with a previous report (1) of a gene-dosage response of several seed-protein genes in common wheat. The results indicate that transcription is the basic rate-limiting step in the expression of these genes. However, the nonlinear mode of response in genes whose dose was increased from zero to six indicated a dosage compensation. The phenomenon was more pronounced in the fraction of HMW gliadins than in that of HMW glutenins (Figs. 2 and 4). These nonlinear relationships between the gene dose and protein level may result

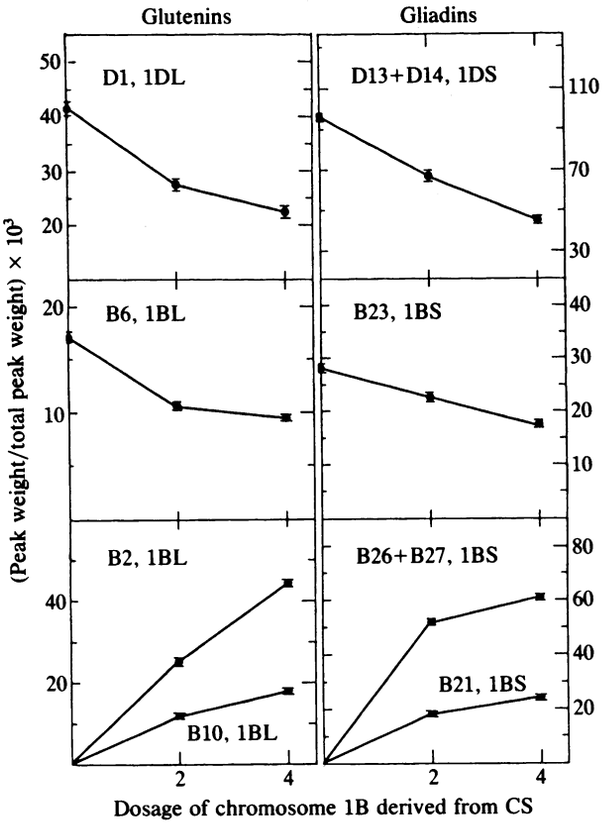

FIG. 4. Quantity of HMW glutenin (*Left*) and HMW gliadin (*Right*) subunits in F_1 hybrids of the crosses Mono 1B × Chinese Spring ('Timstein 1B') and Tetra 1B × Chinese Spring ('Timstein 1B'), containing in their 3*n* endosperm zero, two, and four doses of chromosome 1B of Chinese Spring (CS), respectively. Measurements are given on the basis of total protein. The subunit studied and its controlling chromosome are given for each curve. Bars represent SEM.

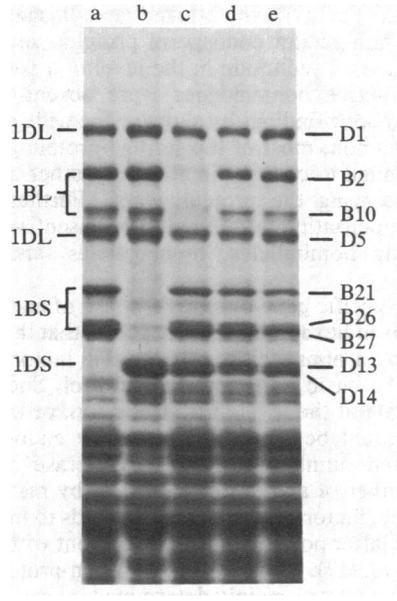

FIG. 5. NaDodSO$_4$/PAGE migration patterns of total endosperm proteins extracted from Chinese Spring (lane d) and from the following aneuploid lines of Chinese Spring: DT1DL (lane a); DT1BL (lane b); DT1BS (lane c); DT1AS (lane e). The controlling chromosome and the subunit designations are given at left and at right, respectively.

from transcriptionally or posttranscriptionally controlled dosage compensation.

Stronger evidence for dosage compensation is derived from the fact that the percentage of total grain protein in the different aneuploid lines was similar, irrespective of the large differences in the dose of HMW glutenin or gliadin genes among these lines. Analysis at the subunit level indicated that indeed the rate of expression of each of the endosperm protein genes depended also on the number of other active HMW glutenin or gliadin genes present in the genome. Hence, when chromosome 1B of Chinese Spring was at six doses, the expression of the HMW gliadin genes encoding subunits B26 and B27 and carried by this chromosome was significantly higher in the absence of 1D than in its presence in three doses (Fig. 2). Moreover, as the dose of the 1B genes of Chinese Spring was increased, a compensating decrease in the expression of those of 1B of Timstein or of those of 1D of Chinese Spring was observed (Figs. 2 and 4). This compensation was generally nonspecific, occurring in genes that code for subunits of both HMW glutenins and HMW gliadins, irrespective of whether they were homoalleles, homeoalleles, or nonrelated to the genes whose dosage was increased. Although most compensation was nonspecific, in this case the fraction of HMW gliadins was somewhat more affected. As the level of chromosome 1B of Chinese Spring was elevated from zero to four (Fig. 4) or six (Fig. 2), the staining intensity of the HMW gliadin subunit B23 of chromosome 1B of Timstein and D13 plus D14 of chromosome 1D of Chinese Spring was slightly more reduced, as compared with that of the glutenin subunit B6 of chromosome 1B of Timstein and D1 of chromosome 1D of Chinese Spring.

Gene-dosage compensation was also observed when some of the HMW gliadin genes were deleted, as in the deficiencies for chromosome arms 1BS or 1DS. Under this reduced gene dosage, the expression of the other HMW glutenin or gliadin genes was increased about 50% (Table 1). Again, this compensation was nonspecific.

Our findings are in general agreement with those of

Table 1. Effect of deficiency for chromosome arms 1AL, 1BL, 1DS, or 1BS of Chinese Spring on the relative amounts of HMW glutenins and HMW gliadins

Line	Deficient arm	Relative peak area			
		HMW glutenin subunits		HMW gliadin subunits	
		D1 + D5	B2 + B10	D13 + D14	B21 + B26 + B27
Chinese Spring	None	100.00[a]	100.00[a]	100.00[a]	100.00[a]
DT1AS	1AL	97.95[a]	101.20[a]	95.96[a]	90.19[a]
DT1BS	1BL	91.87[a]	—	106.45[a]	100.90[a]
DT1DL	1DS	148.38[b]	152.17[b]	—	161.01[b]
DT1BL	1BS	159.42[b]	161.32[b]	152.44[b]	—

The peak areas (determined by peak weight) of the various subunits were standardized to total proteins (total peak weight) and are expressed as percentage of the control line Chinese Spring. Values followed by a different superscript letter differ significantly at the 5% level by the Duncan test.

Aragoncillo *et al.* (1), who studied gene-dosage response in six wheat non-gliadin endosperm proteins and reported in only two cases a reduction in the level of a specific protein when the active homeologues were absent. Our studies extend the above findings by showing the wide occurrence of the phenomenon; most of the storage-protein genes studied here responded to changes in dosage of other chromosomes or arms carrying the protein genes. Furthermore, gene-dosage compensation was found to be essentially nonspecific, affecting homoalleles, homeoalleles, and nonrelated genes.

The nonspecific gene-dosage response of endosperm storage proteins in hexaploid wheat suggests that these genes are regulated by a nonspecific, rate-limiting factor(s), superimposed on the basic transcriptional control. Such a factor(s) could operate at the level of transcription, or translation, or both and could be controlled either by endosperm tissue (e.g., limited number of RNA polymerase molecules or limited number of active ribosomes) or by maternal tissues (for instance, factors providing amino acids to the developing grain). The latter possibility is in agreement with the findings of Millet *et al.* (17), who showed that grain-protein content in tetraploid wheat was mainly determined by maternal tissues.

Regulation of gene expression by a nonspecific, rate-limiting factor(s) could account for the differential degree of compensation exhibited by genes of the HMW glutenins and gliadins. Since the HMW glutenins comprise a smaller fraction of wheat storage proteins, deletion of chromosome arm 1BL, carrying a small gene cluster for these proteins, hardly affected the limitations imposed by the rate-limiting factor(s), resulting in minor changes in the level of other endosperm proteins. On the other hand, deletion of chromosome arm 1BS or 1DS, each carrying a large gene cluster for HMW gliadins, which comprise a larger fraction of the storage proteins, had a considerable effect, presumably by reducing the constraints imposed by the rate-limiting factor(s). The report (18) that higher nitrogen fertilization increased the relative amount of HMW gliadins much more than that of HMW glutenins indicates that nitrogen could prove to be one of the nonspecific rate-limiting factors.

Deficiency for chromosome arm 1AL or for the whole chromosome 1A, carrying no active HMW glutenin or HMW gliadin genes, did not cause any noticeable change in the staining intensity of the HMW glutenin and gliadin subunits encoded by genes of 1B and 1D. Evidently, 1A does not carry repressors, regulators, or other controlling genes that affect the expression of the active HMW glutenin and gliadin genes.

The genetic makeup of hexaploid wheat, comprised of three different genomes, may facilitate permanent heterozygosity of homeoalleles. For genes that encode enzymes, this heterozygosity may be of great physiological and evolutionary advantage. On the other hand, for genes like those coding for storage proteins, the triplicated gene dosage may lead to overproduction and lack of efficiency. One way to overcome gene redundancy is by genetic diploidization, which in wheat is achieved through genetic repression or mutations (19–22). The second way to overcome redundancy is by gene-dosage compensation, which should regulate a desirable level of protein production independently of the number of active structural genes present. This mechanism operates instantly and concurrently with allopolyploidization. However, in cases of posttranscriptional control, it allows the overproduction of mRNA and, therefore, is more wasteful than genetic diploidization. On the other hand, genes whose dosage is being compensated retain the potential of producing different amounts of protein, depending on environmental conditions (e.g., higher nitrogen levels, which enhance grain-protein production) (23). This potential is usually lost when genetic diploidization is brought about through repressions or mutations.

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