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Expression of individual HMW glutenin subunit genes of wheat (*Triticum aestivum L.*) in relation to differences in the number and type of homoeologous subunits and differences in genetic background

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Abstract. The amount of individual high-molecular-weight (HMW) glutenin subunits of bread-wheat has been studied in relation to variation at homoeologous loci and in the general genetic background. The relationships between *Glu-1* loci have been studied using near-isogenic lines (NILs) of the variety Sicco and in the progenies of two crosses. Substitution of the Sicco *Glu-D1* allele by a null-allele resulted in higher amounts of the homoeologous subunits. The presence of a *Glu-A1* null-allele did not have a noticeable effect on the amounts of homoeologous subunits. In three out of four NILs and in the sister-lines of two crosses, the amounts of HMW-subunits did not depend on the allele make-up at homoeologous loci. Only in the NIL which contains the *Glu-D1* allele, encoding subunits 1Dx2.2 and 1Dy12, was the amount of homoeologous subunits lower than the amount of these subunits in Sicco. This study suggests a relation between the amount of HMW-subunits encoded by an allele and its contribution to bread-making quality. The effect of genetic background has been studied using F4 and F5 lines of two crosses. The total amounts of subunits, relative to the total amount of kernel proteins, showed a considerable variation between lines. The ratio between individual subunits did not differ between genetic backgrounds. Because this ratio is also largely independent of differences in environmental conditions, it is concluded that the relative amount of a subunit is a valuable measure for the detection of genetically-determined differences in the expression of HMW-subunit genes.

Key words: Wheat – HMW glutenin subunits – Gene expression – Quantitative variation

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Introduction

The high-molecular-weight (HMW) glutenin subunits, a group of storage proteins of wheat, have been studied extensively in relation to bread-making quality. In the allohexaploid bread-wheat (*Triticum aestivum* L.), these proteins are encoded by genes at three complex loci (*Glu-A1, Glu-B1* and *Glu-D1*) on homoeologous chromosomes 1A, 1B and 1D respectively. At each locus two types of single-copy genes are present (Harberd et al. 1986). These genes encode subunits which are classified as x-type or y-type HMW glutenin subunits according to their higher or lower molecular weight respectively (Payne et al. 1981). A large number of alleles has been found in wheat varieties and in species related to bread-wheat (see Kolster et al. 1988). In wheat varieties, the *Glu-A1* alleles do not encode a y-type subunit, while one of the *Glu-A1* alleles does not result in an x-type subunit either. Alleles which do not encode HMW-subunits are referred to as null-alleles. Some *Glu-B1* alleles do not encode a y-type subunit. The *Glu-D1* alleles which occur in varieties all encode both types of subunits. *Glu-B1* and *Glu-D1* null-alleles are very rare. Variation in the bread-making quality of genotypes has been associated with variation in HMW glutenin subunit genotype (see Payne et al. 1979, 1987a; Moonen et al. 1982; Branlard and Dardevet 1985; Kolster et al. 1991a). Variation in the amounts of HMW glutenin subunits (the total amount as well as the amounts of individual subunits) is also of importance for bread-making quality (Schofield and Booth 1983; Huebner and Bietz 1985; Kruger et al. 1988; Wieser et al. 1989). The presence of null-alleles at each of the *Glu-1* loci of wheat lines resulted in a decreased total amount of HMW glutenin subunits. This was negatively correlated with bread-making quality (Payne et al. 1987b; Lawrence et al. 1988; Halford et al. 1992).

In general, phenotypic variation in the amounts of the HMW glutenin subunits has been reported in the literature. A considerable environmental variation in the amounts of individual HMW-subunits was shown by Kolster et al. (1991 b). There is a lack of knowledge about genetic variation in the expression of HMW glutenin genes. Moreover, there is limited information about the influence of variation in genetic background, including variation at homoeologous loci, on the expression of HMW-subunit genes. In inter-varietal substitution lines, the amounts of HMW glutenin subunits encoded by a substituted chromosome did not differ from the amounts produced in the donor-variety (Galili and Feldman 1985). In aneuploid wheat lines, the expression of HMW subunit genes at the *Glu-D1* locus was not affected by the deletion of a chromosome-arm carrying the *Glu-B1* locus (Galili et al. 1986). These results suggest that HMW-subunit gene expression does not depend on genetic background. However, other studies did show effects of a variation in genetic background. Namely, an increase in the dosage of *Glu-1* loci in compensating nullisomic-tetrasomic lines was related to a decrease in the amounts of homoeologous HMW-subunits and in the amounts of other groups of storage proteins. Furthermore, the deletion of chromosome arms encoding so-called HMW-gliadins resulted in an increase in amounts of HMW-subunits encoded by the remaining arms (Galili et al. 1986). Aneuploid wheat lines were used in most of the studies here referred to. These lines may not be representative of the situation in euploid wheat. Furthermore, allelic variation at the *Glu-1* loci may influence the amounts of *individual* HMW glutenin subunits, but this topic has not yet been studied. The aim of the present investigation was to analyze the expression of HMW glutenin subunit genes in euploid wheat genotypes in relation to:

(1) differences in the number of genes resulting in a HMW glutenin subunit, which in general ranges from three to five in bread-wheat,
(2) differences in the type of alleles present at homoeologous *Glu-1* loci,
(3) differences in genetic background (i.e., in genes other than the HMW glutenin subunit genes).

Near-isogenic lines (NILs) of Sicco, in which HMW glutenin subunit alleles are replaced by allelic variants, have been used in the Sicco genetic background to study the relations between genes of the *Glu-1* loci in determining gene expression (1 and 2). To study the expression of HMW glutenin subunit genes in different genetic backgrounds (3), F4 and F5 lines of two crosses have been used. These lines also gave information on the relations studied under 1 and 2.

Materials and methods

Plant material

Kernels of two subsets of near isogenic lines (NILs) of the Dutch spring-wheat variety Sicco were kindly provided by Dr. P.I. Payne, PBI Cambridge, England (Payne et al. 1987b). Subset A was composed of NILs in which the *Glu-A1* and/or the *Glu-D1* alleles of Sicco are replaced by null-alleles; subset B was composed of NILs in which the Sicco alleles are replaced by allelic variants. Table 1 shows the HMW glutenin subunit genotype of the NILs and the donor varieties. Figure 1 shows SDS-PAGE patterns of the proteins present in kernels of the NILs and Sicco control. The experimental conditions used for this gel differed

Table 1. The HMW glutenin alleles of the *Glu-A1*, *Glu-B1* and *Glu-D1* loci of the near-isogenic lines of Sicco and the donor varieties

Subset	Line	HMW glutenin genotype			Donor genotype
		Glu-A1	*Glu-B1*	*Glu-D1*	
A	NIL-1A null	0+0[a]	7+9	5+10	(Nap Hal × Gabo[b])
	NIL-1D null	1+0	7+9	0+0	–
	NIL-1A/1D null	0+0	7+9	0+0	–
	Control	1+0	7+9	5+10	–
B	NIL-2*	2*+0	7+9	5+10	Glenlea
	Control	1+0	7+9	5+10	–
	NIL-7+8	1+0	7+8	5+10	Chinese Spring[c]
	Control	1+0	7+9	5+10	–
	NIL-2+12	1+0	7+9	2+12	Chinese Spring[c]
	Control	1+0	7+9	5+10	–
	NIL-2.2+12	1+0	7+9	2.2+12	Danchi
	Control	1+0	7+9	5+10	–

[a] The subunits encoded by the x- and the y-type gene, respectively. A silent gene is represented by an 0

[b] Represents a Gabo isogenic line in which the Nap Hal HMW glutenin subunit genotype (0, 1Bx17+1By18, 0) was introduced by four backcross generations. During the backcrossing with Sicco, genotypes heterozygous for 1Ax1 and 1Dx5+Dy10 were selected; after selfing of the BC-5 plants the different types of isogenic lines were selected

[c] For the NIL 1Bx7+1By8 and NIL 1Dx2+1Dy12 the same donor variety was used. The lines were separated at BC-1 (see Materials and methods)

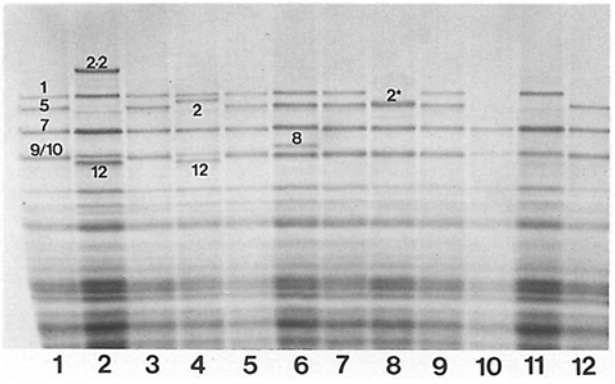

Fig. 1. SDS-PAGE separation (8.3% gels) of the storage proteins in near-isogenic lines of Sicco. *Lanes 1, 3, 5, 7, 9*: Sicco control; *lane 2*, NIL-2.2+12; *lane 4*, NIL-2+12; *lane 6*, NIL-7+8; *lane 8*, NIL-2*; *lane 10*, NIL-1A/1D null; *lane 11*, NIL-1D null; *lane 12*, NIL-1A null

from those used for the quantification of the HMW-subunits. To prevent proteins migrating from the gel, the separation was stopped immediately after the tracking dye reached the end of the gel. In the separation procedure used for quantification, the separation was continued for another 3 hours. Furthermore, in order to obtain intensely-stained protein bands, a lower extraction buffer/fluor ratio (ml/g) was used, resulting in more concentrated protein extracts. The SDS-PAGE patterns of the NILs and Sicco control show no qualitative differences in proteins other than the target HMW-subunits.

Each NIL had been produced by five consecutive backcrosses to Sicco (Payne et al. 1987b). BC-5 plants from kernels heterozygous for the target locus were selfed. The resulting kernels which were homozygous for the donor allele were also NIL-substituted (NIL-sub) in relation to which the Sicco HMW-subunit genotype served as a NIL-control (NIL-con). Each NIL-sub and its specific control were derived from the same BC-5 plant. The NILs are considered to be to a large extent isogenic for loci other than *Glu-1*. However, it should be noted that a considerable proportion of the donor genome linked to the marker gene is introduced in the NILs, even after five backcrosses and selfing at BC-5, as shown by the theoretical study of Stam and Zeven (1981). Therefore, it is likely that proximally located cis- or trans-acting regulatory sequences, or other linked genes which interact with the HMW glutenin subunit genes, are introduced together with the target gene.

A second set consisted of kernels of 42 F4 and 45 F5 lines resulting from the following two crosses in which the parents and their HMW glutenin subunits encoded by alleles at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively, are given between brackets: cross 84024 [SVP-76025111 * Saiga; (1Ax2*, 1Bx7+1By8, 1Dx2+1Dy12) * (1A null, 1Bx7+1By9, 1Dx2+1Dy12)] and cross 83009 [SVP-75016453 * SVP-73003242; (1Ax1, 1Bx6+1By8, 1Dx5+1Dy10) * (1A null, 1Bx6+1By8, 1Dx2+1Dy12)]. The progenies of these crosses were bulk-propagated until the F3. The F4 generation started with randomly chosen single kernels. The kernels used in this study were produced at autumn-sown trial fields of the former Foundation for Agricultural Plant Breeding (now part of the Centre for Plant Breeding and Reproduction Research).

In this study of the effect of variation in genetic background on HMW subunit gene expression, segregating progenies were preferred to material with a wide genetic origin, although the latter have a larger variation in genetic background. This is because, in sister lines, subunits showing identical relative mobilities during SDS-PAGE – which is the criterion used for their genetic identification – can be considered to be encoded by identical genes. This is not the case for subunits in unrelated genotypes where (1) effects on the expression of genes encoding identical subunits of differences in proximal regulatory sequences cannot be distinguished from effects of differences in the genetic background, and (2) allelic subunits may have a similar R_m during SDS-PAGE (Kolster et al. 1988).

Electrophoresis and quantification

Proteins present in extracts of wholemeal flours were separated on 8.3% SDS-PAGE electrophoresis gels. The extraction procedure, electrophoretic conditions, gel staining, and the densitometric analysis were as described previously (Kolster et al. 1992). This method has been developed to enable quantification and identification of individual subunits. In this report, the amount of Coomassie Brilliant Blue (CBB) adsorbed by the HMW glutenin subunits (expressed as 100 * absorbance unit times mm) will be referred to as the amount of that subunit. A systematic study to evaluate this quantification method, including a statistical analysis of the reproducibility of the results obtained, is described by Kolster et al. (1992).

Extracts of each genotype were applied on four different gels. The two subsets of NILs were analyzed separately, each gel contained a complete subset. In the statistical analysis of the quantitative measurements of the NILs (Genstat 5 committe 1987), the gel was treated as a block factor. Because of the large number of lines originating from the two crosses, different sets of four gels were required for the quantification of the subunits. For the statistical analyses, differences in staining intensity between gels of different sets were assumed to be absent. To verify this assumption, an extract of a reference flour was applied in the first and last slot of each gel.

Wholemeal flours are used for the electrophoretic analysis of HMW glutenin subunits. Consequently, genotypes homozygous for the *Glu-A1* alleles, encoding subunit 1Ax1, cannot be distinguished from heterozygous genotypes containing the *Glu-A1* allele, encoding subunit 1Ax1 and the null-allele, as flour of both genotypes contain subunit 1Ax1. This will have contributed to variation in the amount of subunit 1Ax1. Consequenty, the ratio between individual subunits present in a genotype will also be affected. However, when the relative amounts of the *Glu-B1*- and *Glu-D1*-encoded subunits was calculated without taking the amount of subunit 1Ax1 into consideration, the conclusions from these experiments remained the same.

Results and discussion

Differences in the number of HMW glutenin subunits

The relation between the number of HMW glutenin subunits produced by a genotype and the amounts of individual subunits [(1) in Introduction] was studied using near-isogenic lines of Sicco, in which the alleles of the *Glu-A1* and *Glu-D1* loci have been replaced by null-alleles (subset A, Table 1). Table 2 shows the amounts of HMW-subunits present in these NILs. A comparison of the amounts of the subunits present in Sicco, the NIL-con and the NIL-1A null shows that replacing the *Glu-A1* allele encoding subunit 1Ax1 by the null-allele did not result in a significant change in the amounts of the *Glu-B1*- and *Glu-D1*-encoded subunits. In the NIL-1D null and the NIL-1A/1D null, the amounts of the remaining

Table 2. Amounts of the individual CBB-stained HMW glutenin subunits[a] of near-isogenic lines (NILs) of Sicco, in which the alleles of the *Glu-A1* and/or *Glu-D1* locus are replaced by null-alleles

HMW-subunit	Sicco	NILs				LSD[b]
		Control	1A-null	1D-null	1A/1D-null	
1 (*Glu-A1x*)	20	18		25		3.4
7 (*Glu-B1x*)	44	42	47	60	50	5.9
9 (*Glu-B1y*)	18	18	18	28	26	3.3
5 (*Glu-D1x*)	27	25	27			4.2
10 (*Glu-D1y*)	32	35	33			5.8
Protein content (%)	11.1	11.1	10.3	12.3	11.2	

[a] 100 * absorbance units · mm
[b] LSD = least significant difference of the absorbance values at $P = 0.05$, based on the densitometric analysis of eight lanes (two independently-produced extracts of each sample, each applied on four gels)

Table 3. Amounts of the individual CBB-stained HMW glutenin subunits[a] in four genotypic groups of sister lines (cross 83009), denoted A to D

HMW glutenin subunit	A	B	C	D
0 (*Glu-A1x*)[b]	–		–	
1 (*Glu-A1x*)		21		22
6 (*Glu-B1x*)	32	37	34	38
8 (*Glu-B1y*)	31	33	34	38
2 (*Glu-D1x*)			45	48
12 (*Glu-D1y*)			61	65
5 (*Glu-D1x*)	45	50		
10 (*Glu-D1y*)	71	75		
Protein content (%)[c]	13.0	13.1	13.3	13.6
Control samples[d]	128	135	126	129
Number of lines	7	15	7	16

– = no subunit present
[a] 100 * absorbance units · mm
[b] For the quantification of the HMW glutenin subunits of a line, four replicate densitometric analyses were used. Due to the large differences in the staining intensity between lines, none of the differences between a subunit present in different group were significant
[c] The average protein content of the samples belonging to the same genotypic group
[d] Total absorbance of the HMW glutenin subunits present in an extract, used as a control for different gels (see Materials and methods)

subunits was significantly higher ($P \leq 0.05$) than the amounts of these subunits in Sicco or the NIL-con (Table 2). The higher protein content of the flour may have contributed to the higher amounts of subunits in the NIL-1D null (Kolster et al. 1991 b), but this cannot be the cause of the higher amounts of HMW subunits in the NIL-1A/1D null.

The same relationship was also studied in the progeny of the two crosses. Both populations contain the *Glu-A1* null-allele and a *Glu-A1* allele encoding a HMW glutenin subunit (1Ax1 in cross 83009 and 1Ax2* in cross 84024). By calculating the average absorbance of randomly chosen lines with the same HMW glutenin subunit genotype (Table 3), differences in genetic background [(3) in Introduction] are largely removed. The amounts of individual subunits in sister lines which differ only in their *Glu-A1* allele (for 83009 compare group A with B and group C with D in Table 3) were only slightly affected by differences in the type of that allele. The differences observed for the amounts of corresponding subunits in different groups could well be the result of differences in the average protein content of the flours of the lines, and in the average staining-intensity of the gels. The latter is represented by the average absorbance of the HMW glutenin subunits of a control sample applied to each gel (Table 3). The analysis of cross 84024 is in full agreement (data not shown). In conclusion, our experiments with NILs show that in euploid wheat a decrease in the number of subunits by substitution of an active *Glu-D1* allele by a *Glu-D1* null-allele results in an inrease in the expression of the remaining HMW glutenin subunit genes. The presence of a *Glu-A1* null-allele in the NILs did not have a noticeable effect on the amounts of homoeologous subunits. The absence of an effect of the presence of a *Glu-A1* null-allele is also found in the experiments with the sister lines. The presence of a *Glu-A1* null-allele is common in wheat varieties, in contrast to the *Glu-D1* null-allele.

The absence of a significant effect of the presence of the *Glu-A1* null-allele may be caused by the amount of subunit encoded, which is considerably lower than the amount produced by the *Glu-B1* and *Glu-D1* alleles (compare amounts of subunits 1Ax1 and 1Dx5 + 1Dy10, Table 2). Another cause of the differences in the effect of the *Glu-A1* and *Glu-D1* null-alleles may be due to differences responsible for the lack of subunits of the genes. The genes of the *Glu-D1* null-allele are deleted (Payne, personal communication) whereas the HMW glutenin subunit genes of the *Glu-A1* null-allele (Harberd et al. 1986) are probably silent as a result of mutations, as shown by sequence analysis of two *Glu-A1y* subunits

genes (Forde et al. 1985; Harberd et al. 1987). No HMW glutenin subunit transcription products of the silent *Glu-A1x* and *Glu-A1y* genes of Chinese Spring (Thompson et al. 1983) or of the *Glu-A1y* gene of Cheyenne (Forde et al. 1985) could be detected. The results of the present study suggest that the number of genes present, and not the number of transcribed genes, determines the level of expression. The number of flanking sequences of genes which can form complexes with proteins, and can therefore be involved in gene-regulation (see Waugh and Brown 1991), might be rate-limiting for gene expression.

Differences in type of HMW glutenin subunits

The relation between the expression of HMW glutenin subunit genes and the variation in the type of alleles present at homoeologous loci [(2) in Introduction] has been studied by means of four near-isogenic lines, in which HMW subunit alleles of Sicco were replaced by allelic variants (subset B in Table 1). Because the introgressed alleles encoded the same number of subunits as the Sicco alleles, each NIL-sub contained the same number of subunits as Sicco. Table 4 shows that both the amounts of HMW-subunits and the protein content differed between NILs. In this table the amounts of individual HMW glutenin subunit present in a NIL-sub should be compared with the amounts of the corresponding subunits present in its specific control. In the first, second and third pair, the amounts of Sicco-type HMW-subunits was equal or only slightly higher in the NIL-sub than in the NIL-con. In the NIL 1Dx2.2+1Dy12, the amounts of Sicco-type HMW-subunits was significantly lower than the amounts in its control. This difference cannot be explained by differences in protein content. Therefore, the probable cause is the substitution of the *Glu-D1* allele, encoding subunits 1Dx5 and 1Dy10, by an allele encoding 1Dx2.2 and 1Dy12 (including linked genes, see Materials and methods, plant material). The latter allele originates from a Japanese variety (Payne et al. 1983) and is very rare in wheat varieties. The differences *between* pairs of NILs (see Table 4) can be caused by the introduction of genes other than the target genes. Also environmental influences could result in differences between NILS (Kolster et al. 1991 b), so further studies are necessary to separate these causes.

The effect of the substitution of an allele, without changing the number of subunits, can also be studied using the progeny of the two crosses. In groups of lines which differ only in the type of *Glu-D1* allele (encoding 1Dx2 and 1Dy12 or 1Dx5 and 1Dy10; compare A with C and B with D in Table 3), the amounts of *Glu-B1* subunits 1Bx6 and 1By8 were comparable. The small differences observed were probably a result of differences in the average protein content of groups of lines and in the staining intensity of the gels (see Table 3). The results of cross 84024 agree (data not shown).

In conclusion, the *Glu-1* loci did not influence HMW glutenin subunit gene expression except in the case of the substitution of the *Glu-D1* allele encoding subunits 1Dx5 and 1Dy10 by the rare allele encoding 1Dx2.2 and 1Dy12. Payne and coworkers (Payne, personal communication) found this *Glu-D1* allele to be of a "low quality" with respect to bread-making. This low quality may thus result from the overall reduction in the amounts of HMW glutenin subunits.

In passing it may be noted that the absorbance of some of the subunits encoded by alleles introgressed in Sicco differs from the absorbance of the corresponding Sicco-type subunits (Table 4). Assuming that subunits do not differ in the binding capacity of the dye, these differences are considered as differences in the amount of subunit. As far as these limited data go, there is a coincidence between the combined amounts of subunits and the contribution to bread-making quality of the *Glu-B1* and *Glu-D1* alleles, as reported by Payne et al. (1987 a). The same holds for *Glu-D1*-encoded subunits in lines of cross 83009 (Table 3); the amount of the subunit of the high-quality *Glu-D1* allele, encoding subunits 1Dx5 and 1Dy10, is

Table 4. Amounts of the individual CBB-stained HMW glutenin subunits[a] in near-isogenic lines of Sicco, in which alleles of the *Glu-A1*, *Glu-B1* or *Glu-D1* locus are replaced by allelic variants (given in parenthesis)

Subunit	*Glu-A1* 2*		*Glu-B1* 7+8		*Glu-D1* 2+12		*Glu-D1* 2.2+12	
	Control	Substituted[b]	Control	Substituted	Contol	Substituted	Control	Substituted
1 (*Glu-A1x*)	18***	13 (2*)	27 ns	30	27 ns	27	20***	17
7 (*Glu-B1x*)	49 ns	51	67 ns	68 (7)	71 ns	79	67***	47
9 (*Glu-B1y*)	16 ns	16	22***	32 (8)	21*	28	24***	16
5 (*Glu-D1x*)	32 ns	30	43 ns	46	41*	33 (2)	39***	14 (2.2)
10 (*Glu-D1y*)	40 ns	40	65*	70	66 ns	69 (12)	59***	43 (12)
Protein content (%)	11.0	11.1	14.8	15.2	13.4	14.0	15.3	14.8

[a] 100 * absorbance units · mm

[b] The amount of subunits should be compared with the amount present in its specific control

*** $P<0.001$; ** $P=0.01$; * $P=0.05$; ns = not significant

higher than the amount of the allelic pair of subunits 1Dx2 and 1Dy12, which are encoded by a low-quality allele. The present study thus suggests a relationship between the amounts of HMW glutenin subunits encoded by an allele and its contribution to bread-making quality.

Differences in genetic background

The effect of variation in genetic background on HMW subunit gene expression [(3) in Introduction] has been studied in the F4 and F5 lines of the two crosses. Figure 2 shows the total amount of HMW glutenin subunits – relative to the total amount of kernel proteins – for sister lines grouped for HMW glutenin subunit genotype. The proportion of HMW-subunits encoded by identical alleles differed considerably between lines. This variation in proportion exceeds that observed for replicate plots of a variety and for plots of a variety grown at different locations (Kolster et al. 1991 b). In the latter study, genetically determined differences in the proportion of the HMW glutenin subunits between varieties with the same HMW glutenin subunit make-up were also shown. Therefore, it is likely that the variation in genetic background contributes considerably to the variation in the amounts of HMW glutenin subunits.

A second question is whether differences in genetic background differentially affect the expression of individual HMW glutenin subunit genes. Since the amounts of HMW glutenin subunits of a genotype depends on its protein content (Kolster et al. 1991 b), absorbance is not the most optimal measure for this specific purpose. The amount of individual HMW glutenin subunits, relative to the total amount of HMW glutenin subunits, can be used because it is largely independent of differences in the protein content (Kolster et al. 1991 b). However, there are restrictions. Firstly, variation in the relative amount of a subunit may result not only from variation in the amount of the subunit itself but also from variation in the amount of (one of the) other subunits. Secondly, alleles may differ in the number and amounts of subunits they encode. Consequently, comparison is only feasible for lines with identical HMW subunit genotypes. Accordingly, Table 5 shows the maximum and minimum relative amounts of individual subunits observed in groups of sister lines with the same HMW subunit genotype. The variation in relative amount of a subunit within a group of genotypes is slightly higher than the variation observed as a result of differences in growing conditions (Kolster et al. 1991 b). The same holds for cross 84024 (data not shown).

Therefore, these experiments give no clear-cut evidence that individual HMW glutenin genes are differentially affected by differences in genetic background. However, the total amount of HMW glutenin subunits is affected by differences in genetic background. The effects

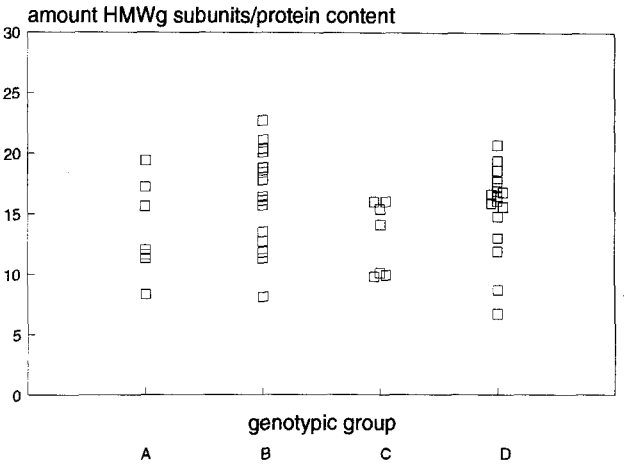

Fig. 2. Variation in ratio between the amount of HMW glutenin subunits (100 * absorbance units times mm) and the protein content of sister-lines of cross 83009. The lines are grouped for HMW glutenin subunit genotype, denoted **A–D**. See Table 3 for HMW subunit genotypes of the groups. The protein content of the kernels of the lines ranges from 9.1 to 14.2%

Table 5. Relative amounts of the individual HMW glutenin subunits (absorbance of HMW glutenin subunits relative to total absorbance of the subunits) in groups of sister-lines of cross 83009. The minimal and maximal relative amount present in the lines is reported[a]

HMW glutenin subunit	A	B	C	D
1 (*Glu-A1x*)		0.02–0.15		0.06–0.16
6 (*Glu-B1x*)	0.17–0.18	0.15–0.19	0.19–0.20	0.16–0.19
8 (*Glu-B1y*)	0.16–0.18	0.15–0.19	0.19–0.20	0.17–0.20
2 (*Glu-D1x*)			0.26–0.29	0.19–0.25
12 (*Glu-D1y*)			0.31–0.37	0.27–0.35
5 (*Glu-D1x*)	0.24–0.28	0.22–0.26		
10 (*Glu-D1y*)	0.39–0.42	0.32–0.40		

[a] Maximum LSD ($P<0.05$) for the relative absorbance of a line, based on four replicates: 0.025

of variation in storage protein genes on the expression of other storage protein genes, as described by Galili and coworkers (see Introduction), is another example of a relationship between genetic background and the expression of HMW glutenin subunit genes.

Concluding remarks

In the relation between individual HMW glutenin subunit genes, effects of gene-dosage and the type of *Glu-D1* alleles occurred. This suggests the presence of regulatory genes linked to the HMW-subunit genes. No effects of variation at the *Glu-A1* and *Glu-B1* locus on the amount of other subunits were found. In the study of Galili et al. (1986), deletion of the chromosome arm carrying the *Glu-B1* locus did not affect the amount of *Glu-D1*-encod-

ed subunits. Lines in which other *Glu-1* loci were removed were not studied by these authors. These results may indicate that regulatory genes of the *Glu-D1* locus are involved in the regulation of expression of HMW-subunit genes. Bittel et al. (1991) in their study of gliadin genes, also found a regulatory effect of the D-genome on the expression of genes of storage protein. In each line used in the present study variation at the *Glu-D1* locus did not affect the amount of other subunits. Therefore, the effect appears to be dependent on the type of *Glu-D1* allele present. The fact that an increase in the dosage of *Glu-B1* genes in aneuploid lines resulted in a decrease in the amount of other HMW-subunits was ascribed to non-specific mechanisms, such as amino-acid limitation (Galili et al. 1986).

The ratio between HMW glutenin subunits seems largely unrelated to differences in genetic background. This suggests that a common regulation mechanism coordinates expression of the genes at the *Glu-1* loci. Such a coordination between HMW glutenin subunit genes has also been shown for wheat grown under different environmental conditions (Kolster et al. 1991 b). Bartels and Thompson (1986) were able to demonstrate that expression of storage protein genes is coordinated, probably at the level of transcription, during kernel development. The present study shows that the total amount of HMW-subunits, relative to the total amount of protein, depends on the genetic background. Therefore, regulation mechanisms, superimposed on the mechanism regulating the coordinated expression of HMW glutenin subunit genes, are probably involved in coordinating the expression of storage protein genes. Finally, environmental conditions are known to affect the total amounts of HMW subunits produced by a genotype (Kolster et al. 1991 b).

The present study has significance for investigations on genetic variation in the expression of HMW glutenin subunit genes. Firstly, for the most commonly occurring HMW glutenin subunit alleles, no interactions were found between the *Glu-1* loci in determining gene expression. Consequently, is possible to use the amount of a subunit as a measure of gene expression, without taking the HMW glutenin subunit genotype into consideration. Secondly, the amount of a given subunit relative to the total amount of subunits is influenced by differences in genetic background only to a limited extent. The same holds for differences in environmental conditions (Kolster et al. 1991 b). Therefore, when in a given genotype a subunit is found which differs strongly in relative amount compared with the amount in other genotypes, it is probably caused by characteristics of the gene itself. A genetic analysis is required for determining the exact nature of this aberrant expression.

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