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Control by Homoeologous Group 1 Chromosomes of the High-Molecular-Weight Subunits of Glutenin, a Major Protein of Wheat Endosperm

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Summary. The electrophoretic mobilities of the high-molecular-weight (HMW) subunits of glutenin from 7 varieties were compared by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (SDS). In total, 12 subunits were clearly resolved and they had nominal molecular weights of between 95,000 and 140,000. The chromosomes which control their synthesis were determined using monosomic lines and inter-varietal substitution lines. All subunits were shown to be controlled by the homoeologous group 1 chromosomes. Each variety contains between 3 and 5 HMW subunits; two are under the control of the 1D chromosome, 1 or 2 are controlled by chromosome 1B and 0 or 1 by chromosome 1A. The segregation of two 1D-controlled subunits of similar electrophoretic mobilities were analysed in the F_2 progeny of crosses between 'Chinese Spring' and 'Holdfast'. The results suggest that the genes which code for the two proteins are allelic.

Key words: Glutenin – *Triticum* – Genetics – SDS-polyacrylamide – Gel-electrophoresis

Introduction

The endosperm of the wheat grain usually contains between 7% and 15% by weight of protein and just under one half of this is glutenin. Glutenin is a large, heterogeneous protein complex ranging in molecular weight from several hundred thousands to several millions (Huebner and Wall 1976). It is built up of 15 or more different subunits (Payne and Corfield 1979) which are probably connected together by a combination of disulphide bonds and hydrophobic interactions. Glutenin plays an important part in the bread-making process, adding strength and elasticity to a dough (Bietz, Huebner and Wall 1973). In order to assist the breeder in developing new varieties of improved bread-making quality, it is important to understand much more of the biochemistry and genetics of the glutenin subunits.

Unlike the other major group of proteins within the endosperm, the gliadins, the identification of the chromosomes which control the synthesis of the glutenin subunits has been little studied, mainly because of the lack of adequate fractionation techniques. The advent of SDS-polyacrylamide gel electrophoresis has now enabled the high-molecular-weight subunits (90,000-150,000) to be well separated, although the smaller subunits (30,000-51,000) are still rather poorly resolved (Payne and Corfield 1979). Using this technique, Bietz, Shepherd and Wall (1975) detected 4 subunits of glutenin from the variety 'Chinese Spring' which had molecular weights greater than 70,000. Nullisomic-tetrasomic, nullisomic-trisomic and ditelocentric lines of 'Chinese Spring' were then analysed. The authors showed that 2 of the subunits were controlled by the long arm of chromosome 1D and the other 2 by the long arm of 1B. This work has recently been confirmed using a two-dimensional electrophoretic procedure (Brown et al., 1980).

In this paper, we have investigated the chromosomal control of several high-molecular-weight subunits of glutenin from 6 varieties. Most of these subunits are not present in 'Chinese Spring' and all were shown to be controlled by either chromosomes 1A, 1B or 1D.

Materials and Methods

Plant Material

a) Aneuploid Lines

Monosomic lines (2n = 41) belonging to homoeologous group 1 (and also in some cases group 6) were used to study the chromosomal control of a number of glutenin subunits. A monosomic

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plant lacks one entire chromosome of an homologous pair. The monosomics of 'Cheyenne' and 'Spica' were developed by and obtained from Dr. R. Morris, University of Nebraska, USA, and Dr. R.A. McIntosh, University of Sydney, Australia, respectively. The monosomic lines of 'Cappelle-Desprez', 'Bersée', 'Hobbit sib' (a sister line of 'Hobbit') and 'Holdfast' were all developed at The Plant Breeding Institute, Cambridge.

b) Inter-varietal Chromosome Substitution Lines

In these lines, a pair of chromosomes from a recipient variety has been replaced by an homologous pair of chromosomes from other, donor, varieties by means of cytogenetical techniques (Law and Worland 1973). 'Chinese Spring' (Hope 1A) was developed by Dr. E.R. Sears, University of Missouri, USA. 'Chinese Spring' (*Triticum spelta* 1A), 'Cappelle-Desprez' ('Cheyenne' 1D) and 'Chinese Spring' (*Aegilops umbellulata* $1C^u$) were produced at The Plant Breeding Institute, Cambridge.

Preparation of Flour Samples

Single grains were ground to a fine flour using a Glen Creston wrist-action, ball mill. Grinding time was 5 min. and there was no significant heating during this period. Larger amounts of flour for column chromatography experiments were hammer-milled.

Preparation of Glutenin by Gel-Filtration Chromatography

In most experiments, 6g flour were mixed with a magnetic stirrer in the presence of 30 ml of AUC (0.1M acetic acid, 3M urea and 0.01M cetyltrimethyl ammonium bromide) at room temperature for 2 h. The suspension was centrifuged at 2,000 × *g* for 10 min. and the supernatant obtained was centrifuged again at 100,000 × g_{ave}. Between 60 and 100 $A_{280\,nm}$ units of this final supernatant (max. volume 8 ml) was loaded onto a column of Sepharose CL-4B equilibrated with AUC. Chromatography was at room temperature at a flow rate of about 2.2 ml $cm^{-2}\,h^{-1}$. Fractions containing proteins were collected, dialysed against water for 48 h, shell frozen and finally freeze-dried. Fuller details of this procedure are given elsewhere (Payne and Corfield 1979). The only difference was that a smaller column, 2.2 cm × 40 cm, was used.

SDS-Polyacrylamide Gel Electrophoresis

Flour samples (4mg) were suspended in 100 µl of a medium containing 2% (w/v) sodium dodecyl sulphate (SDS), 5% (v/v) 2-mercaptoethanol, 0.001% (w/v) pyronin Y, 10% (v/v) glycerol and 0.063 M Tris-HCl, pH 6.8. Freeze-dried protein samples from column chromatography experiments were treated in a similar manner except the weight of sample was reduced from 4 mg to 0.6 mg. Samples were left at room temperature for about 2 h and occasionally shaken. They were placed in a boiling water-bath for 2 min., allowed to cool and then 50 µl of each sample was loaded onto a polyacrylamide gel which measured 16 cm × 13 cm × 1.5 mm and was made up as described by Laemmli (1970). In routine experiments, 17% gels were made and subjected to electrophoresis at 9 mA for about 18 h when the tracking dye, pyronin Y, had reached to within 1 cm of the bottom of the gel. The high-molecular-weight glutenin subunits migrated no more than 2 cm but all the proteins of the sample were retained within the gel. When better resolution of the larger protein subunits was required, 17% gels were subjected to electrophoresis at 10 mA for 18 h and then 15 mA for a further 8 h. Alternatively, 10% gels were run for 18 h at 14 mA. Thirteen or 14 samples were applied to each gel. The gel after the separation was stained with Coomassie Brilliant Blue R and destained as described previously (Payne and Corfield 1979). In some instances when the protein bands were poorly stained, the gel was restrained with a solution of 0.2% (w/v) Coomassie Brilliant Blue G-250 as described by Blakesley and Boezi (1977).

Results

1 Relative Electrophoretic Mobilities of the High-Molecular-Weight Polypeptides from 7 Varieties

A separation of the high-molecular-weight (HMW) polypeptides from the grain of 'Chinese Spring' is shown in Figure 1a. Four are resolved and are numbered 2, 7, 8 and 12. In this particular separation, band 12 is clearly double, the top band of the two being the stronger. This usually

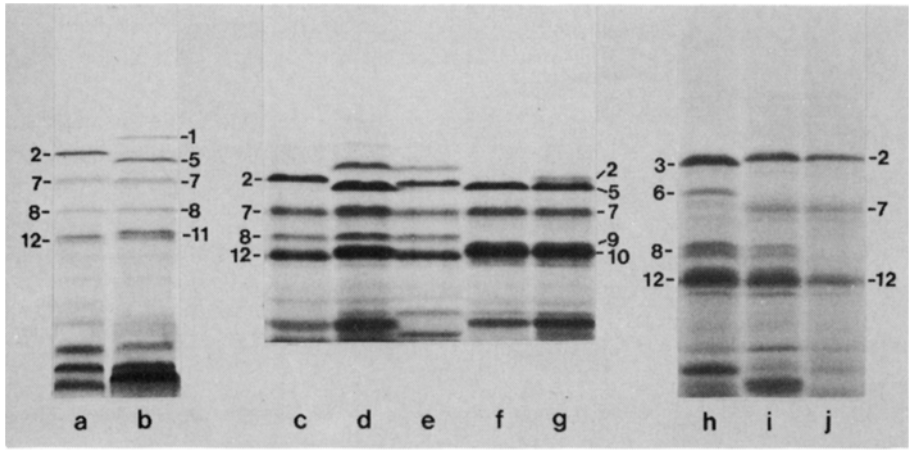

Fig. 1a-j. Analysis by gel electrophoresis of HMW protein subunits from flour of the following: a 'Chinese Spring'; b 'Holdfast'; c 'Chinese Spring' × 'Holdfast', F_2 generation (single grain); d 'Chinese Spring' × 'Holdfast', F_2 generation (single grain); e 'Bersée'; f 'Spica'; g 'Cheyenne'; h 'Hobbit sib'; i 'Chinese Spring'; j 'Cappelle-Desprez'. Samples a, b, h, i and j were fractionated in 17% polyacrylamide gels (24 h) and c-g in 10% gels

occurs using 17% gels but in 10% gels only one diffuse band is obtained (Fig. 1i). The reverse situation occurs with band 8 for it is usually double in 10% gels (Fig. 1i) and usually single in 17% gels. At least some of the bands, therefore, probably consist of more than one protein and this has been confirmed by 2-dimensional fractionation (Brown et al. 1980). For the sake of simplicity, double bands will be considered as one in this communication.

Some of the other varieties have polypeptides which are identical to those found in 'Chinese Spring' and they have therefore been assigned the same band number. The variety 'Holdfast' has 3 proteins not detected in 'Chinese Spring' (Fig. 1b). One of these, band 11, has only a slightly lower mobility than band 12 of 'Chinese Spring'. Band 1 of 'Holdfast' is also present in 'Bersée' (Fig. 1e). 'Bersée' lacks band 2 of 'Chinese Spring' (Fig. 1c) and band 5 of 'Holdfast' (Fig. 1d) but has a band 4 of intermediate mobility (Fig. 1e). The HMW band of fastest mobility from 'Bersée' appears to migrate more rapidly than band 11 of 'Holdfast' but, for the sake of simplicity, it has been assigned the same number. The varieties 'Spica' and 'Cheyenne' have a very similar banding pattern to each other (Fig. 1f, g) except 'Cheyenne' has an additional band of identical mobility to band 2 of 'Chinese Spring' (Fig. 1c). Bands 9 and 10 of 'Cheyenne' and 'Spica' (poorly resolved in Fig. 1f and g but much better resolved in Fig. 3b and c) were not detected in the other varieties. 'Hobbit sib' also has two new bands (Fig. 1h). One of them is band 3, intermediate in mobility between band 2 of 'Chinese Spring' (Fig. 1i) and band 4 of 'Bersée' (Fig. 1e). The other is band 6 which has a somewhat different mobility from subunits from the other varieties studied. The HMW subunits of 'Chinese Spring' and 'Cappelle-Desprez' are similar except the latter variety does not contain the band 8 subunits (Fig. 1j).

The relative mobilities of all 12 numbered subunits are drawn in Fig. 2 and compared to the HMW subunits of 'Chinese Spring'.

2 *Identification of the HMW Polypeptides as Subunits of Glutenin*

The AUC-extracts of 5 varieties were fractionated by gel filtration. In each case the HMW polypeptides eluted at, or just after, the void volume of the column and well before classical gliadin was eluted. A typical fractionation was shown in a previous publication (Payne and Corfield 1979). The results strongly suggest that the polypeptides are HMW subunits of glutenin and this is in agreement with the work of others (Orth and Bushuk 1973a; Bietz, Shepherd and Wall 1975). The varieties extracted contained different combinations of all the polypeptides used in the subsequent genetic analysis other than band 4 (from 'Bersée'). The proteins from two varieties ('Chinese Spring' and 'Holdfast') were also fractionated by differential solubility in different solvents. The HMW subunits were not extracted in water, 0.5 M NaCl, and 70% (w/v) ethanol at room temperature but they did partially dissolve in 0.1 N acetic acid (results not shown). This confirms their identity as subunits of glutenin.

3 *Analysis of Aneuploid Stocks*

a) Genetic Theory

Endosperm tissue of cereals develops from the fusion of a diploid nucleus, formed from the combination of two genetically-identical polar nuclei, with a haploid pollen grain nucleus, so that all endosperm cells are triploid. The aneuploid lines used in this study were obtained by self-fertilising monosomics (2n = 41) that were identified by cytological methods. By selfing a monosomic, the genetic constitution of the embryo and endosperm of the resulting seed will vary depending on the transmission of the monosomic chromosome to the male and female gametes. Because the monosomic chromosome tends to lag behind the other chromosomes during meiosis, only about 25% of nuclei emerging from meiosis carry this chromosome, whereas 75% are deficient (Sears 1954) and so are nulliso-

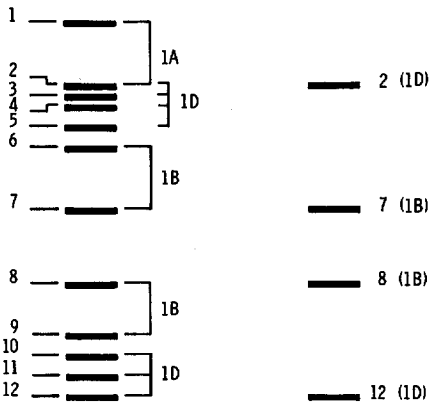

Fig. 2. Relative electrophoretic mobilities of the HMW subunits of glutenin from seven varieties: 'Bersée', 'Cappelle-Desprez', 'Cheyenne', 'Hobbit sib', 'Holdfast' and 'Spica'. The HMW subunits of 'Chinese Spring' are drawn on the right for comparison. The molecular weights of the subunits were determined using 4 protein standards: *E. coli* RNA polymerase, α subunit (160,000), bovine serum albumin (68,000), RNA polymerase, β subunit (39,000) and soybean trypsin inhibitor (21,500), and assuming that electrophoretic mobility is inversely related to the log molecular weight. Subunit 1 had a nominal molecular weight of 145,000; subunit 2, 136,000; subunit 6, 127,000; subunit 8, 106,000 and subunit 12, 95,000

mic. Functional female gametes therefore occur in these frequencies. This is not the case for male gametes however, because 21-chromosome gametes compete more effectively in fertilization and only about 4% of the 20-chromosome gametes are transmitted. The frequencies of the various types of embryo and their endosperms obtainable from selfing a monosomic can therefore be predicted on the basis of these transmission frequencies and these are shown in Table 1.

This indicates that the majority of endosperms of monosomic seed will have only one dose of a critical chromosome compared with 3 for the disomic control. Consequently, if the amount of product is related to gene dosage, it should be possible to screen grains from a range of monosomic lines to determine the chromosomal control of the gene concerned.

Table 1. The predicted frequencies of the various types of embryo and their endosperms obtained from selfing a monosomic plant

		♂ gametes			
		A		–	
Approximate frequencies	♀ gametes	96%		4%	
25%	Endosperm AA	AAA	24%	AA–	1%
	Embryo A	AA		A–	
75%	Endosperm ––	A––	72%	–––	3%
	Embryo –	A–		––	

b) Results

A comprehensive list of the analyses is set out in Table 2. The banding pattern of the HMW proteins from a single grain of a 1B monosomic line of 'Cheyenne' is shown in Fig. 3a. Bands 7 and 9 are depressed in intensity (compared to 'Cheyenne') by more than 50%. This grain must therefore be monosomic and bands 7 and 9 (but not bands 2, 5, 10) are controlled by chromosome 1B. A second grain (Fig. 3b) is disomic and bands 7 and 9 are present in nor-

Table 2. Analysis of aneuploid lines

Sample	Type	Samples analysed	Observations
'Cappelle-Desprez' 1-2	1B monosomic series	Flour from mixed grain	Band 7 depressed
" " 14-1	1A " "	" " " "	No bands depressed
" " 17-5	1D " "	" " " "	Bands 2 and 11 depressed
" " 17-7	1D " "	" " " "	Bands 2 and 11 depressed
'Holdfast' 1-4	1B " "	" " " "	Bands 7 and 8 depressed
" 17-1	1D " "	" " " "	Bands 5 and 11 depressed
" 17-2	1D " "	" " " "	Bands 5 and 11 depressed
" 17-1	1D " "	4 single grains	2 grains had bands 5 and 11 depressed (monosomic) and 2 grains were normal (disomics)
" 1-1	1B " "	4 " "	3 grains had bands 7 and 8 depressed (monosomic) and 1 grain was normal (disomic)
" 6-1	6A " "	4 " "	All 4 grains normal
" 10	6B " "	4 " "	" " "
" 19	6D " "	4 " "	" " "
'Cheyenne' 17-1	1D " "	3 " "	2 grains had bands 5 and 10 depressed (monosomics) and 1 grain was normal (disomic)
" 17-2	1D " "	2 " "	1 grain had bands 5 and 10 depressed (monosomic) and the other was normal (disomic)
" 1-1	1B " "	3 " "	1 grain had bands 7 and 9 depressed (monosomic) and 2 grains were normal (disomic)
" 1-4	1B " "	2 " "	1 grain had 7 and 9 depressed (monosomic) and the other was normal (disomic)
" 14-6	1A nullisomic	Flour from mixed grain	Band 2 was deleted
'Spica' 1-2	1B monosomic series	3 single grains	All three grains had bands 7 and 9 depressed
" 17-2	1D " "	2 " "	Both grains had bands 5 and 10 depressed
'Hobbit' sib 1-1	1B " "	4 " "	1 grain had bands 6 and 8 deleted (nullisomic), 2 had bands 6 and 8 depressed (monosomics) and 1 was normal (disomic)
" 17-1	1D " "	4 " "	3 grains had bands 3 and 11 depressed (monosomics) and 1 grain was normal (disomic)
'Bersée' 14-1	1A " "	4 " "	All grains had band 1 depressed (monosomics)
" 17-1	1D " "	5 " "	3 grains had bands 4 and 11 depressed (monosomics) and 2 grains were normal (disomics)

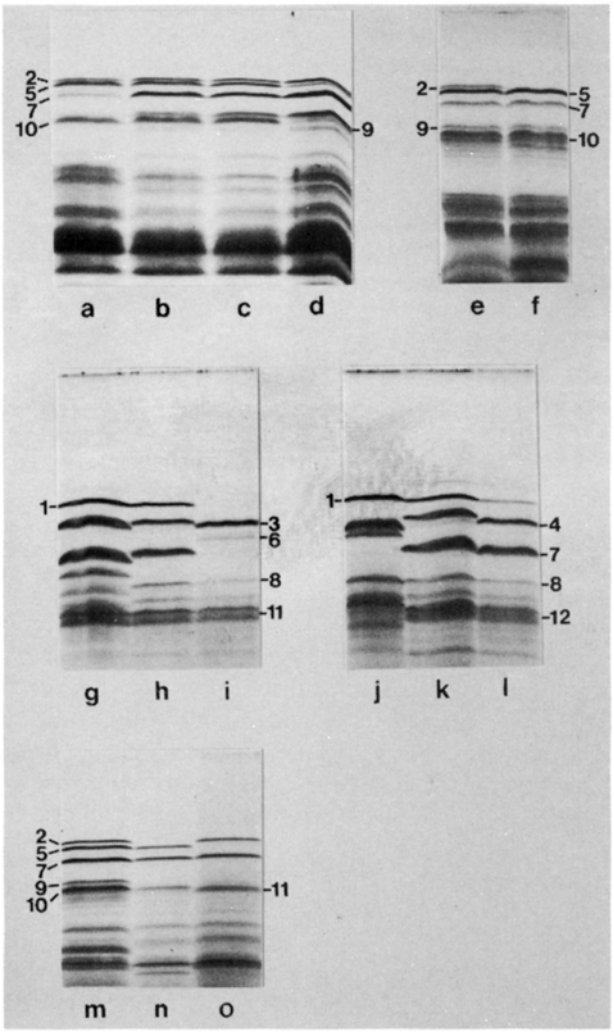

Fig. 3a-o. Analysis of aneuploid and substitution lines.
a 'Cheyenne', monosomic for chromosome 1B; b 'Cheyenne', disomic in a monosomic 1B series; c 'Cheyenne', disomic in a monosomic 1D series; d 'Cheyenne', monosomic for chromosome 1D; e 'Cheyenne'; f 'Cheyenne', nullisomic for chromosome 1A; g *T. spelta*; h Substitution line; chromosome 1A of *T. spelta* into 'Chinese Spring'; i 'Hobbit' sib, monosomic for chromosome 1B; j 'Hope'; k Substitution line; chromosome 1A of 'Hope' into 'Chinese Spring'; l 'Bersée', monosomic for chromosome 1A; m 'Cheyenne'; n Substitution line; chromosome 1D of 'Cheyenne' into 'Cappelle-Desprez'; o 'Cappelle-Desprez'. All samples were fractionated in 17% gels run for either 16 h (a-f, m, n, o) or 24 h (g-l)

mal amounts. The grain analysed in Fig. 3c was taken from a 1D monosomic line of 'Cheyenne' and its HMW bands were of similar intensity to 'Cheyenne'. A second grain (Fig. 3d) had bands 5 and 10 depressed, indicating that the synthesis of these two protein subunits is controlled by chromosome 1D.

By this type of analysis, control by homoeologous group 1 chromosomes was established for all the HMW subunits for the seven varieties except band 2 of 'Cheyenne' and band 1 of 'Holdfast'. Band 2 was shown to be completely lacking in a nullisomic 1A line of 'Cheyenne' (Fig. 3 e, f). Chromosomal control of band 1 from 'Holdfast' was established by more indirect methods. These are described in the next section.

4 *Chromosomal Control of Band 1 from 'Holdfast'*

Band 1 of 'Holdfast' was shown not to be controlled by chromosomes 1B, 1D, 6A, 6B or 6D (Table 1). Because band 1 of 'Bersée' is controlled by chromosome 1A (Table 2), the possibility of control of the 'Holdfast' subunit by the same chromosome was investigated next. Unfortunately, 'Holdfast' 1A aneuploid stocks were not available so instead the grain obtained from the F_1 monosomic hybrid resulting from crosses between 'Chinese Spring' monosomic for chromosome 1A and 'Holdfast' were analysed.

A preliminary analysis of 6 of the grains showed that the 'Holdfast' band 1 occurred either as strongly as in 'Holdfast' itself or, more commonly, at about half the normal intensity. If the subunit is controlled by chromosome 1A then those grains with band 1 faint would be monosomic for this chromosome, grains with a normal dosage of band 1 would be disomic and those with band 1 missing altogether would be nullisomic.

To test this prediction, the 15 remaining grains were each cut into two segments, the smaller segment containing the embryo. The embryos were germinated and chromosomes counted in the dividing root-tip nuclei. The corresponding halves were analysed for band 1. The results of some of the data are shown in Fig. 4. Of the 11 grains illustrated, 3 were identified by gel electrophoresis as disomics, 6 were monosomics and 2 nullisomics. The cytologi-

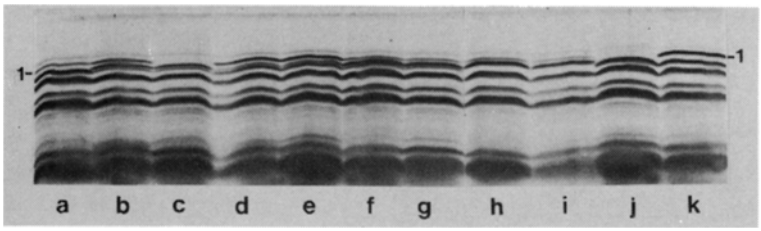

Fig. 4o-k. Single grain analysis of the F_2 progeny of a cross between 'Chinese Spring', monosomic for chromosome 1A, and 'Holdfast'. Grains a, b, f, g, h and i are monosomic, grains d, e and k are disomic and grains c and j are nullisomic

cal chromosome counting procedures were in agreement with these conclusions. We therefore conclude that band 1 of 'Holdfast' is, like band 1 of 'Bersée' and band 2 of 'Cheyenne', controlled by chromosome 1A.

5 Analysis of Inter-Varietal Chromosome Substitution Lines

Several chromosome substitution lines were also analysed and they confirmed several of the conclusions made in the analysis of the aneuploid stocks. One example of this type of analysis is given. The HMW subunits of 'Cappelle-Desprez' ('Cheyenne' 1D) are shown in Fig. 3n. This particular line is identical to 'Cappelle-Desprez' except its 1D chromosomes have been eliminated and replaced by those of 'Cheyenne'. From the above work on aneuploid stocks one would expect the substitution line to have bands 5, 7 and 10 instead of bands 2, 7 and 11 which occur in 'Cappelle-Desprez' (Fig. 3o), bands 5 and 10 being derived from 'Cheyenne' (Fig. 3m). This actually occurred (Fig. 3n) thus confirming that chromosome 1D codes for bands 2, 5, 10 and 11.

In another set of substitution lines (not shown, but see Brown et al. 1980 for a more detailed analysis) the $1C^u$ chromosomes of *Aegilops umbellulata* were substituted into 'Chinese Spring' for chromosome 1A in one stock, chromosome 1B in another stock and 1D in a third stock. The analysis confirmed the findings of Orth and Bushuk (1973b) and Bietz, Shepherd and Wall (1975) that chromosome 1B controls the synthesis of bands 7 and 8 and chromosome 1D controls bands 2 and 12 of 'Chinese Spring'.

Triticum spelta and the variety 'Hope' each contain a polypeptide of identical mobility to band 1 of 'Holdfast' and 'Bersée'. Fig. 3g and h show the HMW polypeptides of the *T. spelta* 1A chromosomes substituted into 'Chinese Spring' and Fig. 3j and k the Hope 1A chromosomes also substituted into 'Chinese Spring'. The results show that the HMW polypeptide in both, like band 1 of 'Holdfast' and 'Bersée', is controlled by chromosome 1A.

6 Segregation of Subunits 2 and 5 in the F_2 Progeny of Crosses Between 'Chinese Spring' and 'Holdfast'

The proximity of the genes on chromosome 1D which control the synthesis of subunits 2 and 5 was studied next. The F_2 progeny of crosses between 'Chinese Spring' (subunit 2 present, subunit 5 absent) and 'Holdfast' (subunit 2 absent, subunit 5 present) were examined by single-grain analysis. Four types of banding pattern were obtained (Fig. 5): (1) subunit 2 only, (2) subunit 5 only, (3) subunits 2 and 5 present at levels of intensity 2: 1, and (4) subunits 2 and 5 present at a ratio of 1 : 2. If the gene for subunit 2 is designated A and that for subunit 5 is B, then the above progeny have genetic complements of AAA, BBB, AAB and ABB respectively (Table 3). None of the progeny had banding patterns expected for recombinants. However, it is possible that some recombinant classes could be indistinguishable from the parental classes. Thus, it could be envisaged that AAAB, ABBB, AA and BB would be difficult to distinguish from AAB, ABB, AAA and BBB respectively. However, all the other recombinant classes should be recognisable since either they would give rise to two intensely stained bands (AAABB), bands of equal intensity (AAABBB, AABB and AB), single faint bands (A or B) or no bands at all (- -). In none of the 138 grains examined were these possible recombinant classes observed. As a consequence it is unlikely that misclassification has occurred.

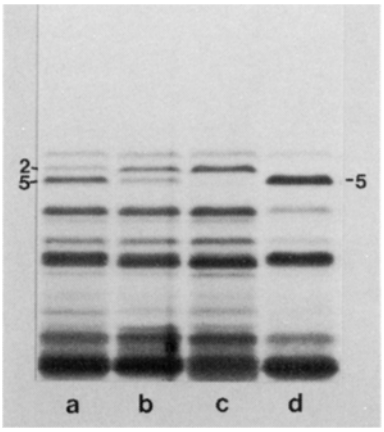

Fig. 5a-d. Single grain analysis of the F_2 progeny of a cross between 'Chinese Spring' and 'Holdfast'. Slot a, bands 2 and 5 present at an approx. ratio of 1:2; Slot b, bands 2 and 5 present at an approx. ratio of 2:1; Slot c, band 2 present, band 5 absent; Slot d, band 5 present, band 2 absent

The frequencies of the 4 parental classes were scored (Table 4) and the results suggest they were present in a 1:1:1:1 ratio. The experimental deviation from this simple ratio was shown to be insignificant $\{\chi^2_3 = 2.17, P\ 0.7\text{-}0.5\}$.

Table 3. Punnet square of the expected F_2 progeny from a 'Chinese Spring', 'Holdfast' cross. The gene for subunit 2 is designated A and that for subunit 5 is B. The progeny in the dashed square are parental types and those outside are recombinant types

		♂ gametes			
		A	B	AB	–
	AA	AAA	AAB	AAAB	AA
	BB	ABB	BBB	ABBB	BB
♀ gametes	AABB	AAABB	AABBB	AAABBB	AABB
	–	A	B	AB	–

It was calculated that the maximum distance that the genes for subunits 2 and 5 can be apart, given a probability level of 0.05, is 0.01cM (Hanson 1959). The genes, therefore, may well be allelic.

Discussion

There is considerable variation in the composition of HMW glutenin subunits in different varieties (Orth and Bushuk 1973a; Bietz, Shepherd and Wall 1975). A selection of these subunits has been examined for chromosomal control in this communication using a range of aneuploid stocks and inter-varietal chromosome substitution lines. Each of the subunits analysed was shown to be controlled by one of the homoeologous group 1 chromosomes (Fig. 2). Unfortunately, we cannot definitely conclude that these chromosomes carry the structural genes for the HMW subunits because the effects observed could conceivably have been caused by the presence of genes whose products affect the synthesis or processing of the subunits. Using monosomic stocks, unambiguous assignment of structural genes to a chromosome can only be made if all the homoeologous groups are analysed and decreased synthesis is demonstrated for individual grains in only one stock.

It was shown that subunits of identical mobilities in different varieties were controlled by the same chromosome. The only exception was band 2; in varieties 'Chinese Spring' and 'Cappelle-Desprez' it was controlled by chromosome 1D and in 'Cheyenne' by chromosome 1A. The 'Cheyenne' band 2 stains rather faintly with Coomassie Blue (Fig. 1g, 3b, e) compared to band 2 of 'Chinese Spring' and 'Cappelle-Desprez' (Fig. 1a, c, j) and a survey of other varieties (unpublished data) suggests that the subunit controlled by chromosome 1D is by far the most common form. This conclusion is supported by the work of Orth and Bushuk (1973b) and Khan and Bushuk (1977). They analysed the HMW glutenin subunits of 4 hexaploid wheats and their derived AABB tetraploids. In 3 of the 4 cases, the tetraploids lacked a subunit in the region where band 2 would migrate to, showing control by the D genome.

Of the seven varieties analysed, two major subunits were always controlled by chromosome 1D and either 1 or 2 subunits by chromosome 1B. In contrast, chromosome 1A controlled only single subunits in just 2 of the 7 varieties and none in the rest. One of the subunits was band 1 of 'Holdfast'. This is a particularly interesting finding because the presence of this subunit in glutenin has been linked to bread-making quality in PBI winter wheat varieties (Payne, Corfield and Blackman 1979). The subunit is somewhat uncommon in glutenin, occurring in 3 of the 8 varieties examined in this study and in 28 of 110 varieties surveyed (unpublished data).

The single-grain analysis of the F_2 progeny from the cross between 'Chinese Spring' and 'Holdfast' suggests that subunits 2 and 5 are coded for by genes which are very close together on the same chromosome and are problably allelic. We envisage just two loci on the 1B and 1D chromosomes and probably 1 on the 1A chromosome which code for the HMW glutenin subunits and that the variation observed in the banding patterns of SDS-polyacrylamide gels of different varieties are simply allelic variants. Thus, bands 2-5 (but not band 2 of 'Cheyenne') are allelic variants of genes at one locus of the 1D chromosome, shown by Orth and Bushuk (1974) and Bietz, Shepherd and Wall (1975) to be located on the long arm. Subunits 6 and 7 are probably allelic variants of a gene or gene cluster on the 1B chromosome. Evidence for this comes from the finding that 19 varieties contain band 6 and 61 varieties band 7 but only two have both and these were shown to be caused by heterogeneous stocks (unpublished data).

Further work is in progress to identify more allelic variants and to determine more precisely the location of the genes which code for the HMW glutenin subunits.

Literature

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Table 4. Segregation of two subunits controlled by chromosome 1D in the F_2 generation of crosses between 'Holdfast' and 'Chinese Spring'

Cross	Subunit 2 (Chinese Spring)	Subunit 5 (Holdfast)	2:1 subunit 2:5	1:2 subunit 2:5
CS 14 × HD 1	4	3	6	4
CS 14 × HD 2	2	1	5	2
CS 21 × HD 2	18	12	17	16
CS 21 × HD 3	11	13	13	11
Total (138)	35	29	41	33

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