Theor Appl Genet (1988) 75:628–641

Linkage mapping of genes controlling endosperm storage proteins in wheat*

1. Genes on the short arms of group 1 chromosomes

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Received August 3, 1987; Accepted September 22, 1987
Communicated by R. Riley

Summary. A translocation mapping procedure was used to map gene-centromere distances for the genes controlling endosperm proteins on the short arm of each of the chromosomes 1A, 1B and 1D in wheat. The genes controlling triplet proteins (tentatively designated *Tri-1*) were found to be closely linked to the centromere on chromosome arms 1AS and 1DS and loosely linked to the gliadin genes (*Gli-1*) on the same arms. The *Gli-1* genes segregated independently or were very loosely linked to their respective centromeres. The *Gli-B1*-centromere map distance on 1BS was also estimated using conventional telocentric mapping and the result was similar to that obtained with the translocation mapping. A simple two-step one-dimensional electrophoretic procedure is described which allows the low-molecular-weight (LMW) glutenin subunits to be separated from the gliadin bands, thus facilitating the genetic analysis of these LMW subunits. No recombination was observed between the genes (designated *Glu-3*) controlling some major LMW glutenin subunits and those controlling gliadins on chromosome arms 1AS and 1DS. However, in a separate experiment, the genes controlling LMW glutenin subunits on 1BS (*Glu-B3*) showed a low frequency of recombination with the gliadin genes.

Key words: Wheat – Triplet proteins – Gliadins – Glutenins – Linkage mapping

Introduction

The short arms of homoeologous group 1 chromosomes in wheat control at least three distinct groups of endosperm storage proteins viz: gliadins (Shepherd 1968; Wrigley and Shepherd 1973), LMW glutenin subunits (Jackson et al. 1983; Payne et al. 1984a; Singh and Shepherd 1984b, 1985) and triplet proteins (Singh and Shepherd 1984b, 1985, 1987). Much effort has been made to determine the linkage relationship between these genes and their map positions on the chromosome. The genes on the short arms of chromosomes 1A, 1B and 1D controlling monomeric gliadins were designated *Gld-1A*, *Gld-1B* and *Gld-1D*, respectively, by Sozinov and Poperelya (1980) and *Gli-A1*, *Gli-B1* and *Gli-D1* by Payne et al. (1982). The genes controlling individual gliadin bands on each of these chromosomes have been shown to segregate as a unit (block) with nil or very rare recombination (Sozinov and Poperelya 1980; Metakovsky et al. 1984). Furthermore, recently it has been shown that the genes controlling monomeric gliadins are also very closely linked with those controlling LMW glutenin subunits, on chromosome arms 1AS and 1BS (Payne et al. 1984a).

The *Gli-1* and *Glu-1* loci, located on the opposite arms of homoeologous group 1 chromosomes, have been shown to segregate independently (Lawrence and Shepherd 1981b; Chojecki et al. 1983) or, sometimes to exhibit loose linkage (Payne et al. 1982). However, more precise information on their location has come from mapping experiments which have included an interstitial locus, or the centromere, between them. The *Gpi-D1* locus, coding for an isozyme of glucose-phosphate isomerase, has been located proximal to the *Gli-D1* locus on chromosome arm 1DS and it showed 34.5% recombination with *Gli-D1* and 36.2% recombination with *Glu-D1* on the long arm (Chojecki et al. 1983). Galili and Feldman (1984) found that the gene controlling a particular protein band (B-30) mapped proximally to the *Gli-B1* locus, showing 25.5% recombination with *Gli-B1*, and 23.5% recombination with *Glu-B1* on the long arm. Recently, Jackson et al. (1985) mapped a new locus, *Glu-B2*, controlling the D group of LMW glutenin subunits, between *Glu-B1* and *Gli-B1*.

Although considerable progress has been made in the last few years towards mapping the endosperm protein genes in wheat, there are still some significant gaps in our knowledge. For example, the genes controlling triplet proteins and those controlling LMW glutenin subunits on chromosome arm 1DS have not

* Portion of the Ph.D. thesis submitted by the senior author

been mapped with respect to each other or the centromere. Furthermore, only one of the seed protein genes located on the short arms of group 1 chromosomes has been mapped directly with respect to the relevant centromere. Thus Rybalka and Sozinov (1978) found 42% recombination between a gliadin gene on 1BS and the centromere.

In this paper a tanslocation mapping procedure described earlier (Singh and Shepherd 1984a) was used to map the gene-centromere distances for the genes controlling triplet proteins, ω-gliadins and LMW glutenin subunits on each of the short arms of group 1 chromosomes. In addition, the map distance between *Gli-B1* and the centromere was estimated using the conventional telocentric mapping procedure (Sears 1962, 1966) for comparison with the translocation mapping results.

Materials and methods

2.1 Genetic stocks

The wheat parents included cultivars Chinese Spring (CS), Hope, Gabo, India 115, and Halberd; intervarietal chromosome substitution lines CS-Hope 1A and CS-Hope 1B with, respectively, chromosomes 1A and 1B of Hope substituted for their counterparts of CS (Sears et al. 1957); CS ditelocentric (Dt) lines Dt 1AL, Dt 1BL and Dt 1BS (Sears 1954; Sears and Sears 1978) and a Warigal 1DL-1RS translocation derived from the original 1DL-1RS translocation isolated in CS (Shepherd 1973). All of these stocks are maintained at the Waite Agricultural Research Institute. Translocation lines 1AS-1RL (Singh and Shepherd, unpublished), 1BS-1RL and 1DS-1RL (Lawrence and Shepherd 1981a) were all selected in a CS wheat background and the chromosome arm 1RL in each of these lines was derived from CS-Imperial rye addition line 'E' (Driscoll and Sears 1971). These translocations are thought to have arisen by the fusion of wheat and rye telocentrics produced after simultaneous misdivision of univalents in double monosomics (Shepherd 1973).

2.2 Gene symbols used for endosperm proteins of wheat and rye

The symbols used to describe the genes controlling endosperm storage proteins, located on group 1 chromosomes of wheat and rye, are listed in Table 1. The genes for high-molecular-weight (HMW) glutelin subunits in wheat and rye (*Glu-A1* etc.) have been assigned the symbols introduced by Payne et al. (1982) and Singh and Shepherd (1984a), respectively. The symbols for gliadin genes (*Gli-A1* etc.) follow Payne et al. (1982) whereas the *Sec-1* symbol for ω-secalins follows Shewry et al. (1984). The genes for LMW glutenin subunits, which are closely linked with the *Gli-1* locus, have been given the symbol *Glu-3* to distinguish them from *Glu-2* already assigned to the genes controlling some LMW acidic subunits of wheat glutenin (Jackson et al. 1985). The designations *Tri-A1* and *Tri-D1* for the genes controlling triplet proteins conform to the nomenclatural system used for the glutelin and gliadin genes.

The allele symbols denoted for the HMW glutelin subunits of wheat and rye follow Payne and Lawrence (1983) and Singh and Shepherd (1984a), respectively. However, in the absence of published symbols for alleles of the other proteins, they have been described by the initial letter(s) of the cultivars possessing them (Table 1).

2.3 Gene mapping procedures

The crossing procedures used for mapping of the *Tri-1*, *Gli-1* and *Glu-3* genes are depicted in Fig. 1.

2.3.1 Translocation mapping. The principle of the translocation mapping procedure used to map the gene-centromere distances on the group 1 chromosomes of wheat was similar to that described earlier when mapping the *Glu-R1* locus on the long arm of rye chromosome 1R (Singh and Shepherd 1984a).

Table 1. Gene and allele symbols used for the endosperm storage proteins in wheat and rye

Protein class	Arm location	Gene symbol	Allele symbol (Cultivar)	References	
				Genes	Alleles
HMW glutelin subunits	1AL	*Glu-A1*	*a* (Hope); *c* (CS)	1	2
	1BL	*Glu-B1*	*b* (CS); *d* (Hope); *e* (Halberd); *i* (Gabo)	1	2
	1DL	*Glu-D1*	*a* (CS, India 115); *d* (Warigal)	1	2
	1RL	*Glu-R1*	*i* (Imperial)	3	3
ω-Prolamins	1AS	*Gli-A1*	*cs* (CS); *h* (Hope); *g* (Gabo)	1	*
	1BS	*Gli-B1*	*cs* (CS); *h* (Halberd); *g* (Gabo, Hope)	1	*
	1DS	*Gli-D1*	*cs* (CS); *i* (India 115); *h* (Halberd)	1	*
	1RS	*Sec-1*	*i* (Imperial)	4	*
LMW glutenin subunits	1AS	*Glu-A3*	*cs* (CS); *h* (Hope)	*	*
	1BS	*Glu-B3*	*cs* (CS); *h* (Hope)	*	*
	1DS	*Glu-D3*	*cs* (CS); *i* (India 115)	*	*
Triplet proteins	1AS	*Tri-A1*	*cs* (CS); *h* (Hope)	5	*
	1DS	*Tri-D1*	*cs* (CS); *i* (India 115)	5	*

CS = Chinese Spring; References: [1] Payne et al. 1982; [2] Payne and Lawrence 1983; [3] Singh and Shepherd 1984a; [4] Shewry et al. 1984; [5] Singh et al. (1986); * new symbols

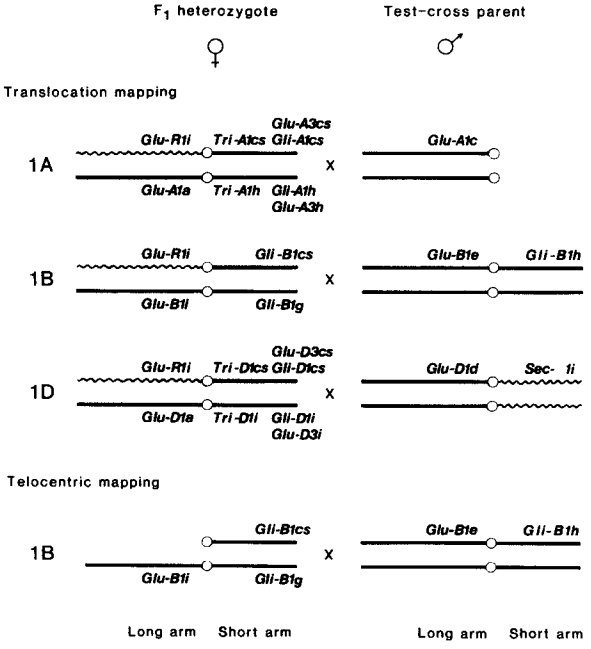

Fig. 1. Crossing procedures for mapping genes controlling wheat endosperm proteins on the short arms of group 1 chromosomes. Translocation mapping: *1A* (CS 1AS-1RL×CS-Hope 1A)×CS Dt 1AL; *1B* (CS 1BS-1RL×Gabo)×Halberd; *1D* (CS 1DS-1RL×India 115)×Warigal 1DL-1RS Telocentric mapping: *1B* (CS Dt 1BS×Gabo)×Halberd. Gene and allele symbols are explained in Table 1. *CS* = Chinese Spring

Each of the translocation lines was first hybridized with another wheat cultivar, chosen to have electrophoretically different protein bands at the loci of interest on both the long and short arms of the group 1 chromosome being analysed. To ensure that all of the electrophoretic differences between the two parents could be recognized in the test-cross progeny, care was taken to choose a male test-cross parent which did not contribute any band overlapping those of interest in the F_1.

The method may be illustrated with reference to mapping *Tri-A1*, *Gli-A1* and *Glu-A3* loci on chromosome arm 1AS. Providing that the translocation arose from centric fusion between 1AS and 1RL, normal pairing and crossing over is expected to occur between the *homologous* short arms but no pairing is expected between the *homoeologous* chromosome arms 1AL and 1RL because of the inhibitory effect of the *Ph-1b* gene on chromosome 5B. Since there was no pairing between the long arms, the Glu-1 proteins could be used as markers for the centromere. Thus the F_1 heterozygote could be used to map the position of any gene on the short arm of 1A with respect to the centromere in a manner analogous to the telocentric mapping method developed by Sears (1962, 1966).

3.2 Telocentric mapping. Telocentric mapping of the *Gli-B1* genes was carried out concurrently with the translocation mapping of this locus to compare these two methods of mapping gene-centromere distances. The crossing procedure used was similar to the translocation mapping except CS Dt 1BS was used in place of the CS 1BS-1RL translocation (Fig. 1, 1B).

3.3 Intrachromosomal mapping. The recombination frequency occurring between the *Gli-B1* and *Glu-B3* genes on chromosome arm 1BS and that between these genes and the *Glu-B1* locus on the long arm of 1B was determined using the triparental cross: (CS×CS-Hope 1B)×CS Dt 1BL.

4 Cytological analysis

Pollen mother cells (PMCs) from F_1 heterozygotes were analysed to find the degree of pairing between the wheat-rye translocation chromosomes and their normal group 1 wheat chromosome homologues. The anthers used in the cytological analysis were collected from the same F_1 plants that were used for producing test-cross seeds. PMCs were analysed both at diakinesis and at metaphase I to estimate the degree of desynapsis (precocious terminalisation of chiasmata) which had occurred between the two stages. Squash preparations of metaphase I PMCs were stained with a C-banding procedure, modified from Vosa and Marchi (1972). Upon C-banding the long arm of chromosome 1R of Imperial rye showed a well-defined telomeric heterochromatin band (Singh and Shepherd 1984a) while none of the wheat chromosomes showed a telomeric C-band. Thus, the pairing of translocation chromosomes with their normal wheat counterparts could be observed directly. The standard Feulgen technique was used for observing chromosome pairing at diakinesis.

5 Seed protein extraction and electrophoretic separation

Total proteins were extracted from endosperm halves of single kernels as described earlier (Singh and Shepherd 1985). Unreduced proteins were extracted using the sample buffer without 2-mercaptoethanol (2-ME) and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine the triplet protein and gliadin phenotypes. These extracts were then reduced with a drop of 2-ME and subjected to further SDS-PAGE to observe the HMW glutelin phenotypes.

The LMW glutenin subunits were separated by a two-step one-dimensional (1-D) SDS-PAGE system as follows: In the first step, 30 µl of unreduced total protein extract from each of 20 individual seeds was loaded in the slots of a 1.4 mm thick slab gel with a separating gel containing 10% (w/v) acrylamide, 0.08% (w/v) bisacrylamide and a stacking gel with 3% (w/v) acrylamide, 0.08% (w/v) bisacrylamide. After electrophoresis for about 1 h at 50 mA/gel, a 1 cm strip of the separating gel adjacent to the origin was cut and removed to an equilibration solution as described for 2-D electrophoresis (Singh and Shepherd 1985). In the second step, this gel strip now containing reduced proteins was first washed with distilled water and then loaded onto the stacking gel (lacking slots) of a new slab gel with either the same composition as used in the first step or a 7.5–15% acrylamide gradient gel. The gel strip was loaded by forcing it between the two glass side plates, and this was facilitated by making a slightly thicker gel (1.8 mm) for the second step electrophoresis. After loading, electrophoresis was carried out at a constant current of 25 mA/gel until the dye front (2–3 drops of 1% (w/v) bromophenol blue added to the top buffer tank) reached the anodal end of the gel. Both the HMW and LMW glutelin subunits could be scored in these two-step gels.

6 Genetic analysis

Recombination fractions (p) were calculated directly by dividing the observed number of recombinants (R) with the total number of progeny analysed (n) excluding the aneuploid progeny. The standard error (S_p) of the recombination frac-

tion was calculated using the formula for the binomial distribution (c.f. Mather 1951)

$$S_p = \sqrt{[p(1-p) \div n]} \quad \text{(i)}$$

Where no recombinants were detected between two protein markers in the test-cross progeny, the upper limit (at the 95% confidence limit) for the recombination fraction (p) was calculated using the method of Hanson (1959).

$$p = [1-(0.05)^{-n}] \quad \text{(ii)}$$

where, n = number of euploid progeny analysed.

Map distances (cM) and their standard errors were calculated from recombination frequencies, using the Kosambi function (Kosambi 1944) as applied earlier in the linkage mapping of wheat proteins by Payne et al. (1982).

$$cM \pm SE = 25 \times \ln [(100+2R) \div (100-2R)] \pm 2{,}500\, S_R \div (2{,}500-R^2) \quad \text{(iii)}$$

where, R = recombination percentage; S_R = standard deviation of R.

Results

1 Cytological analysis of F_1 heterozygotes

The translocation chromosomes 1AS-1RL, 1BS-1RL and 1DS-1RL, possessing chromosome arm 1RL from Imperial rye, could be identified easily in C-banded preparations of PMCs at metaphase I because 1RL has a prominent telomeric heterochromatin band (eg. Fig. 2 a, b). However, these C-bands were not clear at diakinesis and then Feulgen staining was used instead; the bivalents involving translocations were recognized by their heteromorphic configuration (Fig. 2c). Telocentric 1BS was easily identified at both metaphase I and diakinesis because of its much smaller size and the presence of a prominent satellite. The chromosome pairing data are summarized in Table 2.

The glass-house where these plants were grown showed considerable seasonal temperature fluctuations and this was reflected in the chromosome pairing data. In a given season, the data for pairing between the reference chromosomes were homogeneous among F_1 plants of the same genotypic constitution and therefore only pooled data are shown. The pairing frequency at

Table 2. Observed frequency of pairing between complete wheat chromosomes and the homologous short arms of wheat-rye translocations and 1BS telocentric at metaphase I and diakinesis

F_1 combination	season grown	Meiotic stage[a]	No. of PMCs	No. paired	% pairing
CS 1AS-1RL	Spring	MI	209	189	90.4
×CS-Hope 1A	(1982)	Dk	80	79	98.8
CS 1BS-1RL	Summer	MI	207	123	59.4
×Gabo	(1981)	Dk	52	46	88.5
	Spring	MI	217	177	81.6
	(1982)	Dk	101	94	93.1
CS Dt 1BS	Spring	MI	249	122	49.0
×Gabo	(1982)	Dk	63	45	71.4
CS 1DS-1RL	Summer	MI	265	158	59.6
×India 115	(1982)	Dk	45	41	91.1
	Spring	MI	273	191	70.1
	(1982)	Dk	38	36	94.7

[a] MI = metaphase I; Dk = diakinesis

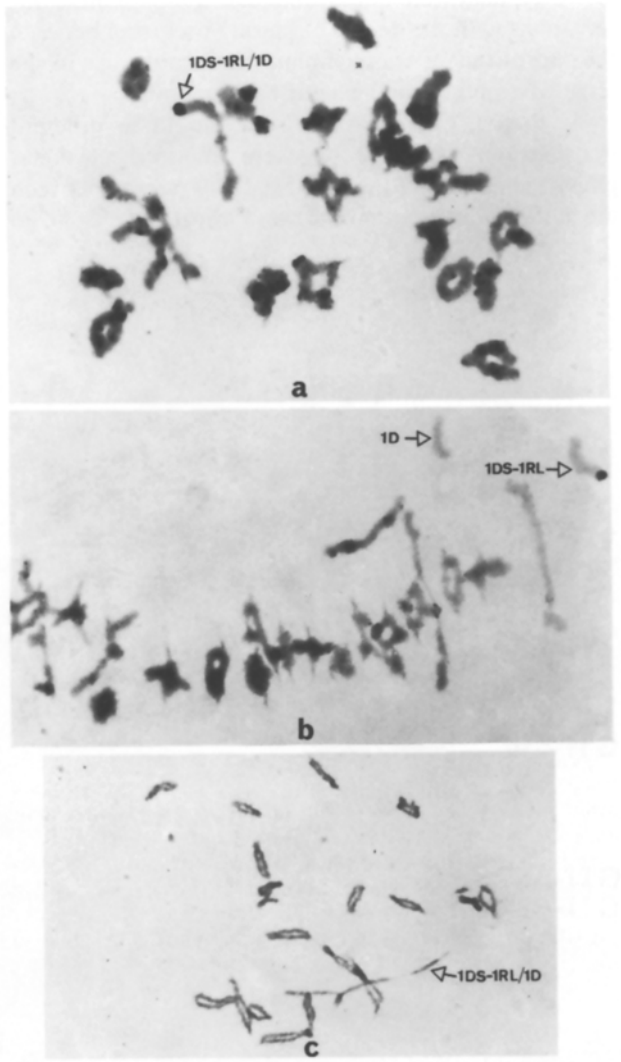

Fig. 2a–c. Squash preparations of PMCs from the F_1 heterozygote (CS 1DS-1RL×INDIA-115) used to map the genes on chromosome arm 1DS. **a** C-banded metaphase I cell showing 1D/1DS-1RL bivalent; **b** C-banded metaphase I cell showing 1D and 1DS-1RL univalents; **c** Feulgen stained diakinesis cell showing 1D/1DS-1RL bivalent

metaphase I was always lower than that at diakinesis suggesting the occurrence of desynapsis. In summer when the average outside temperature was quite high, the metaphase I pairing was significantly reduced even though plants had identical genotypes (Table 2). However, the reduction in pairing frequency was less pronounced at diakinesis. These results are consistent with the findings of Fu and Sears (1973) who also observed reduction in pairing at higher temperature.

The complete 1B chromosome paired much more frequently with the 1BS-1RL translocation than with the 1BS telocentric at both metaphase I and diakinesis (Table 2). Thus, the presence of rye arm 1RL appears to promote pairing between the 1BS arms on the other side of the centromere, rather than interfere with it. However, data were not available to compare these observations with the degree of pairing occurring between the short arms of two complete 1B homologues in the same environment and genetic background.

A total of 1171 PMCs from the three different translocation heterozygotes were analysed at metaphase I and none of them showed any pairing between the homoeologous arms of rye and wheat.

2 Gene mapping

2.1 Mapping the Tri-A1, Gli-A1 and Glu-A3 *loci on chromosome arm 1AS.* The band patterns produced by alleles *Tri-A1cs* and *Tri-A1h* for triplet proteins, *Gli-A1h* for gliadin (Fig. 3A), *Glu-A3cs* for LMW glutenin subunit and homoeoalleles *Glu-R1i* and *Glu-A1a* for HMW glutelin subunits (Fig. 3B) are distinctive in the parents but no distinctive protein band could be associated with alleles *Gli-A1cs* and *Glu-A3h.* Thus the presence of these alleles in the test-cross progeny was only inferred by the absence of the alternative alleles. The *Gli-A1cs* allele produced a band but it had the same electrophoretic mobility as one of the Gli-A1h bands (Fig. 3A). The triplet and gliadin phenotypes (Tri-A1 and Gli-A1) were determined by one-dimensional SDS-PAGE of unreduced extracts (Fig. 3A) whereas, the glutelin proteins (Glu-A1, Glu-R1 and Glu-A3) were scored after two-step1-D SDS-PAGE (Fig. 3B). The normal 1-D SDS-PAGE of reduced proteins allowed the HMW glutelin subunits to be scored but it could not be used for scoring triplet proteins, gliadins or LMW glutenin subunits (Fig. 3C).

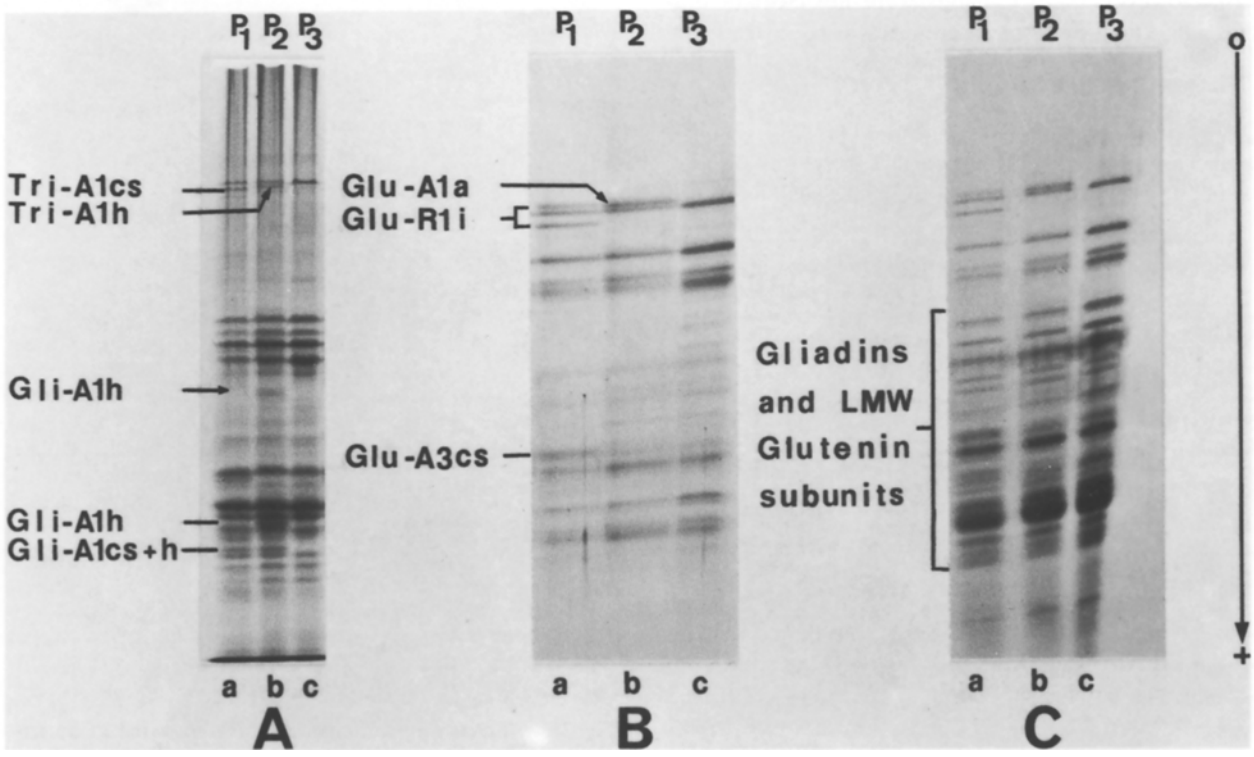

Fig. 3A–C. SDS-PAGE patterns of total seed protein extracts from the parental lines (P_1, P_2, P_3) used to map the *Tri-A1, Gli-A1* and *Glu-A3* genes. The distinctive protein bands for each allele are labelled in roman letters. **A** unreduced proteins; **B** two-step electrophoresis of reduced proteins (see section *2.5* for details); **C** 1-D SDS-PAGE of reduced proteins. *a* (P_1) CS 1AS-1RL translocation; *b* (P_2) CS-Hope 1A substitution; *c* (P_3) CS Dt 1AL

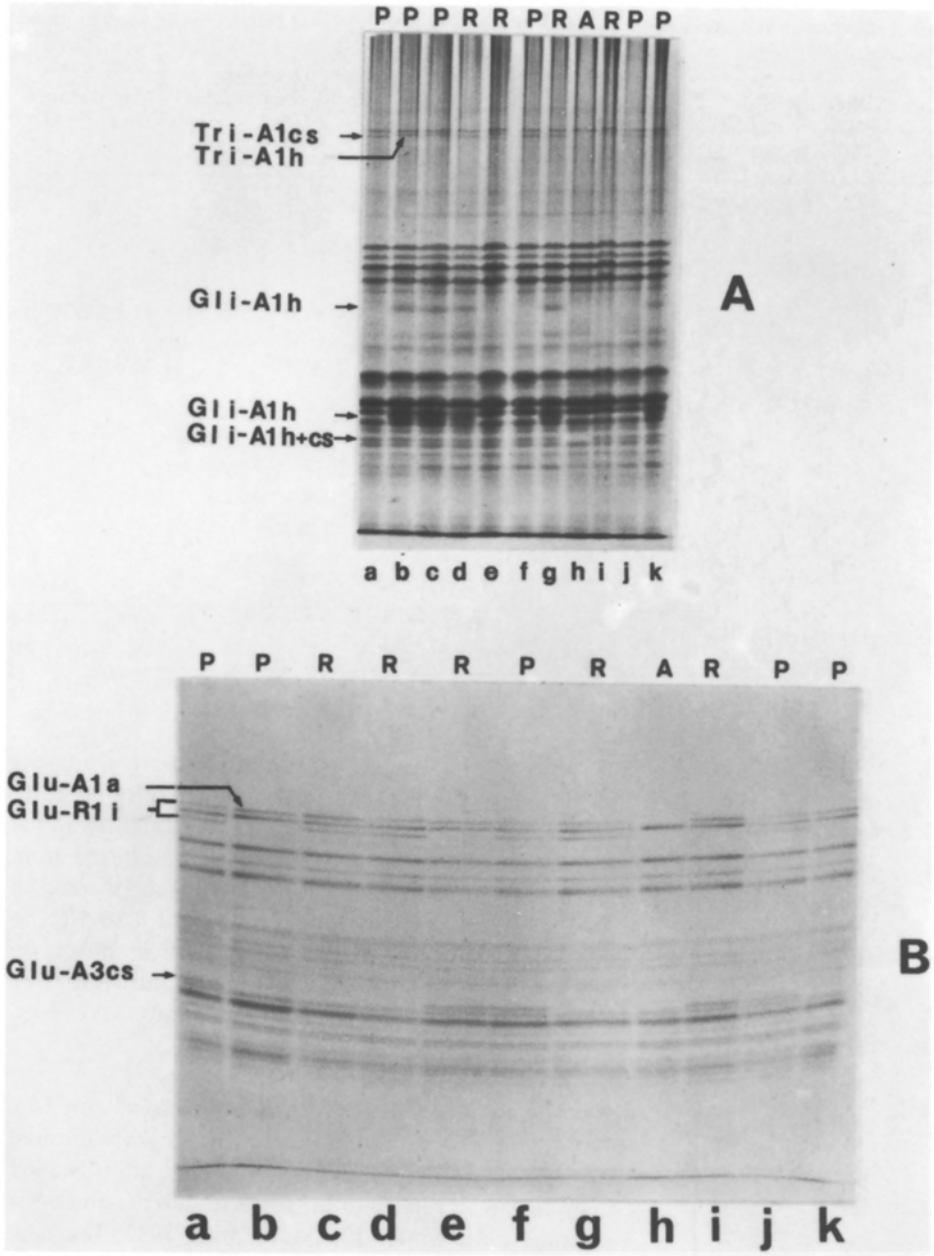

Fig. 4 A, B. SDS-PAGE patterns of total seed protein extracts from some test-cross progeny (a–k) used to map the *Tri-A1*, *Gli-A1* and *Glu-A3* genes. **A** unreduced proteins, showing recombination between the *Tri-A1* and *Gli-A1* genes; **B** two-step electrophoresis of reduced proteins from the same individual seeds analysed in part **A**, showing recombination between the centromere (marked with *Glu-1*) and the *Glu-A3* genes; *P* = parental; *R* = recombinant; *A* = aneuploid (hypoploid)

Since the male parent CS Dt 1AL (P_3 in Fig. 3A, B) used in the test-cross lacked all 1A and 1R protein markers, the test-cross progeny with parental (P), recombinant (R) and aneuploid (A) phenotypes could be easily distinguished (Fig. 4A, B). Altogether 296 test-cross seeds from two F_1 plants were analysed and since the data were homogeneous ($\chi^2_{[6]} = 5.6$, $P > 0.3$) the pooled values are given in Table 3. The presumed homoeoalleles *Glu-A1a* and *Glu-R1i* and the presumed allele pairs *Tri-A1cs/Tri-A1h* and *Gli-A1cs/Gli-A1h* segregated in the expected 1:1 ratio ($\chi^2_{[1]} = 2.4$, 0.0 and 0.01, respectively). Analysis of the joint segregation indicated linkage between the centromere and *Tri-A1* locus ($\chi^2_{[1]} = 171.6$) and between *Tri-A1* and *Gli-A1* loci ($\chi^2_{[1]} = 11.2$) but no linkage between the centromere and the *Gli-A1* locus ($\chi^2_{[1]} = 0.13$). No recombination

Table 3. Protein phenotypes and their frequency in test-cross progeny used to map the *Tri-A1, Gli-A1* and *Glu-A3* loci, grouped into parental, recombinant and aneuploid classes

Progeny class	Protein phenotypes			No. of progeny	Recombination (%)[a]	Map distance (in cM ± SE)
	Glu-1	Tri-A1	Gli-A1/Glu-A3			
Parentals:	Ala	h	h	64		
	Rli	cs	cs	77		
Recombinants:						
c.o. region 1[b]	Ala	cs	cs	8		
	Rli	h	h	20	11.0[b]	11.2 ± 2.0[b]
c.o. region 2[c]	Ala	h	cs	55		
	Rli	cs	h	55	40.1[c]	55.2 ± 8.2[c]
double c.o.[bc]	Ala	cs	h	1		
	Rli	h	cs	2		
Aneuploids: (4.7%)						
hypoploid	–	–	–	11		
hyperploid	Rli + Ala?	h + cs	h + cs	2		
misdivision product	Rli	–	–	1		
Total				396		

[a] Percent of euploid progeny; [b] Between centromere and *Tri-A1;*
[c] Between *Tri-A1* and *Gli-A1;* '–' = protein absent; ? = not confirmed

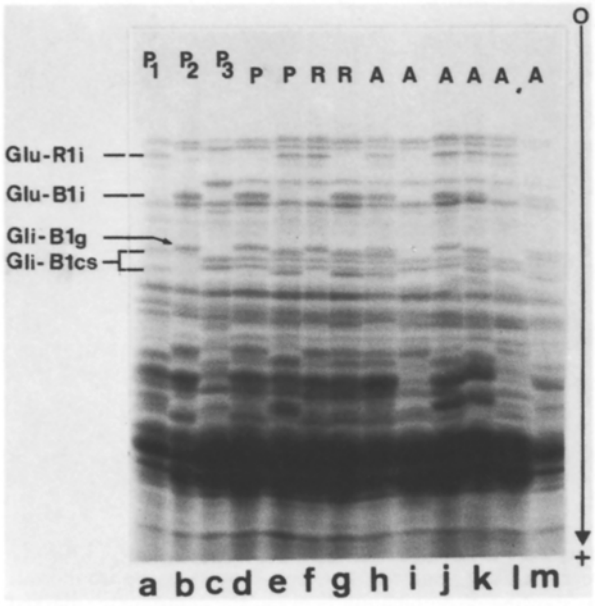

Fig. 5. SDS-PAGE patterns of reduced total protein extracts from the parental lines (P_1, P_2, P_3) and some test-cross progeny from translocation heterozygotes used to map the *Gli-B1* locus in relation to centromere. *a* CS 1BS-1RL translocation; *b* Gabo; *c* Halberd; *d–m* test-cross progeny. *P* = parental; *R* = recombinant; *A* = aneuploid

was observed between the *Gli-A1* and *Glu-A3* genes, and the upper limit of recombination (95% confidence limit, Hanson 1959) was calculated to be 1.1%. The recombination frequencies and the map distances between these loci are shown in Table 3.

A small proportion (4.7%) of the test-cross progeny were aneuploids including hypoploids (Fig. 4A, B, h) hyperploids and misdivision products (Table 3) and it is likely that these arose from gametes produced from megaspore mother cells (MMCs) which showed a similar lack of pairing between 1A and 1AS-1RL to that observed in PMCs (Table 2). Their effect on estimates of the recombination frequencies will not be large and for simplicity these aneuploids have been excluded from the analysis.

2.2 Mapping the Gli-B1 *locus on chromosome arm 1BS.* Both the translocation and telocentric stocks were used to produce test-cross seeds (Fig. 1). The translocation mapping was repeated at three different times viz summer 1981, winter 1982 and spring 1982. The telocentric mapping was carried out concurrently with one of the translocation mapping experiments for comparison of the two procedures. Halberd was usually used as the male parent and no overlap of critical protein bands occurred (Fig. 5).

In translocation mapping the protein phenotypes Glu-R1i, Glu-B1i, Gli-B1cs and Gli-B1g could be easily classified in the parents (Fig. 5a, b) and test-cross progeny and parental (Fig. 5d, e), recombinant (Fig. 5f, g) and several aneuploid types (Fig. 5h–m) could be reliably distinguished. The data obtained from 606 test-cross seeds coming from three families were on the borderline of homogeneity ($\chi^2_{[8]} = 16.71$, $0.05 > P > 0.02$) even though these progeny had been produced in three very different seasons. When the aneuploids

were excluded from the analysis, the data were homogeneous ($\chi^2_{[6]} = 8.43$, $P > 0.2$) and they have ben pooled in Table 4. The segregation of the presumed homoeoalleles *Glu-B1i/Glu-R1i* and the presumed alleles *Gli-B1cs/Gli-B1g* was in close agreement with the expected 1:1 ratio ($\chi^2_{[1]} = 3.60$ and 0.02, respectively). In the joint segregation, there was a significantly greater proportion of parental to recombinant individuals in the progeny ($\chi^2_{[1]} = 16.56$) indicating linkage between *Gli-B1* and the centromere, marked here with the *Glu-1* genes. The recombination frequency was 41.6%.

In the telocentric mapping, parental, recombinant and aneuploid progeny were distinguished as in the translocation mapping, except Glu-R1i bands were lacking in these progeny. The recombination frequency obtained with the telocentric mapping (40.4%) was similar to that obtained with the translocation mapping (41.6%) as was the aneuploid frequency (19.7% and 18.3%, respectively). However, a more valid comparison involves the data from the concurrent mapping experiments (not shown separately in Table 4) and again the recombination values (40.4% vs 46.3%) and the aneuploid frequencies (19.7% vs 16.1%) were similar for the telocentric and translocation mapping, respectively.

A large proportion of progeny in both translocation and telocentric mapping experiments were aneuploids (Table 4) including hypoploids (Fig. 5i), hyperploids (Fig. 5h) and misdivision products (Fig. 5j–m). For simplicity these have been excluded from the initial estimation of map distance. Their possible effect on the estimates of map distance is considered in the "Discussion".

2.3 Mapping the Gli-B1, Glu-B3 *and* Blu-B1 *loci on chromosome 1B.* A triparental test-cross involving (CS × CS-Hope 1B) × CS Dt 1BL was made specially to map the *Glu-B3* locus with respect to *Gli-B1* and *Glu-B1*. The CS × CS-Hope 1B F_1 was heterozygous at each of these three loci and their relevant protein bands are shown in Fig. 6. All of these bands could be scored in the gels after SDS-PAGE of reduced proteins (Fig. 6 B). To determine which of these protein bands were gliadins (Gli-B1cs, Gli-B1g) or LMW glutenin subunits (Glu-B3cs, Glu-B3h) it was necessary to compare the SDS-PAGE patterns of unreduced and reduced proteins from the parental lines. With the unreduced samples the two parents differed only in the ω-gliadin region of the gel (Fig. 6 A, a, b, bands labelled Gli-B1cs, Gli-B1g). Thus the additional band differences which appeared after SDS-PAGE of the reduced proteins (Fig. 6 B, a, b), must represent HMW and LMW subunits of glutenin. This conclusion was confirmed by comparing the difference in the banding patterns of these two parents after two-step SDS-PAGE (Fig. 6 C, a, c), where only the glutenin subunits are present.

After the identity of these bands was confirmed in the parents, the test-cross progeny were analysed by the more simple single-step SDS-PAGE of reduced pro-

Table 4. Protein phenotypes and their frequency in test-cross progeny used to map the *Gli-B1* locus in relation to centromere, grouped into parental, recombinant and aneuploid classes

Progeny class	Protein phenotypes		No. of progeny		Recombination (%)[c]	Map distance (in cM ± SE)
	Glu-1	Gli-B1	(Trans.)[a]	(Telo.)[b]		
Parentals:	Bli	g	156	63		
	Rli	cs	133	*		
	–	cs	*	49		
Recombinants:	Bli	cs	113	44		
	Rli	g	93	*	41.6[a]	59.7 ± 7.1[a]
	–	g	*	32	40.4[b]	56.1 ± 10.3[b]
Aneuploids: (18.3%)[a] and (19.7%)[b]						
hypoploid	–	–	69	31		
hyperploid	Bli + Rli	g + cs	22	*		
	Bli	g + cs	*	10		
misdivision	Bli	–	7	3		
products	Rli	–	1	*		
	Bli	g + cs	1	0		
	–	g + cs	0	2		
	Bli + Rli	g	9	*		
	Bli + Rli	cs	2	*		
Total			606	234		

[a] Translocation mapping; [b] Telocentric mapping; [c] Percent of euploid progeny; '–' = protein absent; * not applicable

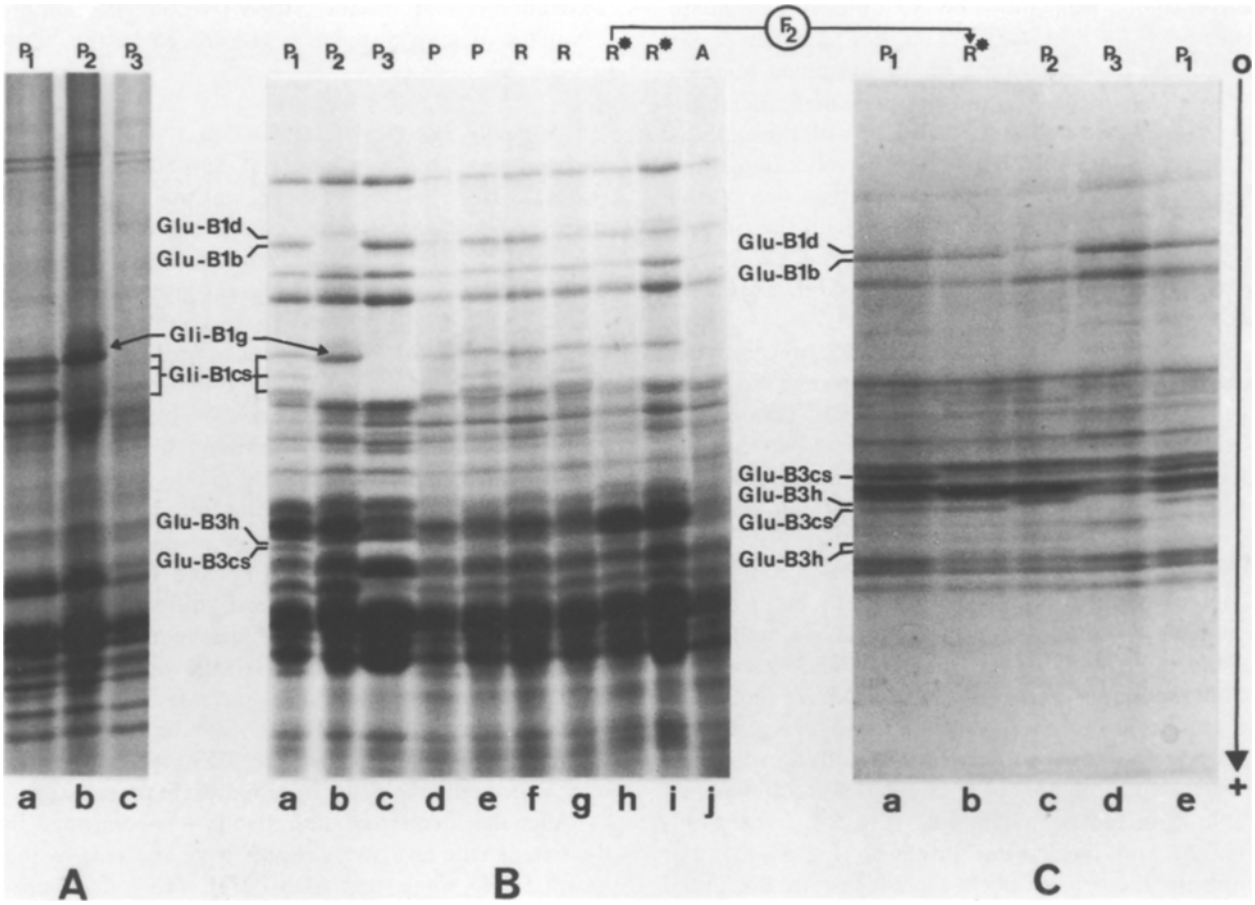

Fig. 6A–C. SDS-PAGE patterns of seed protein extracts from the parental lines (P_1, P_2, P_3), and some test-cross progeny used to map the *Glu-B1*, *Gli-B1* and *Glu-B3* loci. **A** unreduced proteins from the parental lines: *a* CS, *b* CS-Hope 1B substitution, *c* CS Dt 1BL; **B** reduced proteins from parental lines and test-cross progeny *a*, *b* and *c* same as in **A**, *d–j* test-cross progeny; **C** two-step electrophoresis (7.5–15% acrylamide gradient gel) of *a* CS, *b* one of the *Gli-B1/Glu-B3* recombinant homozygotes selected from the F_2 of test-cross progeny, *c* CS-Hope 1B substitution, *d* CS Dt 1BL. *P* = parental; *R* = recombinant between *Glu-B1* and *Gli-B1* genes, *A* = aneuploid, * progeny showing recombination between the *Gli-B1* and *Glu-B3* genes

teins (Fig. 6 B, d–j). Segregation of the *Gli-B1* and *Glu-B3* loci could be directly scored in the test-cross progeny because the male test-cross parent Dt 1BL was deficient for these loci. In addition these two loci were mapped with respect to the *Glu-B1* locus on the long arm of 1B. The allele on Hope 1B produced a distinctive band (Glu-B1d) but CS and Dt 1BL had the same allele (Glu-B1b) and the presence of the CS allele from the F_1 was detected by the increased band staining intensity due to a dosage effect. The dark Glu-B1b bands always occurred as an alternative to the distinctive Glu-B1d band, which confirmed the above classification. Thus, the 234 test-cross progeny could be classified into parental and recombinant types and only one aneuploid was detected (Table 5, Fig. 6 B, d–j). The alleles at the three loci *Glu-B1*, *Glu-B3* and *Gli-B1*, all segregated in a 1:1 ratio ($\chi^2_{[1]} = 0.009$, 0.96 and 1.55, respectively), but the analysis of joint segregation showed that parental types occurred with a significantly higher freqency than the recombinants in both the intervals analysed. The *Glu-B3* gene was mapped between the *Glu-B1* and *Gli-B1* loci and showed 30.5% and 1.7% recombination with these loci, respectively (Table 5). The recombination frequency between *Glu-B1* and *Gli-B1* was estimated to be 31.3% which is significantly less than the values obtained for the centromere-*Gli-B1* interval in the translocation and telocentric mapping experiments (41.6% and 40.4%, respectively). This difference could be due to differences in genetic background of these crosses or to the different environmental conditions. The first possibility seems more likely since Payne et al. (1982) observed widely different recombination values (39% and 47%) for the same gene interval on chromosome 1B in different cultivar crosses.

In contrast to our failure to recombine *Gli-A1* and *Glu-A3* genes, four putative recombinants with the non-parental combination Gli-B1g + Glu-B3cs (Fig. 6 B, h, i)

Table 5. Protein phenotypes and their frequency in test-cross progeny used to map the *Glu-B1, Gli-B1* and *Glu-B3* loci, grouped into parental, recombinant and aneuploid classes

Progeny class	Protein phenotypes			No. of progeny	Recombination (%)[a]	Map distance (in cM ± SE)
	Glu-B1	Glu-B3	Gli-B1			
Parentals:	b	cs	cs	76		
	d	h	g	83		
Recombinants:						
c.o. region 1[b]	b	h	g	40		
	d	cs	cs	30	30.5[b]	35.4 ± 4.8[b]
c.o. region 2[c]	b	cs	g	3		
	d	h	cs	0	1.7[c]	1.7 ± 0.8[c]
double c.o.[bc]	b	h	cs	0		
	d	cs	g	1		
Aneuploid:						
misdivision product	b	–	–	1		
Total				234		

[a] Percent of euploid progeny; [b] Between *Glu-B1* and *Glu-B3;* [c] Between *Glu-B 3* and *Gli-B1;* '–' = protein absent

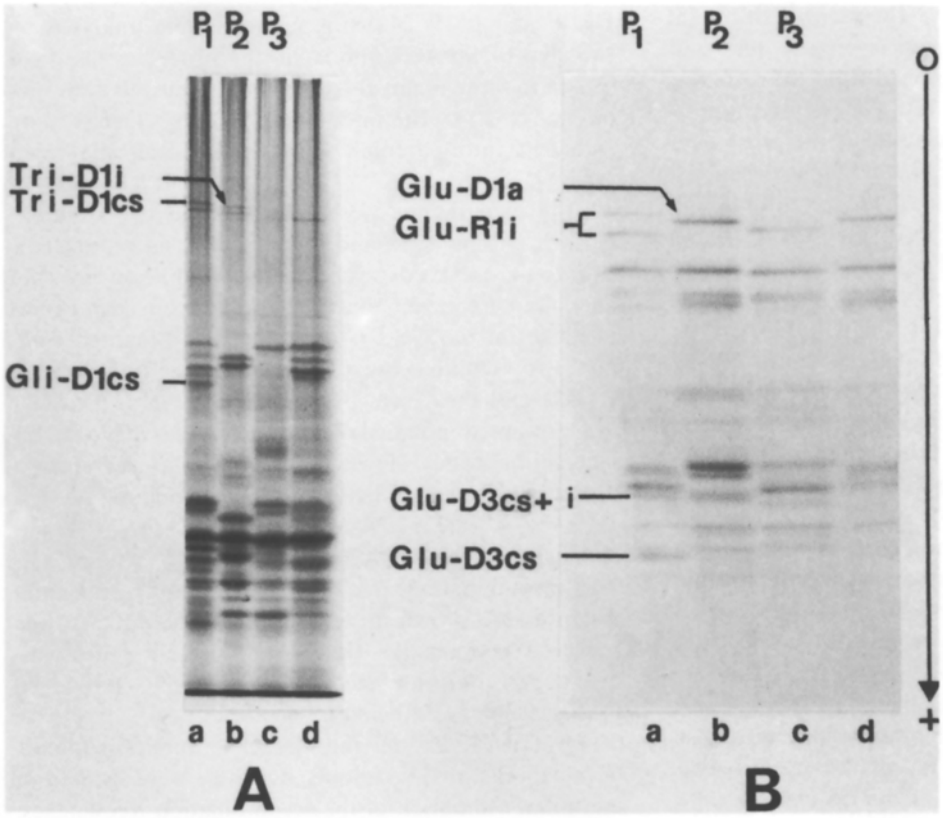

Fig. 7A, B. SDS-PAGE patterns of total seed protein extracts from the parental lines (P_1, P_2, P_3) used to map the *Tri-D1, Gli-D1* and *Glu-D3* genes. **A** unreduced proteins. **B** two-step electrophoresis of reduced proteins: *a* CS 1DS-1RL translocation, *b* India 115, *c* Warigal 1DL-1RS translocation, *d* CS Dt 1DL

Table 6. Protein phenotypes and their frequency in test-cross progeny used to map the *Tri-D1, Gli-D1* and *Glu-D3* loci, grouped into parental, recombinant and aneuploid classes

Progeny class	Protein phenotypes			No. of progeny	Recombination (%)[a]	Map distance (in cM ± SE)
	Glu-1	Tri-D1	Gli-D1/Glu-D3			
Parentals:	Dla	i	i	83		
	Rli	cs	cs	101		
Recombinants:						
c.o. region 1[b]	Dla	cs	cs	15		
	Rli	i	i	17	14.9[b]	15.4 ± 2.1[b]
c.o. region 2[c]	Dla	i	cs	65		
	Rli	cs	i	59	40.3[c]	55.8 ± 7.4[c]
double c.o.[bc]	Dla	cs	i	8		
	Rli	i	cs	14		
Aneuploids: (17.9%)						
hypoploid:	–	–	–	61		
hyperploid?	Rli + Dla	cs + i?	cs + i?	8		
misdivision	Dla	–	–	3		
products:	Rli	–	–	3		
	–	i	i	1		
	–	cs	cs	2		
Total				440		

[a] Percent of euploid progeny; [b] Between centromere and *Tri-D1;* [c] Between *Tri-D1* and *Gli-D1;* '–' = protein absent; ? = not confirmed

were detected in these progeny. The identity of one of these recombinants was confirmed in progeny tests and a homozygous recombinant, selected from the test-cross F_2 is shown in Fig. 6C, b. The LMW glutenin composition of this recombinant indicates that the cross over must have occurred within the *Glu-B3* locus because it has only one band from each of the pairs of LMW glutenin bands controlled by both the *Glu-B3cs* and *Glu-B3h* alleles (bands labelled in Fig. 6C, a, c).

2.4 Mapping the Tri-D1, Gli-D1 *and* Glu-D3 *loci on chromosome arm 1DS.* The band patterns controlled by the presumed homoeoalleles *Glu-R1i* and *Glu-D1a* (HMW glutelin subunits) and the alleles *Tri-D1cs* and *Tri-D1i* (triplet proteins), *Gli-D1cs* (gliadin) and *Glu-D3cs* (LMW glutenin subunits) were easily classified in the parents (Fig. 7A, B, a, b). However, it was not possible to score any protein band corresponding to the *Gli-D1i* and *Glu-D3i* alleles. A band controlled by the *Glu-D3i* locus was present in India 115 but it had similar electrophoretic mobility to one of the Glu-D3cs bands (Fig. 7B, labelled Glu-D3cs + i). This was inferred from our observation that the Glu-D3cs + i band was not present in any of the hypoploid test-cross progeny but it was present in all of the progeny possessing either the India 115 or the CS chromosome arm 1DS.

The segregation of triplet and gliadin bands was analysed by SDS-PAGE of unreduced proteins and HMW and LMW glutenin subunits were analysed by two-step SDS-PAGE similar to the procedure used for separating the equivalent proteins controlled by 1A (section *3 2.1*). The individual test-cross seeds were classified into parental, recombinant and aneuploid types.

The experiments were conducted in two different seasons (spring 1982 and winter 1982) and altogether 440 test-cross seeds were analysed. Except for the aneuploid frequency and the single factor segregation of alleles at the *Gli-D1* locus, the data obtained from the two families were homogeneous ($\chi^2_{[4]} = 9.07$, $P > 0.1$) and they have been pooled in Table 6. All of the presumed homoeoalleles and alleles gave a 1:1 segregation ratio. However, in the joint segregation linkage was detected between the centromere and *Tri-D1* ($\chi^2_{[1]} = 178.22$), *Tri-D1* and *Gli-D1* ($\chi^2_{[1]} = 13.53$) and between the centromere and *Gli-D1* ($\chi^2_{[1]} = 6.91$). The recombination frequencies and equivalent map distances are shown in Table 6. Complete linkage was observed between the *Gli-D1* and *Glu-D3* genes with the upper limit for recombination (95% confidence limit, Hanson 1959) being 1.1%.

Overall, 17.9% of the progeny were aneuploids (Table 6), mainly hypoploids, but these were ignored in the initial estimation of the recombination frequencies. Their possible influence on the estimates of map distance is considered in the Discussion.

Discussion

The validity of the method used to map gene-centromere distances using translocation lines depends on, first, the absence of pairing between the rye arm of the translocation and its homoeologous wheat arm and secondly the wheat arm of the translocation chromosome pairing with its homologous arm on the complete wheat chromosomes with the same frequency as would occur between the two complete homologues. The observations made at metaphase I and diakinesis, provided no evidence of any homoeologous pairing between the wheat and rye chromosome arms. However, the amount of pairing observed between the homologous arms was variable (Table 2) and this was reflected in the number of aneuploids detected in the test-cross progeny.

The 1AS-1RL translocation showed a high level (90.4%) of metaphase I pairing with the complete 1A chromosome and only a small proportion (4.7%) of the test-cross progeny were aneuploids (Table 3). Thus, the estimates of map distance could not have been significantly affected by the lack of pairing. In contrast, the metaphase pairing between the homologous short arms was much lower in the experiments involving the 1BS-1RL translocations and the 1BS telocentric (Table 2), resulting in high frequencies of aneuploids (Tables 4 and 6). The likely influence of a high aneuploid frequency on the estimate of map distance was considered earlier by Singh and Shepherd (1984a) when mapping the centromere-*Glu-R1* map distance. They concluded that the estimates of this small map distance (4.65 ± 1.04 cM) were not greatly affected by aneuploidy since the extreme values only ranged from 3.96 ± 0.89 cM to 4.89 ± 1.10 cM (Singh and Shepherd 1984a). However, a precise correction could not be made to account for desynapsis because they did not have data on the extent of pairing at diakinesis. In the present paper we are dealing with both small and large map distances and since both metaphase I and diakinesis pairing data are available the effect of aneuploidy on the estimates of map distance can be analysed more completely.

To resolve this problem fully it would be necessary to know the total number of test-cross progeny which were derived from MMCs having the reference chromosomes as univalents at metaphase I. This number can be approximated either by measuring the frequency of PMCs showing univalents at metaphase I (Table 2), or by extrapolating from the observed frequency of aneuploids (hypoploids + hyperploids) using the formulae derived earlier (Singh and Shepherd 1984a). The second aproach has the advantage in that it reflects the level of univalency in the same MMCs which produced the test-cross seeds. The next problem is to determine how many of these metaphase I univalents arose from true asynapsis and how many were derived from desynapsis. This is important because these two phenomena are expected to have opposite effects on the estimates of map distance. Asynaptic univalents would have had no opportunity to recombine, and if progeny possessing these are included in the analysis it would lead to an inflation of the parental class and hence an underestimation of the true map distance. In contrast, exclusion of progeny (including aneuploids) resulting from desynaptic univalents is expected to result in an overestimation of map distance as discussed below.

Since the exact level and nature of desynapsis (based on zygotene pairing) is not known, we can not apply an exact correction to the estimates of recombination values (p) to account for both asynapsis and desynapsis. However, it is possible to define the limits of p based on the two extreme assumptions of either maximum asynapsis or no asynapsis. From observations at diakinesis, the maximum amount of asynapsis in the 1BS-1RL translocation heterozygotes is 8.5% (Table 7). The progeny resulting from these 8.5% MMCs will be mostly aneuploids but the few euploids expected should also be excluded from the analysis to avoid inflating the parental class. Out of the total 606 test-cross progeny analysed the expected number of progeny derived from asynapsis is only 51. However, in the initial estimate of the map distance, 111 aneuploid progeny were excluded and presumably these included some gametes arising from MMCs with desynaptic univalents. Thus the initial recombination value is most likely to be an overestimate of the true map distance, and hence it will be the upper limit. If there was no asynapsis, that is 100% zygotene pairing, all univalents observed at metaphase I would have had a chance to cross over so the frequency of recombinants in the gametes derived from them should be the same as in the gametes derived from metaphase bivalents, providing that the probability of desynapsis is independent of the position of chiasmata along the chromosome. However, it is likely that chromosomes with distal chiasmata are more prone to undergo desynapsis than those with proximal ones. On the extreme assumption that all desynaptic univalents resulted from loss of chiasmata distal to the *Gli-B1* locus, all of the aneuploid gametes should be included in the analysis as members of the parental class. This gives a recombination frequency of $34.0 \pm 1.9\%$ between *Gli-B1* and the centromere, which represents the lower limit. However, since it is unlikely that all of the desynapsed chromosomes had chiasmata distal to *Gli-B1*, the true value is likely to be higher than 34%.

The upper and lower limits for the recombination value obtained in the telocentric mapping experiment, assuming maximum asynapsis and no asynapsis, are $46.1 \pm 3.9\%$ and $32.5 \pm 3.1\%$, respectively. With the 1DS-1RL translocation mapping, the initial estimates of 14.9% and 40.3% recombina-

Table 7. Estimates of the relative contributions of asynapsis and desynapsis to the metaphase I univalents in three mapping experiments with high frequency of aneuploids

Mapping experiment	Percent PMCs with univalents[a]		Proportion of univalents due to:	
	Diakinesis (x)	Metaphase I (y)	asynapsis (As) x/y	desynapsis (Ds) $1-(x/y)$
1BS-1RL translocation	8.5	29.5	0.288	0.712
1BS telocentric	28.6	51.0	0.561	0.439
1DS-1RL translocation	7.3	35.2	0.207	0.793

[a] Average data from Table 2

Table 8. Recombination frequencies adjusted for observed asynapsis, desynapsis and aneuploidy

Mapping experiment	No. of progeny resulting from MI univalents					No. of euploid parentals to be excluded	No. of aneuploids to be included as parental	Adjusted recombination (%)
	Aneuploids[a]				Euploids[b] (E)			
	Hypoploids $(1-i)^2\,a$	Hyperploids $(i)^2\,a$	Misdivisions m	Total A	$2i\,(1-i)\,a$*	$E \times As$	$A \times Ds$	
1BS-1RL	69	22	20	111	78	22	79	37.3 ± 2.0[c]
1BS	31	10	5	56	36	20	25	39.4 ± 3.5[c]
1DS-1RL	61	8	9	78	44	9	62	13.0 ± 1.6[d]
								35.2 ± 2.3[e]

[a] Observed values; [b] Estimates based on hypoploid and hyperploid frequency; * See text; *As* and *Ds* are from Table 7; [c] Centromere-*Gli-B1;* [d] Centromere-*Tri-D1;* [e] *Tri-D1-Gli-D1*

tion in the centromere-*Tri-D1* and *Tri-D1-Gli-D1* gene intervals, respectively, are the upper limits and the corresponding lower limits are $12.2 \pm 1.5\%$ and $32.9 \pm 2.2\%$. It is clear from the above analysis that the upper and lower limits are not very different from the initial estimates suggesting that the exclusion of aneuploids does not have a major effect on the estimation of true map distance.

However, the most likely situation is that both asynapsis and desynapsis partly contributed to the observed metaphase I univalents. The relative contributions of these two processes have been estimated from the observed average pairing at diakinesis and metaphase I in each of the three experiments with high aneuploidy (Table 7). The total number of gametes derived from MMCs with metaphase univalents ('*a*'), excluding the number of misdivision products ('*m*'), was calculated according to Singh and Shepherd (1984a) as follows. Assuming that the two critical univalents in the F_1 heterozygote, have an equal probability ('*i*') of inclusion in a functional egg, then the expected number of hypoploid, hyperploid and euploid gametes arising from such MMCs is given by $(1-i)^2a$, i^2a and $2i(1-i)a$, respectively (Singh and Shepherd 1984a). The sum of *a* and *m* gives the total number of test-cross progeny (gametes) derived from MMCs possessing the reference chromosomes as univalents. Based on these data and the proportions of asynapsis and desynapsis (Table 7), the number of euploid parentals that should be excluded from each analysis to allow for asynapsis and the number of aneuploids that should be included in the parental class to allow for desynapsis were calculated (Table 8). The recombination values adjusted for both asynapsis and desynapsis (Table 8) were much closer to the initial estimates obtained by ignoring the aneuploids, than the lower limits given above. Hence, it is concluded that if aneuploid progeny can be identified and excluded, their occurrence in test-cross progeny does not significantly affect the estimation of map distance, particularly when the genes under study are closely linked.

The linkage mapping results presented in this paper confirm the earlier findings of Rybalka and Sozinov (1978) and Payne et al. (1984b) that the gliadin locus *Gli-B1* is located at the distal end of chromosome arm 1BS. Furthermore, it is shown that presumed homoeoloci *Gli-A1* and *Gli-D1* are also distally located and the data clearly reflect the homoeologous relationship of the group 1 chromosomes of wheat. However, genes for triplet-like proteins have not been detected on chromosome 1B so far. Although other loci such as *Gld-B6* (Galili and Feldman 1984) and *Glu-B2* (Jackson et al. 1985) have been mapped between the *Gli-B1* and the *Glu-B1* loci, their relationship with the *Tri-1* genes is not known as yet. The separate location of the genes controlling triplet proteins is a further indication, in addition to their different electrophoretic and solubility properties (Singh and Shepherd 1985, 1987), that they represent a new class of proteins in wheat seeds. The close linkage between the genes controlling LMW glutenins subunits (*Glu-3*) and gliadins (*Gli-1*) suggests that these two groups of genes may have evolved from the same ancestral gene by duplication and mutation. This hypothesis is further supported by the similar amino acid composition and sequence of these two groups of proteins (Shewry and Miflin 1985). However, the LMW glutenin subunits have the propensity to aggregate by interpolypeptide disulphide bonds and the observed 1.7% recombination between *Glu-B3* and *Gli-B1* loci indicates that these proteins are coded by separate genes.

Knowledge of the chromosomal location of the *Tri-1* and *Gli-1* loci has already proven useful in experiments designed to recombine the group 1 chromosomes of wheat and rye by induction of allosyndesis (Koebner and Shepherd 1986). In these experiments, triplet and prolamin markers were used to select for homeologous recombination between wheat-wheat and wheat-rye chromosomes.

Acknowledgement. The senior author gratefully acknowledges the financial support of a University of Adelaide Scholarship for Postgraduate Research.

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