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Mapping of the genes controlling high-molecular-weight glutelin subunits of rye on the long arm of chromosome 1R

By N. K. SINGH and K. W. SHEPHERD

Department of Agronomy, Waite Agricultural Research Institute, The University of Adelaide, South Australia, 5064

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SUMMARY

The gene(s) controlling the high-molecular-weight glutelin subunits in rye (designated as *Glu-R1*) was mapped with respect to the centromere using a 1RL-1DS wheat-rye translocation line and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Analysis of 479 seeds from test-crosses between a 1R/1RL-1DS heterozygote and the cultivar India 115, revealed 14·6% aneuploid and 3·95% recombinant progeny. Excluding the aneuploids, this locus was calculated to be $4{\cdot}65 \pm 1{\cdot}04$ cM from the centromere on the long arm of chromosome 1R, which is comparable to the position of the homoeologous loci in wheat and barley.

1. INTRODUCTION

Conventional inheritance and linkage studies in cereal rye (*Secale cereale* L.) have been restricted mainly because of the difficulty of obtaining homozygous parents due to the cross-pollinating habit of rye which, in turn, is promoted by self-sterility alleles (Lundquist, 1954, 1956). However, with the advent of cytogenetic manipulations allowing the addition (O'Mara, 1940), or substitution (Riley, 1965) of individual rye chromosomes to the wheat genome it has become relatively easy to locate certain rye genes on particular rye chromosomes. For example, Chang, Kimber & Sears (1973) have used addition lines to assign genes controlling some quantitative characters to chromosome 5R of rye and recent work has shown that biochemical characters, such as seed storage proteins and isozymes, are particularly amenable to this form of analysis. Thus genes controlling rye prolamins have been located on chromosome arm 1RS (Shepherd, 1968; Shepherd & Jennings, 1971), rye glutelins on 1RL (Lawrence & Shepherd, 1981) and various isozymes have been assigned to 6 of the 7 rye chromosomes (Tang & Hart, 1975). However, so far none of these minor or major genes in rye has been accurately mapped along the rye chromosomes.

In this paper, a method is described and utilized to map the genes controlling two high-molecular-weight (HMW) subunits of rye glutelin with respect to the breakpoint of a wheat-rye translocation chromosome, thought to be the centromere of rye chromosome arm 1RL. Our results indicate that the genes controlling the HMW subunits of rye glutelin are closely linked with the centromere

and thus they parallel some recent findings on the location of the homoeologous loci controlling HMW glutelins in wheat (Payne *et al.* 1982) and barley (Blake, Ullrich & Nilan, 1982; Shewry *et al.* 1983).

2. MATERIALS AND METHODS

(i) *Parents*

A test-cross procedure utilizing three specially chosen parental lines (Fig. 1), was used to map the HMW-glutelin genes of rye. One parent possesses a wheat – Imperial rye translocation chromosome 1RL-1DS [assumed to have arisen from centric fusion (Lawrence & Shepherd, 1981)] in a Chinese Spring background, and another

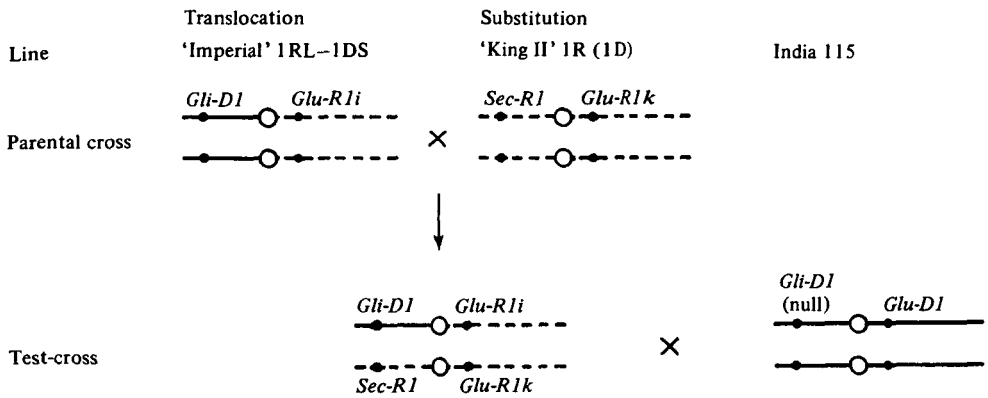

Fig. 1. Test-cross procedure used for mapping the *Glu-R1* locus, showing the arm location of the storage protein genes used as markers.

has a complete chromosome 1R of King II rye substituted for 1D of wheat (Lawrence, 1969) in a mixed Chinese Spring × Holdfast wheat background. One of the glutelin subunits controlled by the *Glu-R1* locus on chromosome arm 1RL of Imperial rye is electrophoretically different from a subunit controlled by the same locus in King II and these alleles have been designated *Glu-R1i* and *Glu-R1k* respectively. The other arms of these two chromosomes are also marked with different prolamin genes (*Gli-D1* and *Sec-R1*) and they can be recognized in the test-cross progeny because the third parent India 115 has a null allele at the *Gli-D1* locus. Thus the presence of recognizably different protein markers on each of the four arms of the bivalent allows progeny to be screened directly on gels for parental and recombinant types and also for aneuploid variants. Furthermore, the presence of the distinctive allele *Glu-D1* on chromosome arm 1 DL of the male parent India 115 (Plate 1) provided a check that each progeny seed analysed resulted from the test cross rather than from selfing of the F_1 hybrid.

Providing that the translocation arose from centric fusion between 1DS and 1RL, pairing and crossing over is expected to occur between the *homologous* long arms of chromosome 1R, but not between the *homoeologous* short arms of 1DS and 1RS. In this way, the hybrid can be used to map the position of any locus on the

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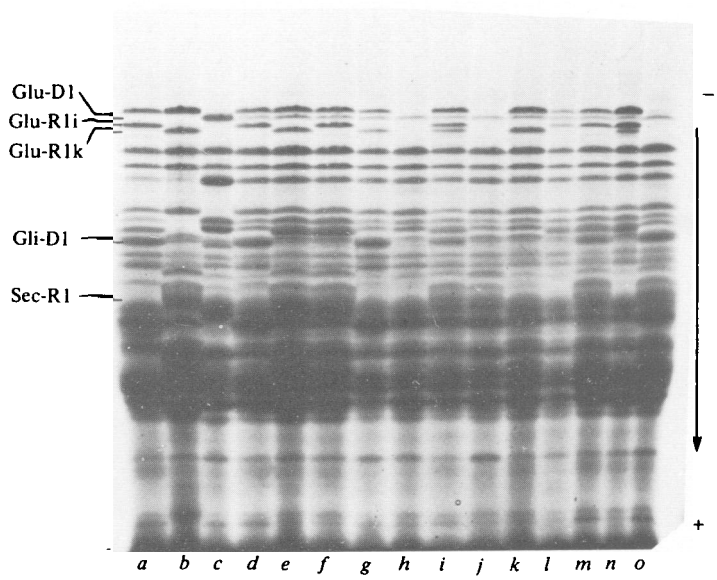

SDS-PAGE phenotypes of parents (*a*)–(*c*) and test-cross progeny (*d*)–(*o*). (*a*) Translocation line Imperial 1RL-1DS, (*b*) substitution line King II 1R (1D), (*c*) India 115, (*d*) Glu-R1i/Gli-D1 (parental), (*e*) Glu-R1k/Sec-R1 (parental), (*f*) Glu-R1i/Sec-R1 (recombinant), (*g*) Glu-R1k/Gli-D1 (recombinant), (*h*) null (hypoploid), (*i*) Glu-R1i/Glu-R1k/Gli-D1/Sec-R1 (hyperploid), (*j*) –(0) misdivision products: (*j*) –/Sec-R1, (*h*)Glu-R1k/–, (*l*)Glu-R1i/–, (*m*) Glu-R1i/– /Gli-D1 /Sec-R1, (*n*)Glu-R1i/Glu–R1k/–/–, (*o*) –/–/Gli-d1/Sec-R1.

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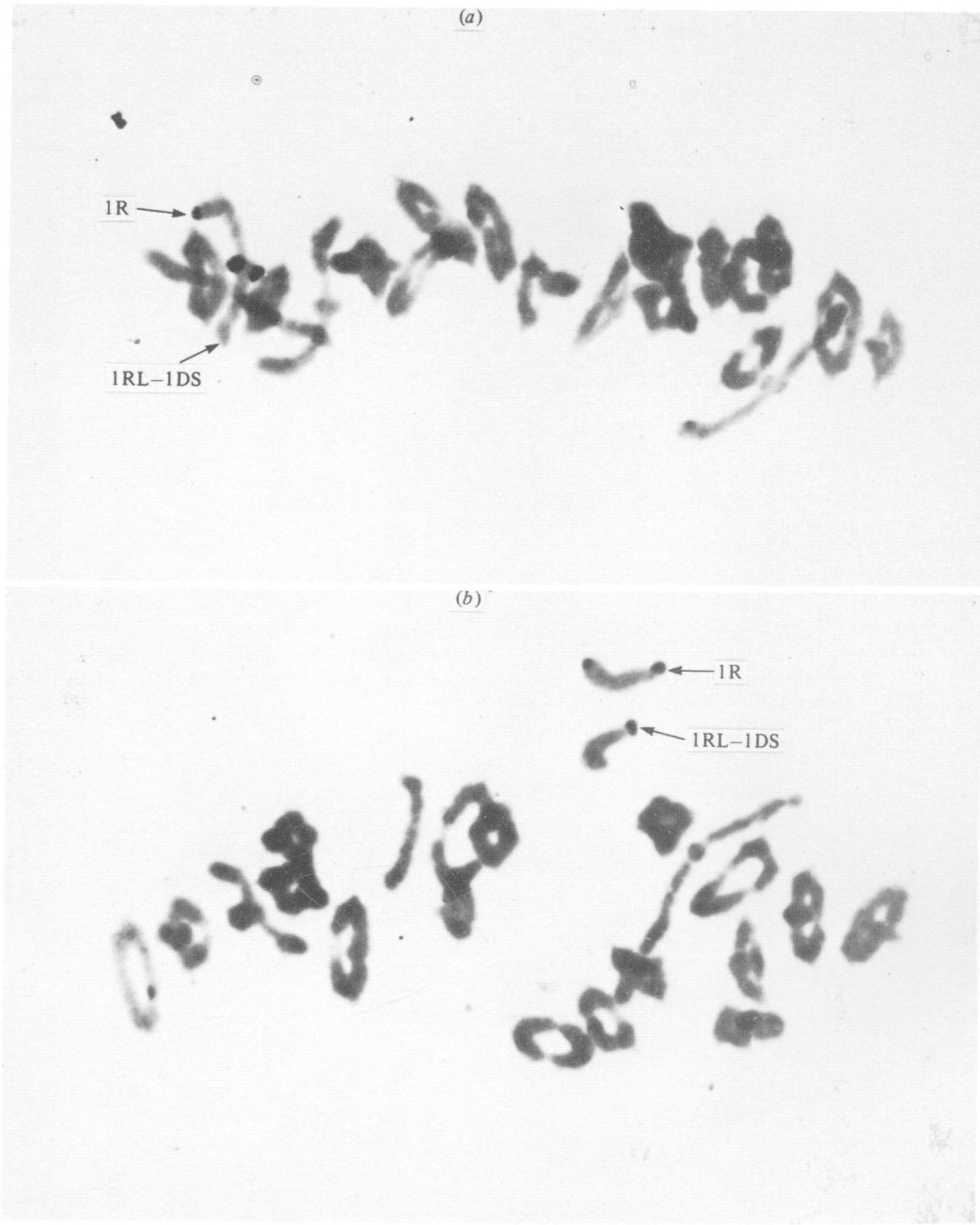

C-banded squashes of PMCs from 1R/1RL-1DS heterozygotes at metaphase I. (*a*) 1R/1RL-1DS as a rod bivalent, (*b*) 1R, 1RL-1DS as univalents.

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long arm of chromosome 1R with respect to the rye centromere in a way analogous to the telocentric mapping method developed by Sears (1966) for mapping wheat genes with respect to their centromeres.

(ii) *Electrophoresis*

Proteins were extracted from a small part (distal $\frac{1}{4}$) of the endosperm of individual test-cross seeds by treatment with sodium dodecyl sulphate (SDS) and 2-mercaptoethanol (2-ME) for 16 h at 37 °C. Because of the small portion of endosperm used it was possible to make replicate runs of any seed where required, and this was especially useful for arranging representative genotypes for photography (e.g. Plate 1). Electrophoresis was carried out in 10 % acrylamide (w/v) gels with dimensions of 145 × 100 × 1 mm using the procedure of Lawrence & Shepherd (1980). The allelic difference between the glutelin subunits of King II and Imperial was best resolved with slower runs using 35 mA/gel for the first 45 min and then 15 mA/gel until the dye front reached the bottom of the separating gel. The rye and wheat prolamins were best scored before destaining the gel because they tended to fade during destaining.

(iii) *Cytology*

To examine the extent of chromosome pairing at metaphase I of meiosis in pollen mother cells (PMCs), individual anthers were selected and fixed in 3 absolute ethanol: 1 glacial acetic acid. In squash preparations the rye chromosomes were easily identified by C-banding using the procedure of Vosa & Marchi (1972).

3. RESULTS AND DISCUSSION

Eight F_1 plants were used to produce 479 test-cross seeds for electrophoretic analysis. The glutelin bands controlled by genes *Glu-R1i*, *Glu-R1k* and *Glu-D1* could be classified without difficulty in the parents (Plate 1*a–c*) and the prolamins controlled by *Gli-D1* and *Sec-R1* (Plate 1*a*, *b*) could also be scored easily. Similarly, all of these protein phenotypes could be reliably determined in the test-cross progeny and parental (Plate 1*d*, *e*), recombinant (Plate 1*f*, *g*) and aneuploid types (Plate 1*h–o*) were distinguished. The data obtained from the separate F_1 hybrids (including the frequency of aneuploids) were homogeneous ($\chi^2_{28} = 24{\cdot}02$, $0{\cdot}7 > P > 0{\cdot}5$) and consequently they have been pooled (Table 1).

Although the aneuploids (14·6 %) detected among the progeny may influence the estimation of map distance, for simplicity they are ignored in the initial analysis. As shown in Table 1, there is a close agreement with the expected 1 : 1 segregation for presumed alleles *Glu-R1i*:*Glu-R1k* and for the presumed homoeoalleles *Gli-D1*:*Sec-R1*. However, it is clear that there is strong linkage between the glutelin and prolamin genes on the opposite arms of the chromosomes. The direct estimate of the linkage between the loci is $4{\cdot}64 \pm 1{\cdot}04$ % recombination which converts to a map distance of $4{\cdot}65 \pm 1{\cdot}04$ cM using the Payne *et al.* (1982) application of the Kosambi (1944) function:

$$\text{cM} = 25 \times \ln\left[(100 + 2R) \div (100 - 2R)\right] \pm 2500\ S_R \div (2500\ R^2),$$

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where R = recombination percentage and S_R = standard deviation of R. Furthermore, if the 1RL-1DS translocation arose from centric fusion of telocentrics then this figure becomes an estimate of the map distance between the *Glu-R1* locus and the centromere.

Table 1. *Protein phenotypes and their frequency among test-cross progeny*

Parental		Recombinant		Aneuploid	Total	R (%)	χ^2 (het) d.f. 28
Glu-R1i Gli-D1	Glu-R1k Sec-R1	Glu-R1k Gli-D1	Glu-R1i Sec-R1	— —	— —	— —	— —
196	194	9	10	70	479	4·64 ± 1·04	24·02 $0{\cdot}7 > P > 0{\cdot}5$

The validity of this method of gene mapping also depends on chromosome arm 1RL of the translocation pairing with the complete 1R of King II with the same frequency as would occur between two complete homologues of 1R and, furthermore, the absence of pairing between chromosome arms 1DS and 1RS. If the translocation reduces pairing between the 1RL arms the map distance will be underestimated, whereas any homoeologous pairing between 1DS and 1RS will lead to an overestimate. To check on these possible sources of error, the pairing between the translocation chromosome and complete 1R was examined at metaphase I of meiosis in PMCs from F_1 plants. Both chromosomes could be easily identified in C-banded preparations since the three rye chromosome arms had prominent telomeric bands whereas 1DS was unbanded (Plate 2). Altogether 607 PMCs were examined from 7 F_1 plants and no pairing was observed between 1DS and 1RS whereas there was an average of 62·2 % pairing between the long arms of these chromosomes, but this frequency was heterogeneous between families ($\chi^2_6 = 34{\cdot}45$, $P \ll 0{\cdot}01$). This heterogeneity for chromosome pairing frequency contrasts with the homogeneity of recombination and aneuploid frequencies obtained with the same F_1 plants. It is assumed that pairing heterogeneity may reflect different degrees of desynapsis in these plants resulting from fluctuations in the glasshouse temperature and the selection of anthers at different stages of metaphase I (cf. Fu & Sears, 1973).

Clearly the high frequency of pairing failure must also have occurred in the megaspore mother cells and contributed to the large number of aneuploids detected in the test-cross progeny. The protein phenotypes of the aneuploids and their frequencies are shown in Table 2. These phenotypes were scored without difficulty and except for two classes of gametes carrying two separate telocentrics which cannot be distinguished from recombinant phenotypes, it is likely that they represent a valid estimate of the frequency of aneuploid female gametes.

Having identified this pairing failure and the occurrence of aneuploids the problem is how to allow for them in the estimation of map distance. The univalents at metaphase I could have arisen from desynapsis or asynapsis or, more likely, some from each process. These two processes have different implications for estimating map distances, but since we do not have data on the degree of pairing between these chromosomes at prophase I we are unable to provide an accurate correction

to the estimate of map distance between *Glu-R1* and its centromere. Instead, to define the lower and upper limits of this estimate of map distance, we have considered the two extreme cases where (i) all univalents are due to desynapsis and (ii) all are due to asynapsis.

Table 2. *Frequency of aneuploid plants detected among the test-cross progeny*

Protein phenotype				No. of progeny	Presumptive aneuploid type	% of population
Gli-D1	Sec-R1	Glu-R1i	Glu-R1k			
+	+	+	+	2	Hyperploid	0·42
−	−	−	−	49	Hypoploid	10·22
−	+	−	−	3	Misdivision products	3·97
−	−	+	−	3		
−	−	−	+	9		
+	+	+	−	2		
+	+	−	−	1		
−	−	+	+	1		
Total				70		14·61

In the former case, all univalents at metaphase I will have paired at prophase I and have had a chance to cross over so that the frequency of recombination in gametes derived from chromosomes paired at metaphase I will be the same as in gametes derived from unpaired chromosomes, providing that the probability of desynapsis is independent of the position of chiasmata along the chromosome. However, it is thought that chromosomes with distal chiasmata are more likely to desynapse than those with proximal ones and it follows that, for proximal loci, the frequency of recombination will be higher among gametes arising from paired chromosomes than those derived from chromosomes unpaired at metaphase I. Taking the extreme case where all gametes derived from unpaired chromosomes are of the parental type, then all of the aneuploid gametes should be added to the parental class and this gives a map distance of $3{\cdot}96 \pm 0{\cdot}89$ cM which represents the lower limit for this distance.

If all of the univalents arose from asynapsis, then they would have had no chance to recombine and they would give rise to parental types only; thus, all these gametes should be excluded from the analysis. Therefore not only the aneuploid progeny should be excluded but also all of those euploid gametes which are derived from previously asynapsed chromosomes. Inclusion of these gametes would inflate the number of parental combinations and lead to an underestimate of the map distance. The very high frequency (49) of hypoploid compared to the paucity of hyperploid (2) progeny indicates that with these chromosomes the chance of inclusion of a univalent in the functional egg is much less than 25 % – the average for wheat chromosomes (Sears, 1954). Furthermore, Tsunewaki (1964) has observed significant differences among the transmission of the univalents in the 21 monosomic lines of wheat. Therefore it is better to calculate the transmission rate for univalents directly using the observed frequencies of hyperploids and hypoploids (Table 2) rather than the average for wheat chromosomes.

It can be assumed that these univalents are transmitted independently since a

homogeneous 1:1 segregation ratio is observed for these chromosomes in the test-cross progeny. Thus they have an equal probability i of inclusion in a functional egg. If the number of gametes arising from asynapsis is a, excluding the misdivision products m, then the frequency of hyperploid, euploid and hypoploid gametes arising from asynapsis will be $i^2/_a$, $2i(1-i)/_a$ and $(1-i)^2/_a$, respectively. Since the observed frequencies for hyperploid and hypoploid progeny are 2 and 49, respectively, the value of a is estimated to be 71. The adjusted recombination percentage R' was calculated using the formula: R' (%) $= [R \div (n-a-m)] \times 100$, where R = observed frequency of recombinants and n = total number of progeny. This gives an upper limit to the map distance of $4{\cdot}89 \pm 1{\cdot}10$ cM. Thus the real map distance must lie somewhere between the two extremes of $3{\cdot}96 \pm 0{\cdot}89$ cM and $4{\cdot}89 \pm 1{\cdot}10$ cM.

It is of interest to compare the location of gene(s) coding for *Glu-R1* polypeptides with those coding for related proteins in wheat and barley. The HMW-subunits of wheat which are presumed to be homoeologous with *Glu-R1* are coded for by homoeoalleles *Glu-A1*, *Glu-B1* and *Glu-D1* which have been mapped 9·0 cM from the centromere on the long arms of group 1 chromosomes (Payne *et al.* 1982). However, translocation mapping similar to the present analysis (Singh & Shepherd, unpublished) gave a much higher map distance for the *Glu-B1* and *Glu-D1* loci. The *Hor-3* locus, controlling the production of D hordein in barley, is also located on the long arm of barley chromosome 5 which is thought to be homoeologous to group 1 of wheat (Lawrence & Shepherd, 1981) and maps very close to the centromere (Blake, Ullrich & Nilan, 1982; Shewry *et al.* 1983). In summary, therefore, it would seem that the ancestral Triticeae genome possessed a chromosome with genes controlling HMW seed proteins on its long arm located very close to the centromere and, despite the genetic divergence during the course of speciation, as evident from different map distances for wheat and rye, the synteny of genes is grossly conserved. The higher map distance for wheat could be due to one or more of the following factors: greater physical distance of the locus *Glu-1* from the centromere, structural changes in some DNA segments between the locus and the centromere or localization of chiasmata in this region in wheat.

Besides its evolutionary significance, knowledge of the chromosomal location and the linkage map of biochemical markers in wheat and related species is of practical value. These markers can be used as effective tags for the controlled transfer of useful homoeoallelic characters from alien species into wheat by induction of homoeologous pairing (Koebner & Shepherd, unpublished).

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