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Mapping the nucleolus organizer region, seed protein loci and isozyme loci on chromosome 1R in rye

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Summary. The nucleolus organizer region located on the short arm of chromosome 1R of rye consists of a large cluster of genes that code for ribosomal RNA (designated the *Nor-R1* locus). The genes in the cluster are separated by spacer regions which can vary in length in different rye lines. Differences in the spacer regions were scored in two families of F_2 progeny. Segregation also occurred, in one or both of the families, at two seed protein loci and at two isozyme loci also located on chromosome 1R. The seed protein loci were identified as the *Sec 1* locus controlling ω-secalins located on the short arm of chromosome 1R and the *Sec 3* locus controlling high-molecular-weight secalins located on the long arm of 1R. The two isozyme loci were the *Gpi-R1* locus controlling glucose-phosphate isomerase isozymes and the *Pgd 2* locus controlling phosphogluconate dehydrogenase isozymes. The data indicated linkage between all five loci and map distances were calculated. The results indicate a gene order: *Pgd 2* ... *Sec 3* ... [centromere] ... *Nor-R1* ... *Gpi-R1* ... *Sec 1*. Evidence was obtained that rye possesses a minor 5S RNA locus (chromosome location unknown) in addition to the major 5S RNA locus previously shown to be located on the short arm of chromosome 1R.

Key words: Ribosomal DNA – Seed proteins – Isozymes – Genetic mapping – 5S DNA sequence – *Secale cereale*

Introduction

In wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and rye (*Secale cereale*) the chromosomal regions coding for the ribosomal RNA (rRNA), which are the sites of the nucleolus organizers, consist of a unit of DNA 9.0–10.0 kb in length repeated several thousand times in long tandem arrays in head-to-tail configuration (Flavell and O'Dell 1976; Appels et al. 1980). Each unit contains a gene coding for the large (26S) rRNA molecule, a gene coding for the small (18S) rRNA molecule and a gene coding for the 5.8S rRNA molecule separated from each other by spacer regions. All or most of the units which comprise a particular array possess spacer regions of the same length. Investigations of the largest of the spacer regions (located between the 26S and 18S rRNA genes) in wheat and other *Triticum* species have revealed the presence of considerable length variation (Appels and Dvorak 1982; Dvorak and Appels 1982). This variation occurs not only between species, but also among individuals of the same species. Differences in the spacer regions between individuals are conveniently assayed using the restriction endonuclease Taq I since, in most grasses examined to date, including rye, this enzyme releases a 1.5–3.0 kb spacer region segment in which most length variation is found (Appels and Dvorak 1982; Dvorak and Appels 1982). The required DNA fragments are visualized, after electrophoresis, denaturing and transferring the DNA to a membrane, by probing with a 2.7 kb spacer Taq fragment from the wheat pTA 250 rDNA unit clone present in the plasmid pTA 250.4 (Gerlach and Bedbrook 1979; Appels and Dvorak 1982).

This paper reports the segregation of spacer-length differences among individuals of two families of F_2 progeny in rye. Segregation also occurred, in one or both families, at two seed protein loci and at two isozyme loci located on the same chromosome (chromosome 1R) as the region coding for the rRNA. From

these data a linkage map of the five loci was obtained. A Hae III restriction endonuclease fragment assayed using a rye 5S DNA probe segregated independently of the other markers and was deduced to originate from a minor 5S RNA locus rather than the major 5S RNA locus known to be distal to the rRNA coding region on the short arm of 1R (Appels et al. 1980).

Materials and methods

Plant material

Three self-fertile lines of rye, designated Rye 2 a, Rye 26 and R14 were used as parents in the inheritance study. Rye 2 a and Rye 26 are inbred lines obtained from T. Lelley (Göttingen) and G. J. Scoles (Saskatoon), respectively. Line R14, obtained from N. H. Luig (Sydney), was derived from a crossing programme in which the self-fertility character originated from *Secale vavilovii.*

Crossing scheme and testing procedure

Two families of F_2 progeny were obtained from the following crosses: Rye 26 as female to Rye 2 a (cross 1) and Rye 2 a as female to R14 (cross 2). Individual F_1 plants were isolated in separate glass-houses at anthesis to ensure that no out-crossing occurred. All F_1 plants were highly self-fertile. Two ears were selected from an F_1 plant of each cross and every seed in these ears scored for the genetic markers segregating in that family. About a third of each grain was removed for seed protein tests after which the embryo part was grown to provide leaf tissue for isozyme tests and for DNA extraction.

Electrophoresis of seed proteins

The seed proteins were extracted into a buffered solvent containing 4% (w/v) sodium dodecyl sulphate (SDS) and 2% (v/v) 2-mercaptoethanol (2-ME) and fractionated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (Lawrence and Payne 1983); the separating gel contained 10% acrylamide.

DNA extraction and analysis

Two tillers from approximately one-month-old plants were used for isolating DNA following the procedure detailed by Appels and Moran (1984). Generally 0.5–1 mg of DNA was recovered and frozen as a stock solution of approximately 1 μg/μl in TE buffer (0.001 M EDTA 0.01 M Tris-HCl, pH 8). Samples for analysis (10 μg) were incubated with the restriction enzyme Taq 1 at 65 °C in a standard buffer (6 mM $MgCl_2$, 6 mM Tris-HCl pH 8, 70 mM NaCl, 12 mM 2-ME, 100 μg/ml bovine serum albumin (BSA)) at 65 °C for 1 h. The Taq 1 restriction endonuclease was prepared from 50 g aliquots of *Thermus aquaticus* using the procedures described by Green et al. (1978). Digested samples were electrophoresed in 1% agarose gels containing TAE buffer (0.02 Na acetate, 0.002 M EDTA, 0.04 M Tris-HCl, pH 8.4) so that the bromophenol blue marker migrated 18 cm. The segment of the gel from the origin to 12 cm was set up to transfer the DNA to Gene-screen (NEN) following manufacturers instructions. The filter was then baked for 2 h at 80 °C under vacuum, prehybridized in PH buffer (3×SSC, 50% formamide, 0.1% SDS, 0.1% polyvinylpyrrolidone, 0.1% BSA, 0.1% ficoll, 0.01 M Tris-HCl pH 8, 0.001 M EDTA) for 2–3 h at room temperature. 1×SSC = 0.15 M NaCl, 0.015 M Na citrate. Hybridization of the filters was carried out in PH buffer containing 0.01 μg/ml ^{32}P-labelled pTA250.4 insert DNA (specific activity ca. 10^7 cpm/μg) at 37 °C for approximately 10 h; this solution was heated to 95 °C/5 min, before use, to denature the probe. Following hybridization the filters were washed at room temp. in 2×SSC, 0.1% SDS which was initially at 65 °C for approximately 5 h with 3 changes of the wash solution.

Isozyme assays

Two isozymes, glucosephosphate isomerase (E.C. 5.3.1.9, GPI) and phosphogluconate dehydrogenase (E.C. 1.1.1.44, PGD) were assayed after starch gel electrophoresis. For each plant about 2 cm of juvenile leaf was crushed in 0.05 ml of 0.05 M sodium phosphate buffer, pH 7.0, containing dithiothreitol (1.5 mg · ml^{-1}). The crude extract was then absorbed in a paper chromatography wick (6×4 mm) which was inserted into a cut 4.5 cm from the cathodal end of the horizontal gel, with each gel accommodating 20 wicks. The gels contained 9.6% Connaught starch in 5 mM histidine – HCl (pH 8.0). The electrode buffer was 0.4 M sodium citrate titrated to pH 8.0 with 1 M citric acid (Brewer and Sing 1970). Electrophoresis was at a constant current of 25 mA for 6 h with the wicks being removed after the first 15 min. Assays for isozymes were as described by Brewer and Sing (1970) except that the solution for GPI was made in 1% agar and applied as an overlay.

Cloning of rye 5S DNA

DNA for cloning was enriched for 5S DNA by actinomycin-D/CsCl bouyant density gradients as described by Gerlach

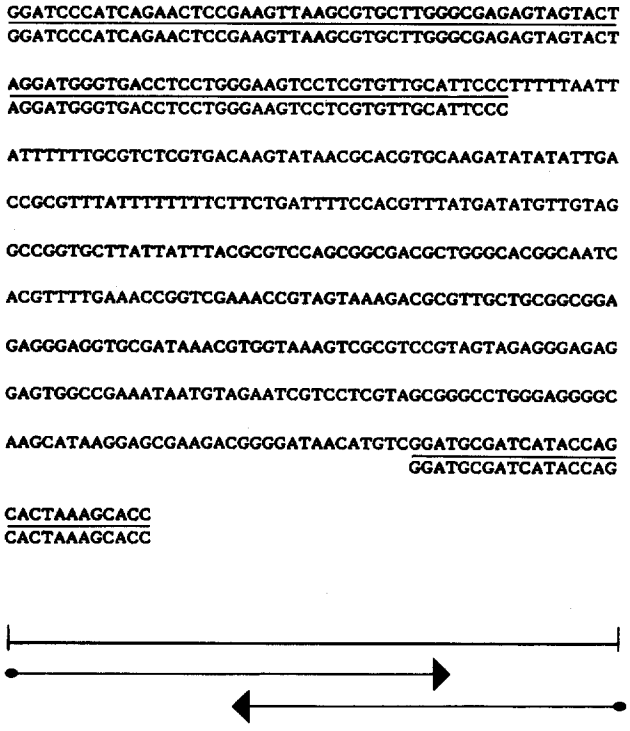

Fig. 1. Rye 5S sequence. The underlined portion of the sequence is the gene region as determined by comparison with published data. Published data are shown underneath the line. The sequencing strategy in shown below the sequence

and Dyer (1980). The starting density of the gradient was 1.63 g · cc^{-1}. The required fractions were extracted with isopropanol (saturated with salt) to remove actinomycin-D, dialysed against TE buffer and ethanol precipitated. Following Bam H1 digestion of these pooled fractions the DNA was centrifuged in a 10%–40% glycerol gradient (made up in TE plus 0.25 M NaCl) in an SW41 rotor, 27,000 rpm, 22 h, 10 °C. The 0–1 kb DNA was ligated to Bam H1 digested and alkaline phosphatase treated pBR322 (Biolabs) and used to transform *E. coli* RRI by standard procedures. Approximately 1,500 clones with rye DNA inserts were screened using a radioactive probe from pTA794 (Gerlach and Dyer 1980) and 13 positive colonies isolated. The sequence, determined using standard M13/dideoxy techniques, of the 462 bp insert in one the clones is shown in Fig. 1. The underlined sequence has perfect homology to the published 5S RNA sequence (Vanderberghe et al. 1984) confirming that the clone was 5S DNA.

Statistical analysis

Recombination fractions and their standard errors were calculated using the method of maximum likelihood. These were converted to map distances in centiMorgans (cM) using Jensen and Jorgensen's (1975) application of the Kosambi (1944) function:

$$cM = 25 \times \ln[(100+2r) \div (100-2r)] \pm 2{,}500\, s_r \div (2{,}500-r^2)$$

where r = recombination percentage, s_r = standard error of r

Results

1 Phenotypes of parents and progeny and locus nomenclature

a) The ribosomal RNA coding region. An autoradiograph which visualizes the spacer segments in the rDNA region of the parent lines, Rye 2a and Rye 26, of cross 1 is shown in Fig. 2. The two bands in Rye 2a (bands 1 and 2) have distinctly slower mobility than the two bands in Rye 26 (bands 3 and 4). The F_2 progeny either possessed one or other of the parental banding patterns, or possessed all four bands (Fig. 2). The segregation ratios of the phenotypes 1, 2 : 1, 2, 3, 4 : 3, 4 (see Table 1) fitted a 1 : 2 : 1 ratio consistent with the 1, 2 combination being allelic to the 3, 4 combination. Bands 1 and 2 always occurred together as did bands 3 and 4, and no new bands appeared among the progeny. A novel band was found in one individual among the seed recovered from the Rye 2a parent used in the cross and this was traced to cross-pollination by rye plants from another breeding programme which were inadvertedly placed in the same small glass house (Fig. 2). It is proposed that the locus controlling the above bands be designated *Nor-R1* following the nomenclature used for wheat by Payne et al. (1984). Alleles will be indicated by showing band numbers in brackets e.g. Nor-R1 (1, 2).

In cross 2, one parent (R14) possessed a single band (band 1) which had the same mobility as one of the two bands of the other parent, Rye 2a (Fig. 3). The F_2 progeny possessed either the Rye 2a pattern (bands 1 and 2) or the R14 pattern (band 1) in agreement with a 3 : 1 ratio (see Table 2) again consistent with the assumption that the differences are controlled by alleles at the same locus. Presumptive heterozygotes were often distinguishable on the basis of quantitative differences in the relative amounts of bands 1 and 2 but scoring was occasionally ambiguous and not used in this analysis.

b) Seed protein loci. The segregation of seed protein differences in the two F_2 families was examined because chromosome 1R is known to carry genes controlling urea-soluble "secalins" (rye endosperm storage proteins) on its short arm (Shepherd 1968; Shepherd and Jennings 1971) and genes controlling high-molecular-weight (HMW) secalins on its long arm (Lawrence and Shepherd 1981). The locus on the short arm has been designated *Sec 1* and its products referred to as the ω- and 40 K γ-secalins (Shewry et al. 1984). The products of the locus on the long arm have been called either the HMW subunits of rye glutelin and the locus

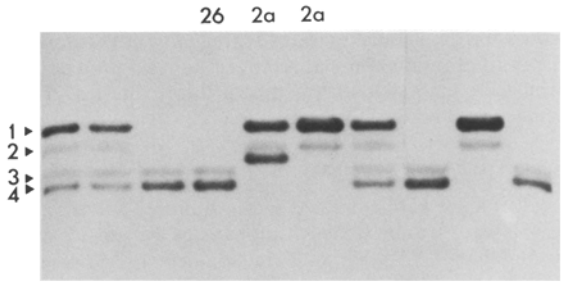

Fig. 2. Segregation of rDNA spacer variants in the Rye 2a × Rye 26 cross. Shown are the parents used in cross 1, Rye 26 with *bands 3* and *4* and Rye 2a with *bands 1* and *2* (the right hand side of the two lanes marked 2a). Other lanes show F_2 progeny. The left hand side of the two lanes marked 2a shows a novel band migrating just ahead of *band 2* found in one seed recovered from the 2a parent (discussed in text). *Bands 1, 2, 3* and *4* were estimated to be approximately 2.8, 2.5, 2.2 and 2.15 kb long, respectively. The origin is at the top of the gel

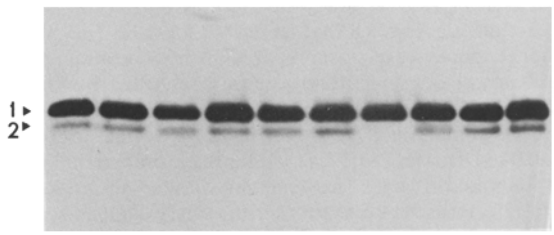

Fig. 3. Segregation of rDNA spacer variants in the R14 × Rye 2a cross. R14 possesses *band 1* alone and Rye 2a *bands 1* and *2*. Lane 7 from the left hand side shows an F_2 individual lacking *band 2*. The origin is at the top

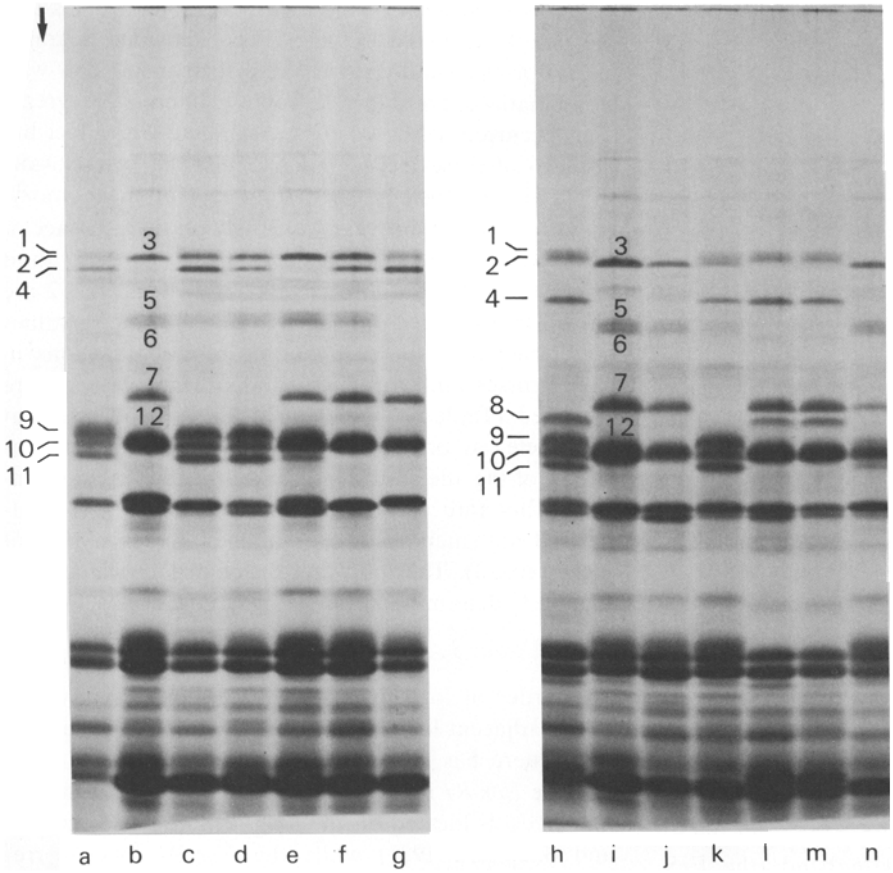

Fig. 4. Fractionation by SDS-PAGE of seed proteins. The analyses show the parents used in cross 1 (*a* = Rye 26, *b* = Rye 2a) and F_2 progeny from this cross (*c, d, e, f, g*) together with parents used in cross 2 (*h* = R14, *i* = Rye 2a) and F_2 progeny from this cross (*j, k, l, m, n*). The *arrow* shows direction of protein migration

designated *Glu-R1* (Singh and Shepherd 1984), or the HMW secalins and the locus designated *Sec 3* (Shewry et al. 1984). The *Sec 3* designation has been adopted in this paper as this was used in a recent review (Miller 1984).

Examples of the banding patterns obtained by SDS-PAGE of the seed proteins of the parents and several F_2 progeny in both cross 1 and cross 2 are shown in Fig. 4. Rye 2a, which is a parent in both crosses, possesses three slow-moving bands, designated 3, 5 and 6, which were always inherited as a unit. Bands 1, 2, and 4 in Rye 26 and R14 were also inherited as a unit. In both F_2 families (see Tables 1 and 2) the segregations of the 1, 2, 4 : 1, 2, 4/3, 5, 6 : 3, 5, 6 phenotypes were in agreement with a 1 : 2 : 1 ratio, consistent with the 1, 2, 4 combination in both Rye 26 and R14 being allelic to the 3, 5, 6 combination in Rye 2a. The mobility of these bands in SDS gels indicates that they are the high molecular weight (HMW) secalins (= HMW glutelin subunits) and thus were assigned to the *Sec 3* locus located on the long arm of 1R.

Bands 7 and 12 in Rye 2a were inherited as a unit as were bands 9, 10, 11 in Rye 26 and R14 (see Fig. 4). In both families the segregation of the 7, 12 : 7, 12/9, 10, 11 : 9, 10, 11 phenotypes was in agreement with a 1 : 2 : 1 ratio, consistent with the 7, 12 combination being allelic to the 9, 10, 11 combination (data not shown). The mobilities of these bands suggests that they are the 75 K γ-secalins controlled by the *Sec 2* locus on chromosome 2R (Lawrence and Shepherd 1981; Shewry et al. 1984). Consistent with this is the finding that the segregation of these bands was independent of the segregation at other loci examined in this study and known to occur on 1R. Further data pertaining the *Sec 2* locus have not been included in this paper.

In cross 2, band 8 from R14 (see Fig. 4) segregated independently of the 9, 10, 11 combination but, as shown below, was loosely linked to bands 1, 2, 4. This band was therefore assigned to the ω-secalin class of protein (Shewry et al. 1984) controlled by the *Sec 1* locus located on the short arm of 1R. Among the F_2 progeny from cross 2, segregation for the presence or absence of band 8 (see Table 2) was in agreement with a 3 : 1 ratio.

c) Isozyme loci. Banding patterns for the isozymes of glucosephosphate isomerase (GPI) and phosphogluconate dehydrogenase (PGD) were examined in the F_2 progeny from cross 1. Both GPI and PGD isozymes were present in two well-separated migration zones and, in each case, obvious segregation of the isozymes

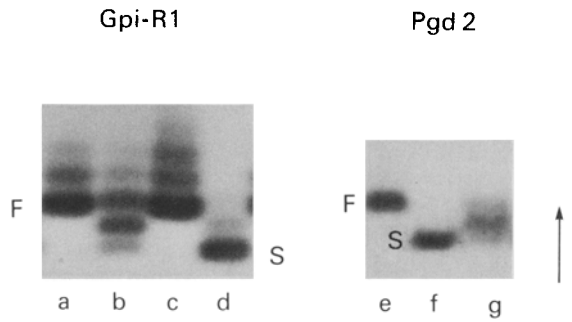

Fig. 5. Zymograms showing isozyme variation. Shown are the banding patterns of glucosephosphate isomerase isozymes controlled by the *Gpi-R1* locus and phosphogluconate dehydrogenase isozymes controlled by the *Pgd 2* locus segregating in cross 1. The origin is at the bottom – *F* designates the fast-moving isozyme and *S* the slow moving isozyme. For *Gpi-R1*, parental patterns are *a* = Rye 2a, *d* = Rye 26 and *b* = heterozygous F_2 individual, *c* = F_2 individual homozygous for fast allele. For *Pgd 2*, parental patterns are e = Rye 26 f = Rye 2a and g = heterozygous F_2 individual. The arrow shows direction of protein migration

occurred in the zone of least mobility. Within this zone, for both isozymes, each of the parents possessed (essentially) a single band (some conformer bands were present for GPI) of different mobility; the fast-moving band was designated F and the slower band S (see Fig. 5). The F_2 progeny possessed either the F, or the S, or both bands plus an intermediate band (the F/S phenotype), as shown in Fig. 5. The segregation ratios of the F : FS : S phenotypes for both GPI and PGD (see Table 1) fitted a 1 : 2 : 1 ratio consistent with the F and S isozymes being controlled by allelic genes. The band of intermediate mobility in the heterozygotes (F/S) indicates that GPI and PGD are both dimeric enzymes.

The loci controlling the GPI and PGD isozymes in the zone of slower mobility were both found to be linked to the other 1R chromosome loci (see below). The GPI alleles were therefore assigned to the Gpi-R1 locus which Chojecki and Gale (1982) showed to be located on chromosome 1R. A locus controlling PGD isozymes has not been reported on chromosome 1R. It is proposed that this locus be designated *Pgd 2* because it almost certainly corresponds to a locus on barley chromosome 5 which has been designated *Pgd 2* (Brown and Munday 1982); barley chromosome 5 and rye chromosome 1R are homoeologous, since both are homoeologous to the group 1 chromosomes in wheat (Shepherd 1973; Lawrence and Shepherd 1981; Brown 1983; Miller 1984).

2 Joint segregations and map distances

In cross 1 the F_2 progeny segregated at four of the loci under examination, namely, *Nor-R1, Sec 3, Gpi-R1* and *Pgd 2* and, at each of the loci, both homozygotes and heterozygotes could be identified. The joint segregations for the genotypes at these loci, in all pair-wise combinations, are shown in Table 1. In cross 2, segregation occurred at the *Nor-R1, Sec 3* and *Sec 1* loci but for two of these loci (*Nor-R1, Sec 1*) the heterozygote could not be distinguished from one of the homozygotes; scoring in this case was solely on the presence or absence of a band from one of the parents. Joint segregations for the three loci examined in cross 2 are shown in Table 2. Contingency table chi-square values were calculated for each of the joint segregations in both crosses and, in all cases, significant values were obtained (Table 3). The data in Table 3 indicate that the alleles at one locus were not inherited independently of the alleles at the second locus under examination. Therefore recombination percentages were calculated and map distances obtained for each pair of loci (Table 3). The following order of loci was unambiguously determined from the map distances:

Pgd 2 . . . Sec 3 . . . Nor-R1 . . . Gpi-R1 . . . Sec 1

This order of loci, together with distances in cM between adjacent loci, is shown in Fig. 6. In this map the centromere has been located between the *Sec 3* locus and the *Nor-R1* locus on the basis of previous evidence that *Sec 3* is located on the long arm of 1R (Lawrence and Shepherd 1981) while the *Nor-R1* locus corresponds to the secondary constriction observed on the short arm of 1R (Appels et al. 1980).

3 The 5S RNA coding region

In situ hybridization studies by Appels et al. (1980) placed a major site for the 5S RNA genes distal to the nucleolus organizer region on chromosome 1R, the site of the rRNA genes. In an attempt to obtain a genetic linkage estimate between these two loci the three parental rye lines used in the preceding section were digested with a number of restriction endonucleases to see if a useful polymorphism could be detected. The restriction endonuclease Hae III gave a number of bands, some of which clearly derived from a unit of the same type used as the probe (see "Materials and methods") since this contained two Hae III sites in the spacer region as well as one in the gene. Rye 2a and Rye 26 were distinguishable on the basis of the presence or absence of one of the Hae III bands (Fig. 7) and thus the DNA samples analysed with the rDNA probe were reanalysed with the 5S DNA probe (pSc T7). However the 5S RNA band difference segregated independently of *Nor-R1* (data not shown). It would appear therefore that rye, as is the case in wheat, has a minor location for genes controlling 5S RNA which was not previously detected by in situ hybridization but which gives rise to

Table 1. Joint segregation of genotypes at specific pairs of loci located on chromosome 1R of rye amongst F_2 progeny from cross 1

		Sec 3	*Nor-R1*	*Gpi-R1*	*Pgd 2*
Female: Rye 26		1,2,4/1,2,4	3,4/3,4	S/S	F/F
Male parent: Rye 2a		3,5,6/3,5,6	1,2/1,2	F/F	S/S

		Sec 3			*Pgd 2*			*Gpi-R1*		
		1,2,4/1,2,4	1,2,4/3,5,6	3,5,6/3,5,6	F/F	F/S	S/S	F/F	F/S	S/S
	3,4/3,4	24	4	0	22	4	2	0	3	25
Nor-R1	3,4/1,2	2	52	7	6	47	8	5	51	5
	1,2/1,2	0	3	24	1	6	20	23	4	0
	F/F	0	6	22	1	9	18			
Gpi-R1	F/S	5	44	9	9	40	9			
	S/S	21	9	0	19	8	3			
	F/F	24	4	1						
Pgd 2	F/S	1	52	4						
	S/S	1	3	26						

Table 2. Joint segregation of genotypes at specific pairs of loci located on chromosome 1R of rye amongst F_2 progeny from cross 2

		Sec 3	*Nor-R1*	*Sec 1*
Female parent: Rye 2a		3,5,6/3,5,6	1,2/1,2	–/–
Male parent: R14		1,2,4/1,2,4	1/1	8/8

		Sec 3			*Nor-R1*	
		1,2,4/1,2,4	1,2,4/3,5,6	3,5,6/3,5,6	1,2/1,2; 1,2/1	1/1
Sec 1	8/8,8/–	28	64	25	88	29
	–/–	5	16	18	37	2
Nor-R1	1,2/1,2; 1,2/1	7	76	42		
	1/1	26	4	1		

Table 3. Contingency table χ^2 values for independent segregation, recombination percentages and map distances for each pair-wise combination of loci located on chromosome 1R of rye at which segregation occurred amongst the F_2 progeny in two crosses

Cross	Marker association	χ^2	Degrees of freedom	*P*	Recombination % (± SE)	Map distance in cM (± SE)
1	*Sec 3 – Nor-R1*	147.8	4	<0.001	7.2±1.8	7.3± 1.8
	– Gpi-R1	94.9	4	<0.001	13.4±2.4	13.7± 2.6
	– Pgd 2	153.3	4	<0.001	7.2±1.8	7.3± 1.8
	Pgd 2 – Nor-R1	95.1	4	<0.001	14.0±2.5	14.4± 2.7
	–Gpi-R1	55.5	4	<0.001	20.7±3.1	22.0± 3.7
	Gpi-R1 – Nor-R1	141.9	4	<0.001	7.6±1.8	7.7± 1.8
2	*Sec 3 – Sec 1*	9.3	2	<0.01	36[a] ±4.6	45.4± 9.6
	– Nor-R1	91.4	2	<0.001	9[a] ±2.4	9.1± 2.5
	Nor-R1 – Sec 1	5.9	1	<0.05	26.5±7.4	29.5±10.2

[a] Calculated only to nearest whole percentage point using tables provided by Allard (1956)

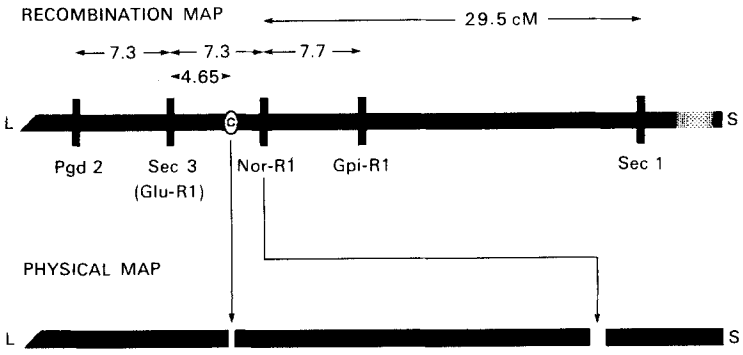

Fig. 6. Comparison of recombination and physical maps of the short arm (*S*) and part of the long arm (*L*) of chromosome 1R of rye. The two maps have been aligned at their centromeres. The *bent arrow* highlights the disparity in the positions of the ribosomal RNA genes on the maps. The recombination map is broken (*grey area*) between the *Sec 1* locus and the end of the short arm to indicate that the map distance between these points is unknown. The map distance between the *Sec 3* locus and the centromere (4.65 cM) is taken from Singh and Shepherd (1984)

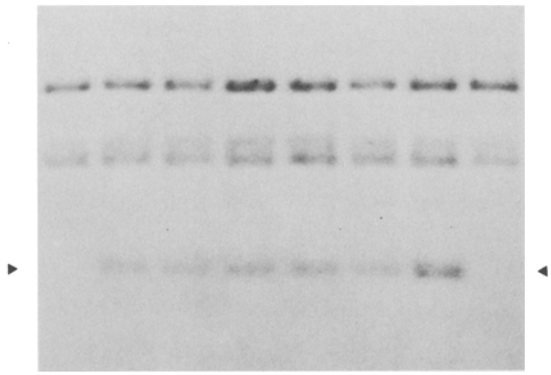

Fig. 7. Segregation of 5S DNA band in the Rye 26 × Rye 2a cross. The *arrow* indicates a band ca 250 bp long which is present in Rye 26 but absent from Rye 2 a

the Hae III band assayed. This is presently under further investigation.

Discussion

The linkage map obtained for the five loci investigated in this study (Fig. 6) is consistent with previous observations that have assigned the *Sec 3* locus to the long arm of 1R and the *Nor-R1* and *Sec 1* loci to the short arm. While the *Gpi-R1* locus has previously been assigned to 1R (Chojecki and Gale 1982) its arm location has not been reported. The linkage map suggests Gpi-R1 is located on the short arm and this has recently been directly established by R. M. D. Koebner (personal communication) using a wheat line possessing a 1RS-1BL translocation chromosome. The *Pgd 2* locus has not been previously reported on chromosome 1R. Loci controlling phosphogluconate dehydrogenase isozymes have been identified on chromosomes 2R, 4R and 6R (Rao and Rao 1980; Salinas and Benito 1983) and we assume that these loci control the isozymes in the faster-moving zone which were not scored in this study.

The map distance between the *Sec 3* and *Sec 1* loci obtained in this work, namely 45.4 ± 9.6 cM (cross 2, Table 3) is comparable to the estimate of 57.4 ± 11.3 cM obtained by Shewry et al. (1984), given that the standard errors of both estimates are large and that recombination frequencies can be influenced by genetic background and environmental conditions. The only other mapping study reported so far for loci on chromosome 1R is that of Singh and Shepherd (1984) where the distance between the *Sec 3* locus and the centromere was estimated to be 4.65 ± 1.04 cM.

The order of four of the loci examined in this study, *Sec 3*, *Nor-R1*, *Gpi-R1* and *Sec 1* is identical to the order of the corresponding loci on chromosome 1B in wheat, namely *Glu-B1*, *Nor 1*, *Gpi-B1* and *Gli-B1*, respectively. Relative map distances between equivalent loci are also comparable (Chojecki et al. 1983; Payne et al. 1984; Ainsworth et al. 1984; Snape et al. 1985). However, in absolute terms, the map distances reported for the 1B loci in wheat are two to three times greater than the values found in this study for equivalent regions on chromosome 1R of rye. e.g. *Sec 3* to *Nor-R1* = 7.3 cM c.f. *Glu-B1* to *Nor 1* = 22 cM and *Sec 3* to *Gpi-R1* = 13.4% recombination c.f. *Glu-B1* to *Gpi-B1* = 36.2% recombination. Similarly Singh and Shepherd (1984) found a map distance in rye to be less than half the equivalent map distance in wheat. Thus an inherently higher frequency of crossing-over may occur in wheat than in rye. Alternatively, if a greater amount of heterozygosity occurs in rye than in wheat, this might account for the lower level of crossing-over in rye, since there is evidence that less crossing-over occurs between heterozygous homologues than between homozygous homologues (e.g. Dvorak and Chen 1984; Crossway and Dvorak 1984).

The mapping data in this study add a further example to the growing list of instances where map distances based on recombination frequency bear little relationship to the physical distance between loci. Thus the mapping data indicate little if any crossing-over occurs between the centromere and the *Nor-R1* locus (by subtraction, 7.3–4.65, a value of 2.65 cM is obtained, see Fig. 6). Physically this distance is 65–75% of the length of the short arm (Sybenga 1983). By contrast, a map distance of 29.5 cM was obtained between the *Nor-R1* locus and the *Sec 1* locus even though this represents less than 25% of the short arm. The disparity is highlighted in Fig. 6 where a cytological map of 1R, based on the morphology of 1R as shown in Sybenga (1983), is included for comparison with the recombina-

tion map. Similar evidence, in cereals, for little or no crossing-over between the centromere and *Nor* loci has been reported for chromosomes 1B and 6B of wheat (Payne et al. 1984; Dvorak and Chen 1984; Snape et al. 1985) and barley chromosomes 6 and 7 (Linde-Laursen 1979). It is thus possible that many chromosomes in wheat and related species possess large regions on either side of the centromeres where little or no crossing-over occurs.

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