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A cytogenetic map on the entire length of rye chromosome 1R, including one translocation breakpoint, three isozyme loci and four C-bands

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Summary. A cytogenetic map of the whole 1R chromosome of rye has been made, with distances between adjacent markers shorter than 50% recombination. Included in the map are isozyme loci *Gpi-R1*, *Mdh-R1* and *Pgd2*, the telomere C-bands of the short arm (*ts1*) and the long arm (*tl1*), two interstitial C-bands in the short arm proximal to the nuclear organizing region (NOR) (*is1*) and in the middle of the long arm (*il1*), respectively, and translocation *T273W* (Wageningen tester set). By means of electron microscope analysis of spread pachytene synaptonemal complexes, the breakpoint of this translocation was physically mapped in the short arm of 1R, proximal to NOR, and in the long arm of 5R (contrary to previous assumptions). The data indicated the marker order: *ts1* – *Gpi-R1* – *is1* – *T273W*/*Mdh-R1* – *il1* – *Pgd2* – *tl1*. A comparison between genetic and physical maps revealed that recombination is mainly restricted to the distal regions of both arms. For the translocation *T273W*, in heterozygotes no recombinants were observed between the translocation breakpoint and its two adjacently located markers (*is1* and *Mdh-R1*), but recombination was not reduced in the distal regions of the chromosome. The segregations of several other isozyme and C-band markers also analyzed in the investigation presented here were consistent with observations of earlier authors concerning chromosome asignment and linkage relationships.

Key words: Rye – Cytogenetic maps – Isozymes – C-heterochromatin bands – Translocation

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Introduction

In recent years, an increasing number of investigations have been carried out on the construction of genetic maps in rye. These have involved different combinations of markers, including those for morphological traits, isozymes, RFLPs, endosperm proteins, disease resistances, the nucleolus organizer region (NOR), the centromeres, translocation breakpoints, and C-bands (for references see Figueiras et al. 1989; Benito et al. 1990, 1991; Vaquero et al. 1990; Carrillo et al. 1990; Sybenga et al. 1990; Gustafson et al. 1990; Singh et al. 1990; Wang et al. 1991).

The best known chromosome in this species is most likely chromosome 1R, in which integrations between genetic and physical maps, providing relevant information about the frequency and distribution of crossing-over along the chromosome length, have been attempted in several instances. Cytological markers as the nucleolus organizer (Lawrence and Appels 1986) and the telomeric C-band of 1RS (Singh et al. 1990) have been used to construct cytogenetic maps for the short arm of this chromosome. Structural changes, including telocentrics and translocations, have also been used as cytological markers (Figueiras et al. 1985, 1989; Sybenga et al. 1990). However the comparison between the genetic distances in these cases with the ones obtained in karyotypically normal plants can present some problems, and in most cases the exact locations of the breakpoints of the translocations used have not been clearly determined. The genetic distances between six C-bands located along the whole length of chromosome 1R were reported by Kaltsikes et al. (1986), with values lower than 50% recombination not being observed between all pairs of consecutive C-bands.

The present article reports on a map of the whole 1R chromosome i.e., from telomere to telomere, with all consecutive markers located at distances of lower than 50% recombination. The map includes three isozyme loci (*Pgi-R1, Mdh-R1* and *Pgd2*), the C-bands of the two telomeres and two interstitial C-bands located, respectively, in the short and long arm of this chromosome. In addition, a comparison has been made between the maps including these markers in plants heterozygous for a translocation (*T273W*) involving chromosomes 1R and 5R and in normal, non-translocated plants. The translocation breakpoint has been physically mapped with respect to other features of chromosome 1R by means of electron microscope analysis of spread pachytene synaptonemal complexes.

Material and methods

Plant material

Different crosses involving plants from cv 'Ailés', cv 'Merced', inbred line E (Giraldez et al. 1979) and a line homozygous for translocation *T273W* (involving chromosomes 1R and 5R; translocation tester set; Sybenga and Wolters 1972) were made in order to obtain plants heterozygous for different markers, including those for isozymes, C-bands and translocation *T273W*.

From these crosses, six multiple heterozygous plants were selected (Table 1). The segregation analysis was made in the six progenies (backcross-like) that were obtained from the crosses between these multiple heterozygous plants and homozygous plants for all markers (the line homozygous for translocation *T273W* indicated above or a line double ditelocentric for chromosome 3R obtained from J. Sybenga). The multiple heterozygous plants were used as females in plants 9-15, 13-13 and 14-25, and as both males and females in plants 5-22, 13-14 and 13-24. In these three plants differences between male and female segregations were not significant.

A line ditelocentric for chromosome 1R obtained from J. Orellana was also used in the synaptonemal complex analyses.

All plants were grown in a climate chamber under identical conditions during their life cycle. During meiosis, a temperature of 18–20 °C and a photoperiod of 14 h light/10 h dark were maintained.

Isozyme assays

Six isozymatic systems from extracts of leaf tissue, phosphoglucose mutase (PGM, EC 2.7.5.1), glucosephosphate isomerase (GPI, EC 5.3.1.9), malate dehydrogenase (MDH, EC 1.1.1.37), phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), glutamic oxaloacetic transaminase (GOT, EC 2.6.1.1) and aconitase (ACO, EC 4.2.1.3), were assayed following electrophoresis of the extract on horizontal 12% starch gel. The buffers and staining methods were as described by Figueiras et al. (1985) and Chenicek and Hart (1987).

Mitotic and meiotic metaphase C-banding analysis

Root tips were immersed in tap water at 0 °C for 24 h to shorten the chromosomes and fixed in acetic acid: alcohol 1:3. Anthers having PMCs at metaphase I were fixed in acetic acid: alcohol

Table 1. Genotypes of the multiple heterozygous plants used as parents of the six segregating progenies analyzed. These plants were crossed by the lines ditelo-3R and/or hom-T273W, whose genotype for the different markers is also shown

Plant[a]	Isozyme loci[b]						C-heterochromatin bands[b]								Translocation *T273W*[b]	Number of plants of the progeny
	Pgm-R1	*Gpi-R1*	*Mdh-R1*	*Mdh-R2*	*Pdg2*	*Got-R3*	*Aco-R2*	*ts1*	*is1*	*il1*	*tl1*	*ts2*	*il2*	*tl2*	*ts6*	
5-22	1/2	1/3	1/3	2/2	1/2	?	1/1	−/+	+/−	−/+	−/+	?	−/−	?	+/+	T/N	44
9-15	1/2	1/3	1/3	1/2	1/2	1/2	1/1	+/+	+/−	−/−	−/+	−/+	−/+	+/+	+/+	T/N	69
13-13	1/2	1/3	1/3	1/2	1/2	2/2	1/2	−/+	+/−	−/−	+/+	?	−/−	?	+/+	N/N	30
13-14	1/2	1/3	1/3	?	1/2	2/2	1/1	−/+	+/−	−/−	−/+	?	−/−	?	+/+	N/N	48
13-25	1/1	1/3	1/3	1/2	1/2	?	1/2	−/+	+/−	+/+	+/+	?	−/−	?	+/+	T/N	146
14-25	1/1	1/3	1/3	2/2	1/2	1/2	1/2	−/+	+/−	−/−	−/+	+/+	+/−	−/+	+/+	N/N	74
ditelo-3R	1/1	1/1	1/1	1/1	1/1	2/2	1/1	+/+	−/−	−/−	+/+	?	−/−	?	+/+	N/N	
hom-T273W	1/1	1/1	1/1	2/2	1/1	2/2	1/1	+/+	−/−	−/−	+/+	?	−/−	?	+/+	T/T	

[a] The number before the hypen refers to the cross from which the plant was obtained

[b] The order in which the different alleles are written (before or after the bar) indicates their parental origin

1:3. The fixed materials were maintained in the fixative for 1–4 months at 3–4 °C and then squashed and stained following the Giemsa C-banding technique described by Giraldez et al. (1979).

Synaptonemal complex analysis

The pachytene synaptonemal complexes (SCs) of 1 plant heterozygous for translocation *T273W* (belonging to the analyzed progenies) and of 1 plant ditelocentric for chromosome 1R (obtained from J. Orellana) were analyzed following the spreading and staining procedures of de Jong et al. (1989) as modified by Naranjo et al. (1989).

The SCs were studied in the electron microscope, photographed and printed at final magnification of × 7000. The lengths of the SCs were measured on the photographic prints by means of a digitizing tablet connected to a computer.

Statistical analysis

Recombination fractions and their standard errors were calculated using the method of maximum likelihood.

Jensen and Jorgensen's (1975) application of the Kosambi (1944) function was used in order to convert recombination fractions to map distances in centiMorgans (cM).

Results and discussion

Plant pheno- and genotypes and marker nomenclature

Table 1 shows the genetic constitution of the heterozygous plants used as parents of the backcross-like offsprings analyzed.

Isozyme loci

Phosphoglucomutase (PGM) and glucosephosphate isomerase (GPI) appeared in the same electrophoretic gel (Fig. 1a) in two well-separated migration zones. The monomeric isozyme PGM had the faster mobility and showed a band pattern similar to that described by Wehling et al. (1985), Salinas and Benito (1985a) and Vaquero et al. (1990). According to the catalogue of gene symbols for wheat (McIntosh 1988), the locus controlling this isozyme is designated *Pgm-R1* (alleles 1 and 2).

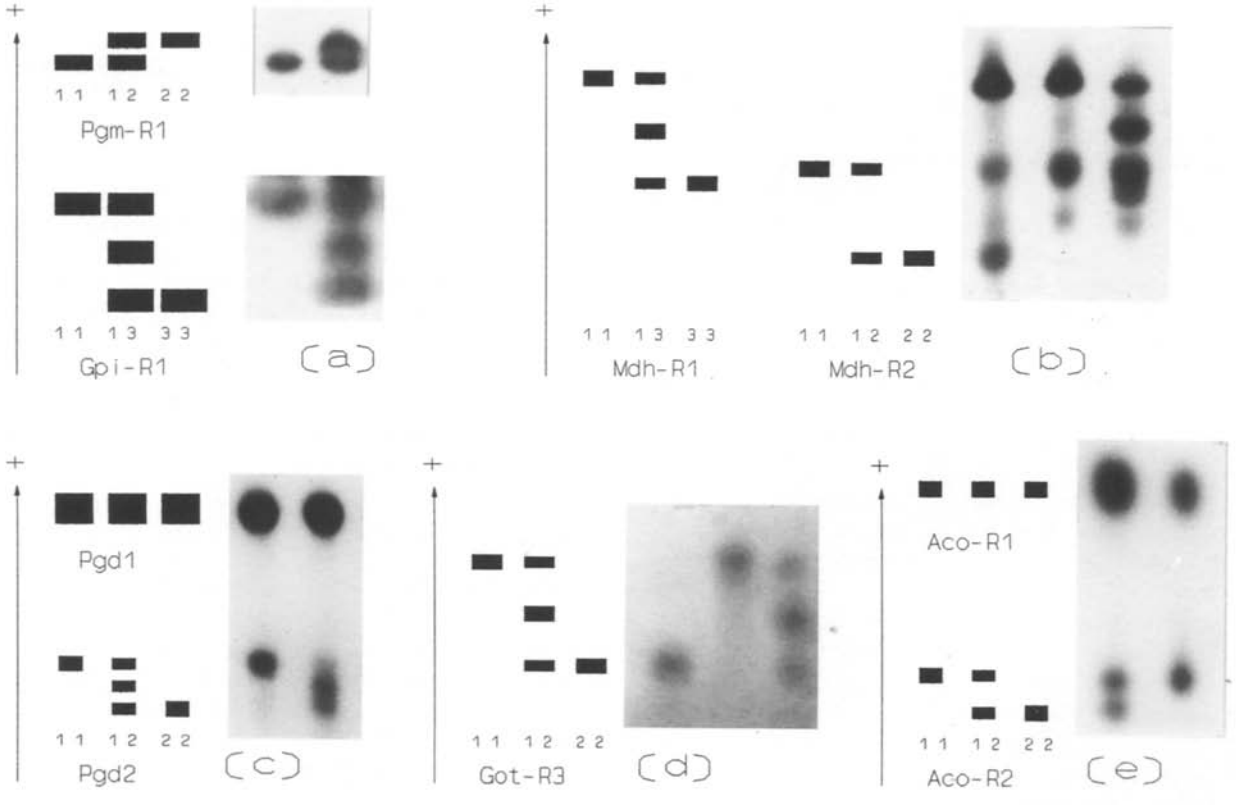

Fig. 1a–e. Electrophoretic patterns and zymograms of the leaf isozyme systems analyzed. **a** Phosphoglucomutase (*PGM*) and glucosephosphate isomerase (*GPI*), **b** malate dehydrogenase (*MDH*), **c** phosphogluconate dehydrogenase (*PGD*), **d** glutamic oxaloacetic transaminase (*GOT*), **e** aconitase (*ACO*). The *arrows* show the direction of protein migration

The electrophoretic pattern of the dimeric isozyme GPI was similar to the one corresponding to a locus designated *Gpi-R1* by Chojecki and Gale (1982), *Pgi2* (alleles 1 and 3) by Vaquero et al. (1990) and *Gpi-1* by Benito et al. (1990). In this work, the locus is designated *Gpi-R1* (McIntosh 1988), alleles 1 and 3.

The isozyme system malate dehydrogenase (MDH; Fig. 1b) shows two migration zones (overlaping in part) that correspond to loci designated *Mdh2-1* (fast mobility; dimeric isozyme pattern) and *Mdh2-2* (slow mobility; monomeric isozyme pattern) by Salinas and Benito (1985b) and *Mdh1* (alleles 1 and 3) and *Mdh2* (alleles 1 and 2), respectively, by Vaquero et al. (1990). According to McIntosh (1988), the loci are designated *Mdh-R1* (fast mobility zone; alleles 1 and 3) and *Mdh-R2* (low mobility zone; alleles 1 and 2).

The segregation for phosphogluconate dehydrogenase (PGD; Fig. 1c) occurred in the zone of lower mobility, showing a band pattern (dimeric) that fits the one corresponding to the locus designated *Pgd2* by Lawrence and Appels (1986) and *6Pgd2* (alleles 1 and 2) by Vaquero et al. (1990). In this work it is designated *Pgd2* (McIntosh 1988), alleles 1 (fast mobility) and 2 (slow mobility).

The isozyme system glutamic oxaloacetic transaminase (GOT; Fig. 1d) was analyzed in four progenies, two of which showed segregation in the zone of lower mobility. This enzyme showed a band pattern (dimeric) that corresponds to the locus designated *Got3* by Vaquero et al. (1990). In this work, the locus is designated *Got-R3* (McIntosh 1988), alleles 1 (fast mobility) and 2 (slow mobility).

In the isozyme system aconitase (ACO; Fig. 1e), segregation occurred in the zone of slower mobility, with an electrophoretic pattern similar to the one described by Chenicek and Hart (1987) for locus *Aco-2*. In this work, the locus is designated *Aco-R2* (McIntosh 1988), alleles 1 (fast mobility) and 2 (slow mobility).

The inheritance of each individual isozyme locus fitted the expected 1:1 segregation.

C-heterochromatin bands

The plants used as parents in this study had different C-banding patterns (Table 1). Figure 2 shows some examples of segregating chromosomes in the progenies analyzed.

Heterozygosis for the amount of C-heterochromatin was clearly established in the telomere C-bands of the short (*ts1*) and long (*tl1*) arms, respectively, of chromosome 1R, in the C-band adjacent to the nucleolar organizer region (*is1*) and in the C-band located in the middle of the long arm (*il1*).

Two of the plants used as parents were also heterozygous for a large interstitial C-band (*il2*) located in the middle of the long arm of chromosome

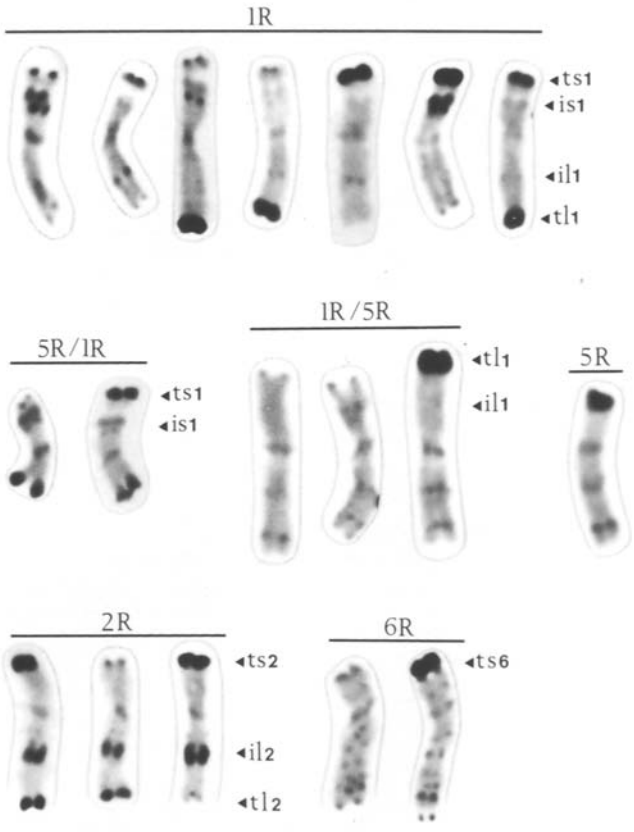

Fig. 2. Some examples of chromosomes 1R, 2R, 5R, 5R/1R, 1R/5R and 6R appearing in the progenies analyzed. The different segregating C-bands are indicated

2R. These two plants segregated for the 2RS telomere C-band (*ts2*) and for the 2RL telomere C-band (*tl2*), respectively. Since chromosomes 2R and 3R have a quite similar banding pattern, in order to avoid misidentifications only those chromosomes having the *il2* band were positively identified as chromosome 2R. Another plant was heterozygous for the telomeric C-band (*ts6*) of the short arm of chromosome 6R.

The inheritance of each individual C-band fitted the expected 1:1 segregation.

Translocation T273W

At mitosis, the two translocated chromosomes were easily identified (Fig. 2) as they fit the morphology and C-banding pattern described by de Vries and Sybenga (1976). This C-banding pattern indicates that the relocated telomere constitutions of the two translocated chromosomes are 1RS-5RS (chromosomes 5R/1R of Fig. 2) and 1RL-5RL (chromosomes 1R/5R of Fig. 2), respectively.

In order to determine the physical location of the translocation breakpoint (TB) with respect to the

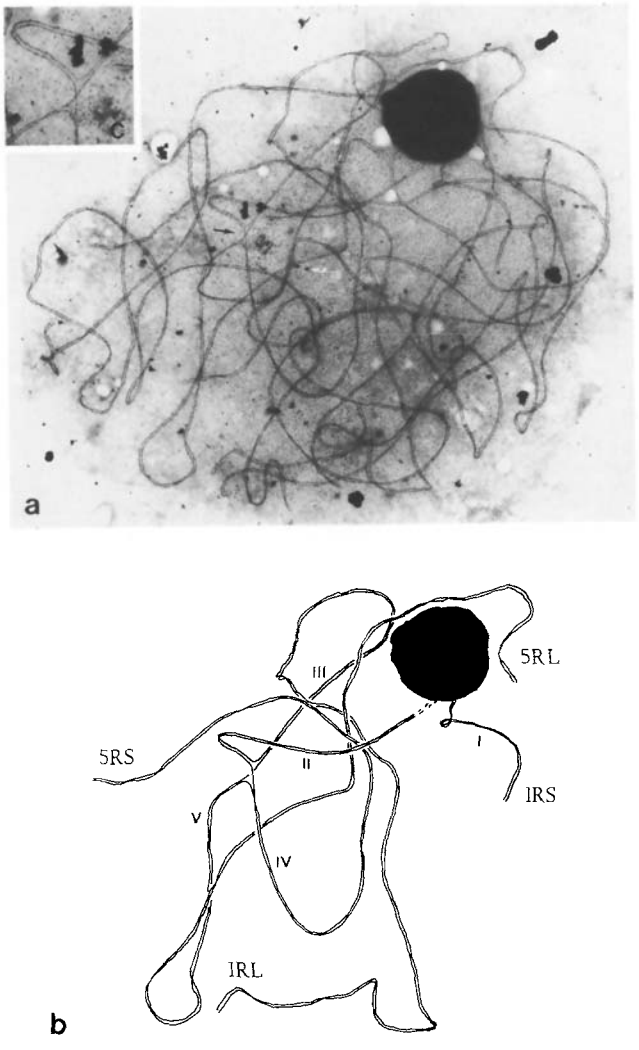

Fig. 3a–c. Electron micrograph **a** of the spread synaptonemal complexes from a pachytene nucleus of a heterozygote for translocation *T273W*, with the corresponding drawing of the quadrivalent **b** in which regions I–V correspond to the ones indicated in the diagram of Fig. 4. The *arrow* in **a** shows the partner exchange at the translocation breakpoint, the region being enlarged in **c**

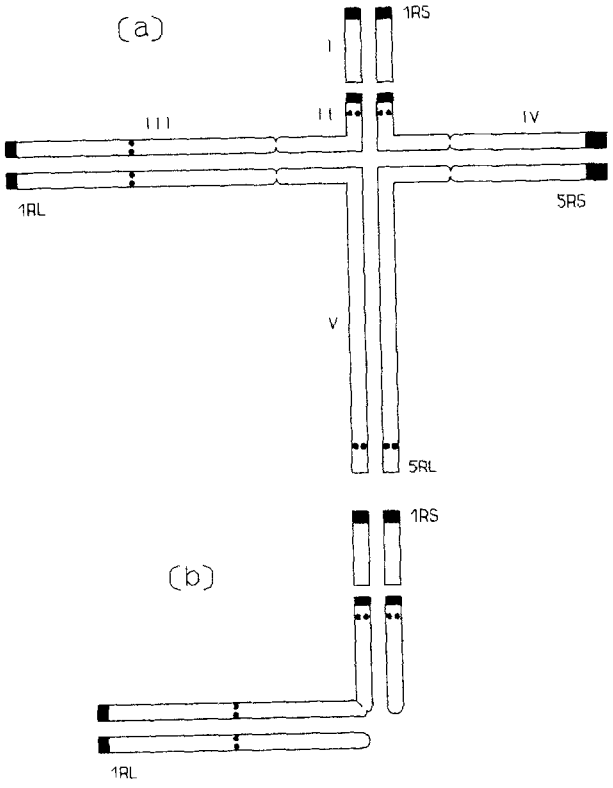

Fig. 4a, b. Pairing diagram of the multivalents formed by the heterozygotes for translocation T273W **a** and by plants ditelocentric for chromosome 1R **b**

NOR the synaptonemal complexes of 5 pachytene cells of heterozytes for translocation *T273W* were analyzed.

In the quadrivalent formed by the chromosomes involved in the translocation, the following five SC segments were considered (Figs. 3 and 4): I, corresponding to the satellite; II, corresponding to the segment between the NOR and the TB; and III, IV and V, corresponding to the segments between the TB and the telomeres of 1RL, 5RS and 5RL, respectively. The last three segments can also be unequivocally determined since segment V is located in the quadrivalent opposite to the satellite (see Fig. 4) and segment IV is shorter than segment III (segments I + II + IV constitute the short translocated chromosome 5R/1R, whereas segments I + II + III constitute chromosome 1R, which is longer; see Fig. 2).

The relative position of the centromere of chromosome 1R was determined by analysis of the SCs of the trivalents formed in eight pachytene cells of one plant ditelocentric for this chromosome (Fig. 4).

Table 2 shows the length of the different synaptonemal complex segments of the *T273W* quadrivalent and those of the trivalent formed in the ditelocentric plant. The relative length of segment II in the quadrivalent (between the NOR and the TB) is much shorter than that of the segment between the NOR and the centromere in the trivalent.

These results clearly reveal that, contrary to the location claimed by de Vries and Sybenga (1976) and Sybenga et al. (1990), the breakpoint of translocation *T273W* is in 1RS (between the NOR and the centromere). The translocated chromosomes having the telomere constitutions 1RS-5RS and 1RL-5RL can only appear if the other chromosome arm affected by the translocation is 5RL.

The metaphase-I configurations formed by the chromosomes involved in the translocation were analyzed in two plants of the progeny 13-25 (Table 1); the four chromosome arms could be identified on the

Table 2. Length (μ) of the different synaptonemal complex (SC) segments of the multivalent formed in pachytene cells of heterozygotes for translocation T273W and plants ditelocentric for chromosome 1R

	Multivalent segments							SC mean length per bivalent	Mean total SC length	Number of cells
	Chromosome 1R					Chromosome 5R				
	ts1-NOR	NOR-TB	NOR-Cen	TB-*tl1*	Cen-*tl1*	*ts5*-TB	TB-*tl5*			
Heterozygotes for translocation T273W										
SC mean length	10.86	9.64	–	60.02	–	36.53	52.47	84.17	590.36	5
±SE	±0.78	±2.72		±2.24		±3.11	±3.73	±5.74	±37.02	
Mean % of the corresponding chromosome	13.48 ±0.62	11.59 ±2.87	–	74.93 ±2.80	–	40.95 ±1.10	59.05 ±1.10			
Plants ditelocentric for chromosome 1R										
SC mean length	9.21	–	16.41	–	37.86	–	–	73.62	505.25	8
±SE	±1.43		±1.55		±3.24			±5.44	±36.57	
Mean % of the corresponding chromosome	14.13 ±1.71	–	26.48 ±2.56	–	59.39 ±2.13	–	–			

ts1, Telomere of 1RS; NOR, nucleolar organizer region; TB, translocation breakpoint; Cen, centromere; *tl1*, telomere of 1RL; *ts5*, telomere of 5RS; *tl5*, telomere of 5RL

Table 3. Metaphase-I association frequency (%±SE) in the different segments of chromosomes involved in translocation *T273W*

Plant	Quadrivalent segments				Remaining bivalent arms (pooled)	Number of PMCs
	1RS	5RS	1RL	5RL		
18-15	65.4±3.9	67.5±3.8	98.0±1.1	100.0±0.0	95.6±0.5	151
18-18	56.4±4.4	75.0±3.9	99.1±0.8	100.0±0.0	97.9±0.4	124
18-15+18-18 (pooled)	61.4±2.9	70.9±2.7	98.5±0.7	100.0±0.0	96.7±0.3	275

basis of their different C-banding pattern. Table 3 shows the metaphase-I association (bond) frequencies of these chromosome arms.

The inheritance of translocation *T273W*, considered separately, fitted the expected 1:1 segregation.

Linkage relationships

Isozyme locus *Pgm-R1* (assigned to 4R by Wehling et al. 1985) and by Salinas and Benito (1985a) and C-bands *ts2, il2, tl2* (chromosome 2R) and *ts6* (chromosome 6R) segregated independently from each other as well as from the remaining markers.

The isozyme locus *Aco-R2* also showed an independent segregation from all other markers. This locus was assigned to chromosome arm 5RL by Chenicek and Hart (1987). The translocation breakpoint of *T273W* is on 5RL, and the association frequency of chromosome arm 5RS in heterozygotes for this translocation is smaller than 100% (Table 3). Since there were no chiasmata in the interstitial segments, the recombination fraction between the telomere of 5RS and the translocation breakpoint must be lower that 50%. Therefore, from the independent segregation of locus *Aco-R2* and the translocation *T273W*, it can be concluded that this isozyme locus is not on 5RS. If located on 5R, it must occupy a distal position (probably near to the telomere) on 5RL.

Joint segregation of isozyme loci *Mdh-R2* and *Got-R3* was obtained only in the progeny of plant 9-15, in which 16 recombinant and 52 parental gametes were recovered (in one plant of this progeny the phenotype for *Got-R3* could not be determined). This segregation deviates significantly from the independence assumption ($\chi^2 = 19.06$; $P < 0.001$) and corresponds to a recombination fraction (%) of 23.5 ± 5.1 and to a distance of 25.5 ± 6.6 cM. The two loci have been previously assigned to chromosome 3R (Salinas and Benito 1985a, b). The distance obtained in the present investigation is higher than the estimate of 13.0 ± 3.0 obtained by Figueiras et al. (1985) and that of 16.6 ± 1.7 obtained by Vaquero et al. (1990). These two loci segregated independently from the remaining markers.

The joint segregations of isozyme loci *Gpi-R1*, *Mdh-R1* and *Pgd2*, C-bands *ts1*, *is1*, *il1* and *tl1* and translocation *T273W* were analyzed in all possible pair-wise combinations amongst the different progenies (detailed data can be sent by the authors on request). From these results together with the mitotic and SC observations the order of the markers located on chromosomes 1R could be determined as:

$$ts1 \ldots Gpi\text{-}R1 \ldots is1 \ldots \frac{Mdh\text{-}R1}{T273W} \ldots il1 \ldots Pgd2 \ldots tl2$$

Since 0% recombination (absolute linkage) between *T273W* and *Mdh-R1* was observed in translocation heterozygotes, the precise order of these two markers could not be determined.

This linkage map is consistent with the observations of Lawrence and Appels (1986), who consequently assigned *Gpi-R1* to the satellite and *Pgd2* to the long arm of chromosome 1R, and with the observations of Figueiras et al. (1989), who established (by indirect means) the loci order *Gpi-R1...Mdh-R1...Pgd2* and suggested that locus *Mdh-R1* is located near to the centromere.

Figure 5 is a comparison between the physical map, based on synaptonemal complex and mitotic C-banding analyses, and distances based on recombination frequency between adjacent markers in the six progenies studied in this work. From these results, two main conclusions can be derived.

1) There are differences between physical and recombination-based relative distances, indicating that recombination is mainly restricted to the distal regions of both chromosome arms. This loss of direct proportionality between physical and genetic distances has been observed previously for the short arm of

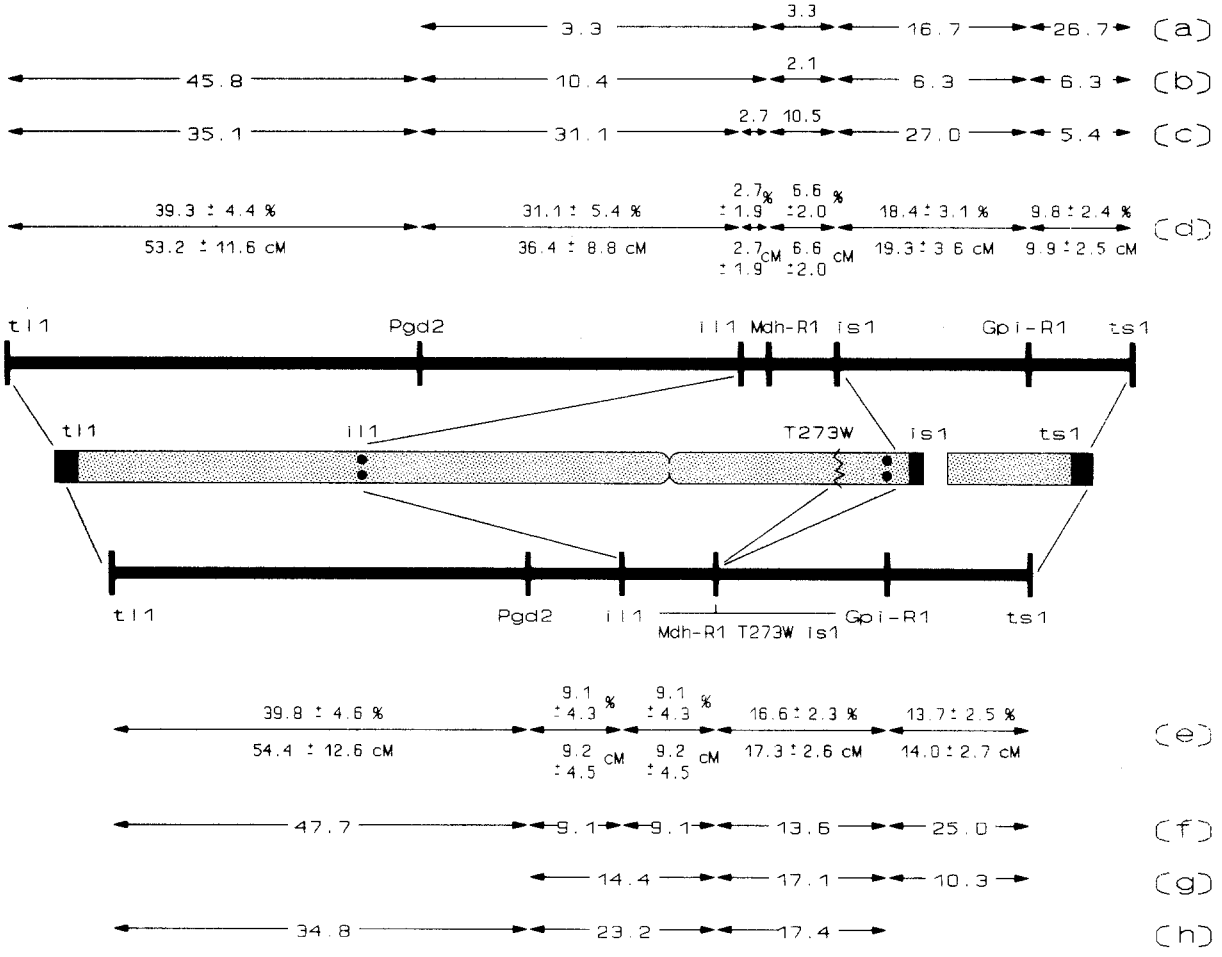

Fig. 5. Comparison of the physical map of chromosome 1R of rye, based on synaptonemal complex and mitotic C-banding analyses, and the genetic maps, based on recombination frequencies (%) between adjacent markers, in the six progenies studied. *a–d* Progenies of plants without the translocation *T273W*: *a* plant 13-13, *b* plant 13-14, *c* plant 14-25, *d* pooled data. *e–h* Progenies of plants with the translocation *T273W*: *e* pooled data, *f* plant 5-22, *g* plant 13-25, *h* plant 9-15. The lengths of the different segments of the genetic maps are proportional to the recombination frequencies indicated in *d* and *e*, respectively, whereas the length of chromosome 1R (physical map) is proportional to 100

chromosome 1R as well as in other chromosome arms of rye and wheat (Dvorak and Chen 1984; Snape et al. 1985; Lawrence and Appels 1986; Goicoechea et al. 1987; Curtis and Lukaszewski 1991). It is worth mentioning that in the present work the physical distances on 1RS determined from synaptonemal complex analysis are directly proportional to the corresponding distances in mitotic chromosomes. Then, pairing seems not to be involved in the differences found between physical and genetic maps.

2) Although from the results obtained on each individual plant the same loci order indicated above could be deduced, there are also between-plants differences in the recombination frequencies of the different chromosome segments. These differences are not related to the presence of translocation *T273W* except in the segment around the breakpoint (including markers *is1*, *T273W* and *Mdh-R1*), for which no recombinants were recovered in the progeny of translocation heterozygotes.

Table 4 shows the frequency of the different 1R chromosomes recovered according to the regions for which they show recombination. The results obtained from the progenies analyzed (Table 5) indicate that there are significant differences between plants in crossing-over frequency in the short arm of 1RS (five progenies compared) and in the regions between markers *Gpi-R1* and *Pgd2* (proximal regions), whereas in the long arm, differences in crossing-over frequency between the 4 progenies for which data could be compared were not significant. There are significant differences in crossing-over frequency in the whole chromosome 1R between some plants, the heterogeneity χ^2 test (three progenies could be compared) approaching the limit of significance.

The data (Fig. 5, Table 5) show a trend towards an increase in proximal crossing-over localization with increasing values in the recombination frequency of the whole chromosomes; this trend is similar to that deduced previously in rye by Giraldez and Lacadena

Table 4. The frequency of the different 1R chromosomes recovered according to the regions for which they show recombination, in the six progenies analyzed. Chromosome 1R was divided into the regions shown below

Markers	*ts1*	*Gpi-R1*	*is1*	*T273W*	*Mdh-R1*	*il1*	*Pgd2*	*tl1*
Regions[a]	1	2	3		4		5	
Recovered chromosomes	Plants without the translocation T273W				Plants with the translocation T273W			
	13-13	13-14	14-25		5-22	9-15	13-25	
Parentals	18	19	18		13	29	91	
Single crossover								
Region 1	6	2	2		4	–	11	
Region 2	4	1	7		1	5	21	
Region 3	0	0	2		0	0	0	
Region 4	0	3	12		2	9	17	
Region 5	–	19	11		13	16	–	
Double crossover								
Regions 1, 2	0	0	0		1	–	2	
Regions 1, 4	1	0	0		1	–	2	
Regions 1, 5	–	1	1		5	–	–	
Regions 2, 4	0	0	5		1	2	2	
Regions 2, 5	–	1	5		3	3	–	
Regions 3, 4	0	1	2		0	0	0	
Regions 3, 5	–	0	3		0	0	–	
Regions 4, 5	–	0	2		0	3	–	
Trible crossover								
Regions 1, 2, 3	1	0	0		0	–	0	
Regions 1, 4, 5	–	0	1		0	–	–	
Regions 2, 4, 5	–	1	2		0	2	–	
Quadruple crossover								
Regions 2, 3, 4, 5	–	0	1		0	0	–	

[a] The segment between markers *is1* and *Mdh-R1* has been considered to be a single region because there was no recombination in this segment in translocation heterozygotes (having the marker *T273W*). The segment between markers *Mdh-R1* and *Pgd2* has been considered to be a single region in order to compare the results of the different plants (see Table 5)

Table 5. Comparison between the frequencies of the different 1R chromosomes recovered according to the number and distribution of recombination events (c.o.) in the six progenies analyzed. Recombination between the centromere and locus *Mdh-R1* was not taken into consideration

Recovered chromosomes	Plants without the translocation *T273W*			Plants with the translocation *T273W*			Heterogeneity χ^2
	13-13	13-14	14-25	5-22	9-15	13-25	
Short arm (*ts1* to *Mdh-R1*)							
Parentals	18	41	43	28	–	108	13.657 (p)
Single c.o.	11	7	30	15	–	36	$df=4$
Double c.o.	1	0	1	1	–	2	$0.01>P>0.001$
Mean c.o./arm	0.43 (a, d)	0.14 (b, c)	0.43 (d)	0.39 (a, d)	–	0.27 (a, c)	
Long arm (*Mdh-R1* to *tl1*)							
Parentals	–	22	29	19	34	–	1.544 (p)
Single c.o.	–	25	39	25	30	–	$df=3$
Double c.o.	–	1	6	0	5	–	$0.7>P>0.5$
Mean c.o./arm	–	0.56 (a)	0.69 (a)	0.57 (a)	0.58 (a)	–	
Proximal regions (*Gpi-R1* to *Pgd2*)							
Parentals	24	40	32	35	45	102	30.981 (q)
Single c.o.	5	6	32	8	20	42	$df=5$
Double c.o.	1	2	9	1	4	2	$0.001>P$
Trible c.o.	0	0	1	0	0	0	
Mean c.o./ Proximal region	0.23 (a, c)	0.21 (a)	0.72 (b)	0.23 (a, c)	0.41 (c)	0.32 (a, c)	
Total (*ts1* to *tl1*)							
Parentals	–	19	18	13	–	–	8.722 (r)
Single c.o.	–	25	34	20	–	–	$df=4$
Double c.o.	–	3	18	11	–	–	$0.1>P>0.05$
Trible c.o.	–	1	3	0	–	–	
Quadruple c.o.	–	0	1	0	–	–	
Mean c.o./ chromosome	–	0.71 (a)	1.12 (b)	0.95 (b)	–	–	

(a–d) Within each row, plants with the same letter do not significantly differ according to the corresponding contingency χ^2 test
(p) Single and double c.o. have been added
(q) Single, double and triple c.o. have been added
(r) Double, triple and quadruple c.o. have been added

(1978) and suggests that this variation is independent of the presence of translocation *T273W* (except in the region around the translocation breakpoint) and also from the C-heterochromatin constitution (see Table 1). On the other hand, since all plants grew in a climate chamber under similar conditions, the possibility of environmental factors being the main reason for this between-plants variation can probably be excluded.

The variation that was found can explain the differences in map distances between the same markers that has been obtained by different authors (see Benito et al. 1990 for a discussion on chromosome 1R) or by the same author in different materials (Singh et al. 1990). It can also explain the contradictory claims of some authors concerning the relative positions of several loci. For instance, Vaquero et al. (1990) compared the three distances between loci *Gpi-R1*, *Mdh-R1* and *Pgd2*, estimated independently from different plants (belonging to several cultivars) that did not segregate simultaneously for the three loci, and concluded [against the also indirect results of Figueiras et al. (1989)] that locus *Pgd2* is the central one. The cytogenetic techniques employed in the study reported here have clarified the controversy, unequi-

vocally by determining that *Gpi-R1* maps on the satellite, *Mdh-R1* near to the centromere (absolutely linked to *T273W*) and *Pgd2* on the distal half of 1RL.

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References

Benito C, Frade JM, Orellana J, Carrillo JM (1990) Linkage and cytogenetic maps of genes controlling endosperm storage proteins and isozymes in rye (*Secale cereale* L.). Theor Appl Genet 79:347–352

Benito C, Zaragoza C, Gallego FJ, de la Pena A, Figueiras AM (1991) A map of rye chromosome 2R using isozyme and morphological markers. Theor Appl Genet 82:112–116

Carrillo JM, Vazquez JF, Orellana J (1990) Linkage relationship between the loci *Sec-1* and *Sec-3* in rye (*Secale cereale* L.). Heredity 64:125–130

Chenicek KJ, Hart GE (1987) Identification and chromosomal locations of aconitase gene loci in Triticeae species. Theor Appl Genet 74:261–268

Chojecki AJS, Gale MD (1982) Genetic control of glucose phosphate isomerase in wheat and related species. Heredity 49:337–347

Curtis CA, Lukaszewski AJ (1991) Genetic linkage between C-bands and storage protein genes in chromosome 1B of tetraploid wheat. Theor Appl Genet 81:245–252

Dvorak J, Chen K-C (1984) Distribution of nonstructural variation between wheat cultivars along chromosome arm 6Bp: evidence from the linkage map and physical map of the arm. Genetics 106:325–333

Figueiras AM, Gonzalez-Jaen MT, Salinas J, Benito C (1985) Association of isozymes with a reciprocal translocation in cultivated rye (*Secale cereale* L.). Genetics 109:177–193

Figueiras AM, Elorrieta MA, Benito C (1989) Association of four isozyme loci with a reciprocal translocation between 1R/4R chromosomes in cultivated rye (*Secale cereale* L.). Theor Appl Genet 78:224–228

Giraldez R, Lacadena JR (1978) Relationships between frequency, localization and errors in chiasma formation in desynaptic rye. Chromosoma 66:193–204

Giraldez R, Cermeño MC, Orellana J (1979) Comparison of C-banding pattern in the chromosomes of inbred lines and open-pollinated varieties of rye. Z Pflanzenzuecht 83:40–48

Goicoechea PG, Roca A, Naranjo T, Giraldez R (1987) Interstitial chiasmata and centromere orientation in heterozygotes for a translocation in rye. Genome 29:647–657

Gustafson JP, Butler E, McIntyre CL (1990) Physical mapping of a low-copy DNA sequence in rye (*Secale cereale* L.). Proc Natl Acad Sci USA 87:1899–1902

Jensen J, Jorgensen JH (1975) The barley chromosome 5 linkage map. I. Literature survey and map estimation procedure. Hereditas 80:5–16

Jong JH de, van Eden J, Sybenga J (1989) Synaptonemal complex formation and metaphase-I configuration patterns in a translocation heterozygote of rye (*Secale cereale*). Genome 32:72–81

Kaltsikes PJ, Lukaszewski AJ, Gustafson JP (1986) Cross-over frequencies in chromosomes 1R and 2R or rye (*Secale cereale* L.). In: Darvey NL (ed), Proc. Int. Triticale Symp. The Australian Institute of Agricultural Science, Sydney, pp 321–326

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lawrence GJ, Appels R (1986) Mapping the nucleolus organizer region, seed protein loci and isozyme loci on chromosome 1R in rye. Theor Appl Genet 71:742–749

McIntosh RA (1988) Catalogue of gene symbols in wheat. In: Miller TE, Koebner RMD (eds), Proc VII Int Wheat Genet Symp. Cambridge, pp 1225–1322

Naranjo T, Roca A, Goicoechea PG, Jong JH de, Smilde WD (1989) Comparison between synaptonemal complexes at pachytene and chromosome association at metaphase I in heterozygotes for a reciprocal translocation of rye. Genome 32:983–991

Salinas J, Benito C (1985a) Chromosomal locations of phosphoglucomutase, phosphoglucose isomerase, and glutamate oxaloacetate transaminase structural genes in different rye cultivars. Can J Genet Cytol 27:105–113

Salinas J, Benito C (1985b) Chromosomal location of malate dehydrogenase structural genes in rye (*Secale cereale* L.) Z Pflanzenzuecht 94:208–217

Singh NK, Shepherd KW, McIntosh RA (1990) Linkage mapping of genes for resistance to leaf, stem and stripe rusts and ω-secalins on the short arm of rye chromosome 1R. Theor Appl Genet 80:609–616

Snape JW, Flavell RB, O'Dell M, Hughes WG, Payne PI (1985) Intrachromosomal mapping of the nucleolar organiser region relative to three marker loci on chromosome 1B of wheat (*Triticum aestivum*) Theor Appl Genet 69:263–270

Sybenga J, Wolters AHG (1972) The classification of the chromosomes of rye (*Secale cereale* L.): a translocation tester set. Genetica 43:453–464

Sybenga J, Parmar S, van Eden J, Shewry P (1990) Mapping seed-storage protein loci *Sec-1* and *Sec-3* in relation to five chromosomal rearrangements in rye (*Secale cereale* L.) Theor Appl Genet 79:201–208

Vaquero F, Rebordinos L, Vences FJ, Pérez de la Vega M (1990) Genetic mapping of isozyme loci in *Secale cereale* L. Theor Appl Genet 80:88–94

Vries JM de, Sybenga J (1976) Identification of rye chromosomes: the Giemsa banding pattern and the translocation tester set. Theor Appl Genet 48:35–43

Wang ML, Atkinson MD, Chinoy CN, Devos KM, Harcourt RL, Liu CJ, Rogers WJ, Gale MD (1991) RFLP-based map of rye (*Secale cereale* L.) chromosome 1R. Theor Appl Genet 82:174–178

Wehling P, Schmidt-Stohn G, Wricke G (1985) Chromosomal location of esterase, peroxidase and phosphoglucomutase isozyme structural genes in cultivated rye (*Secale cereale* L.). Theor Appl Genet 70:377–382