Theor Appl Genet (1993) 87:321–327

Genetic linkage between cytological markers and the seed storage protein loci *Sec2*[*Gli-R2*] and *Sec3*[*Glu-R1*] in rye

C. Alonso-Blanco, P. G. Goicoechea, A. Roca, R. Giraldez

Departamento de Biología Funcional, Area de Genética, Universidad de Oviedo, 33071 Oviedo, Spain

Received: 10 April 1993 / Accepted: 20 April 1993

Abstract. In order to reach a higher accuracy concerning the cytological locations of the rye seed storage protein loci *Sec2*[*Gli-R2*] and *Sec3*[*Glu-R1*] located within chromosome arms 2RS and 1RL, respectively, the linkage relationships between the following loci were analyzed: isozyme loci *Gpi-R1*, *Mdh-R1*, and *Pgd2*, translocation *T273W* (Wageningen tester set, involving chromosome arms 1RS and 5RL), the telomere C-bands of chromosome arms 1RL (*tL1*), 2RS (*tS2*), and 5RS (*tS5*), and three interstitial C-bands in chromosome arm 1RS (*iS1*), in the middle of chromosome arm 1RL (*iL1*), and in the middle of chromosome arm 2RL (*iL2*), respectively. The data indicated that locus *Sec3* is located in the distal half of chromosome arm 1RL (between C-band *iL1* and locus *Pgd2*), while locus *Sec2* is located a short distance (2.9 ± 1.4%) from the telomere C-band of chromosome arm 2RS.

Key words: Rye – Seed proteins – C-heterochromatin bands – Isozymes – Translocation – Genetic mapping

Introduction

Considerable attention has been given to the localization and mapping of the genes encoding the four major groups of seed storage proteins in rye. *Sec1*(*Gli-R1*), which encodes the ω-secalins and 40K γ-secalins, is probably a complex locus with families of closely linked genes and has been located in the short arm of chromosome 1R (Shewry et al. 1984); *Sec2*(*Gli-R2*), encoding the 75K γ-secalins, has been located in the short arm of chromosome 2R (Shewry et al. 1986); and *Sec3*(*Glu-R1*), encoding the high-molecular-weight secalins, has been located in the long arm of chromosome 1R (Singh and Shepherd 1984).

The cytological location of *Sec1* within chromosome arm 1RS was established by Lawrence and Appels (1986), who found that this locus mapped between the ribosomal RNA genes (*Nor-R1* locus) and the telomere of the short arm of chromosome 1R and concluded that *Sec1* is located in the satellite of chromosome arm 1RS. This was later confirmed by Singh et al. (1990) using wheat rye translocation lines and by Sybenga et al. (1990) using chromosomal rearrangements.

Attempts to determine a more precise cytological location of *Sec3* within chromosome arm 1RL have not been totally successful. From the recombination observed between this locus and translocation *T273W*, Sybenga et al. (1990) concluded that *Sec3* is located distally on 1RL since it was assumed that the breakpoint of this translocation in 1RL is at a considerable distance from the centromere. However, after an analysis of synaptinemal complexes in heterozygotes for translocation *T273W*, Alonso-Blanco et al. (1993) established the precise location of its breakpoint in the short arm of chromosome 1R, between the nucleolar organizer region (NOR) and the centromere. Recently, Orellana et al. (1993) have found that *Sec3* is loosely linked to the telomere C-hetetrochromatin band of the long arm.

Linkage analyses between *Sec1* and *Sec3*, and other genetic markers such as isozymes or RFLPs have also been carried out by Shewry et al. (1984), Carrillo et al. (1990, 1992), Benito et al. (1990), and Wang et al. (1991), but *Sec2* has not been genetically linked to any marker.

Communicated by F. Salamini
Correspondence to: R. Giraldez

In the investigation reported here, the linkage relationships between *Sec2*, *Sec3*, three isozyme loci located in chromosome 1R, translocation *T273W*, and six C-bands belonging to chromosomes 1R, 2R and 5R were analyzed. The purpose was to reach a higher degree of precision concerning the cytological locations of *Sec3* and *Sec2* within chromosome arms 1RL and 2RS, respectively.

Material and methods

Plant material

In order to obtain plants heterozygous for different markers, including isozymes, storage proteins, C-bands and a translocation, different crosses were made involving plants from cvs 'Ailés' and 'Merced', inbred line E (Giraldez et al. 1979), and a line homozygous for translocation *T273W* (Translocation Tester Set; Sybenga and Wolters 1972).

The segregation analysis was made in 81 plants of the progeny (obtained by self pollination) of a multiple heterozygous plant selected from these crosses.

Storage protein assays

Secalin patterns from the non-embryo halves of the seeds were determined by SDS-PAGE using 12% gels in the discontinuous system of Laemmli (1970) as modified by Payne et al. (1980). Proteins were extracted following the procedure of Orellana et al. (1993): unreduced proteins were extracted from endosperm halves of single kernels using sample buffer without 2-mercaptoethanol (2-ME). The unreduced extracts were later reduced with one drop of 2-ME. The unreduced and reduced extracts were loaded in different gels.

Apparent molecular weights of rye proteins were determined from the mobilities of the following proteins included in the MV-SDS-200 kit (Sigma): myosin (205,000), β-galactosidase (116,000), phosphorilase B (97,400) bovine albumin (66,000), egg albumin (45,000), and carbonic anhydrase (29,000).

Isozyme assays

Isozyme analyses were carried out on extracts of 12-day-old leaves from the plants obtained after the germination of the half-grains with the embryo. Three isozymatic systems, glucosephosphate isomerase (GPI, EC 5.3.1.9), malate dehydrogenase (MDH, EC 1.1.1.37), and phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), were assayed after horizontal 12% starch gel electrophoresis using the buffers and staining methods described by Figueiras et al. (1985).

Karyotype and C-banding analysis

Root tips from the germinating half-grains were immersed in tap water at 0 °C for 24 h to shorten the chromosomes and fixed in acetic acid- alcohol 1 : 3. The fixed material was maintained in the fixative for 1–4 months at 3°–4 °C. It was then squashed and stained following the Giemsa C-banding technique described by Giraldez et al. (1979).

Statistical analysis

Recombination frequencies were derived from the maximum likelihood equations of Ritter et al. (1990).

Results and discussion

Plant phenotypes and genotypes and marker nomenclature

The progeny analyzed segregated for the following markers.

Storage proteins

Figure 1 shows examples of segregating banding patterns of seed proteins in the progeny analyzed. In the slow mobility region of SDS gels corresponding to the high-molecular-weight secalins (locus *Sec3*, located on chromosome arm 1RL), bands 1, 2, and 4 were always inherited as a unit. This combination behaved as an allelic block of bands 3, 5, and 6, which were also inherited as a unit. The two alleles segregating in this progeny have been designated $Sec3^1$ (bands 1, 2, and 4) and $Sec3^2$ (bands 3, 5, and 6).

Band 7 behaved as an allele of band 8. The mobility of these bands in the SDS gels suggests that they are either 75K γ-secalins corresponding to locus *Sec2* (located on chromosome 2R) or ω-secalins corresponding to locus *Sec1* (located on chromosome 1R). However, their linkage relationships (Table 2) indicate

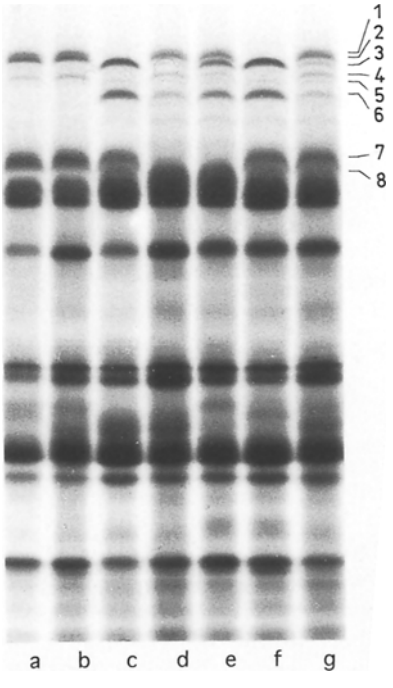

Fig. 1. SDS-PAGE patterns of reduced secalin extracts of some plants of the progeny analyzed. Lanes *a*, *b* homozygous for $Sec3^1$ and for $Sec2^1$, *c*, *f* homozygous for $Sec3^2$ and heterozygous $Sec2^1$ $Sec2^2$, *d*, *e* heterozygous $Sec3^1$ $Sec3^2$ and homozygous for $Sec2^2$, *g* double heterozygous $Sec3^1$ $Sec3^2$ and $Sec2^1$ $Sec2^2$

that the locus involved is *Sec2*. The two alleles present in the plants analyzed have been designated $Sec2^1$ (band 7) and $Sec2^2$ (band 8).

Isozyme loci

According to Mclntosh (1988), the loci for isozymes GPI, MDH (fast mobility), and PGD, all three located on chromosome 1R, are designated *Gpi-R1* (alleles Gpi^1 and Gpi^3), *Mdh-R1* (alleles Mdh^1 and Mdh^3), and *Pgd2* (alleles Pgd^1 and Pgd^2), respectively. Their electrophoretic patterns have been described previously (Alonso-Blanco et al. 1993).

C-heterochromatin bands

Figure 2 shows some examples of segregating chromosomes in the progeny analyzed. Differences in the amount of C-heterochromatin were observed in the telomere C-band of the long arm (*tL1*; "alleles" $tL1^+$ and $tL1^-$), in the C-band adjacent to the nucleolar organizer region (*iS1*; "alleles" $iS1^+$ and $iS1^-$), and in the C-band located in the middle of the long arm (*iL1*; "allels" $iL1^+$ and $iL1^-$) of chromosome 1R; in the telomere C-band of the short arm of chromosome 5R (*tS5*; "alleles" $tS5^+$ and $tS5^-$); and in the telomere C-band of the short arm of chromosome 2R (*tS2*; "alleles" $tS2^+$ and $tS2^-$). Chromosome 2R also segregated for the presence or absence of a large interstitial C-band (*iL2*; "alleles" $iL2^+$ and $iL2^-$) located in the middle of the long arm. The constitution of C-bands *tL1*, *iS1*, *tS5*, and *iL2* was determined in all of the plants analyzed. However, in 11 plants the low quality of the C-banding did not allow the unequivocal determination of the cell constitution for the thin *iL1* C-band nor for C-band *tS2*, since the 2R chromosomes not having the *IL2* C-band have a banding pattern quite similar to that of 3R.

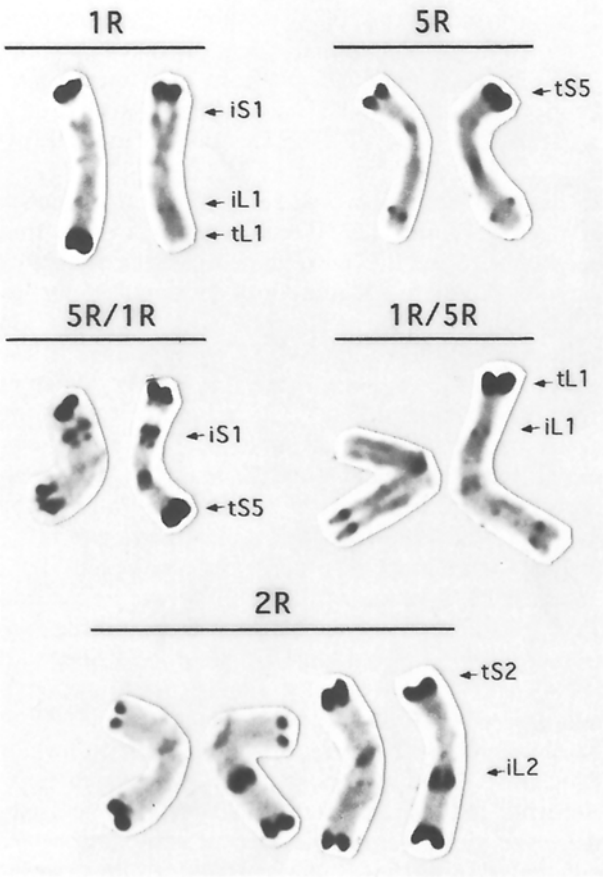

Fig. 2. Some examples of chromosomes 1R, 5R, 5R/1R, 1R/5R, and 2R appearing in the progeny analyzed. The different segregating C-bands are indicated

Translocation T273W

This translocation involves chromosomes 1R and 5R, translocation-derived duplications and deficiencies being inviable. By means of synaptinemal complex analysis (Alonso-Blanco et al. 1993) the precise location of the breakpoint was determined in chromosome arms 1RS (between the NOR and the centromere) and 5RL. At mitosis, the two translocated chromosomes (1R/5R and 5R/1R) can be easily distinguished from the standard ones (Fig. 2). The alternative structural arrangements have been designated 273^N (1R and 5R) and 273^T (1R/5R and 5R/1R).

Parental genotype

On the basis of the alleles described above and after analysis of their parentals, it was concluded that the plant from which the segregating progeny was obtained had the following genotype:

1) Chromosomes involved in translocation *T273W* (1R and 5R):

$Gpi^1\ iS1^+\ 273^T Mdh^1\ iL1^-\ Sec3^1\ Pgd^1\ tL1^-\ tS5^+ / Gpi^3\ iS1^-\ 273^N\ Mdh^3\ iL1^+\ Sec3^2\ Pgd^2\ tL1^+\ tS5^-$

2) Chromosome 2R:

$tS2^-\ Sec2^1\ iL2^- / tS2^+\ Sec2^2\ iL2^+$.

Segregation of individual markers

Table 1 shows the segregations of the three possible phenotypes for each marker compared to the expected 1:2:1 ratio. Differences are neither significant for the markers located in chromosome 2R nor for markers *tL1*, *Sec3*, and *tS5* (located in the chromosomes involved in translocation 1R and 5R). The remaining markers deviated from the expected segregation, a

Table 1. The frequencies of the different phenotypes for each individual marker compared to the expected 1:2:1 segregation

Marker	Alleles		Phenotypes of progeny			Total number of plants	χ^2 (1:2:1)
	A	*a*	*AA*	*Aa*	*aa*		
Gpi-R1	Gpi^3	Gpi^1	29	40	12	81	7.148*
iS1	$iS1^-$	$iS1^+$	32	36	13	81	9.914**
Mdh-R1	Mdh^3	Mdh^1	32	36	13	81	9.914**
T273W	273^N	273^T	32	36	13	81	9.914**
iL1	$iL1^+$	$iL1^-$	28	31	11	70	9.171**
Sec3	$Sec3^2$	$Sec3^1$	27	39	15	81	3.667ns
Pgd2	Pgd^2	Pgd^1	31	35	15	81	7.815*
tL1	$tL1^+$	$tL1^-$	22	38	21	81	0.333ns
tS5	$tS5^-$	$tS5^+$	17	50	14	81	4.679ns
Sec2	$Sec2^1$	$Sec2^2$	23	37	10	70	5.057ns
tS2	$tS2^-$	$tS2^+$	20	41	9	70	5.514ns
iL2	$iL2^-$	$iL2^+$	14	35	21	70	1.400ns

* $0.05 > P > 0.01$; ** $P < 0.01$; ns, non-significant

significant excess of alleles Gpi^3 $iS1^-$ 273^N Mdh^3 $iL1^+$ and Pgd^2 (the parental combination in the non-translocated chromosome 1R) being observed. The deviation is higher for translocation *T273W* and for the markers totally linked to it, and it decreases for markers located more distantly (see next section). It can therefore be concluded that a low viability of the chromosomal rearrangement itself is probably the only reason for the deviations found.

Linkage relationships and cytological location of Sec3 and Sec2

Table 2 shows the joint segregations and the recombination percentages for the most relevant pairs of markers of chromosomes 1R, 5R, and 2R. From these results, the following orders can be established.

1) Chromosome 1R:

$$Gpi-R1\ldots\underline{iS1\ldots T273W\ldots}\underline{Mdh\text{-}R1}\ldots iL1\ldots Sec3\ldots Pgd2\ldots tL1$$

2) Chromosome 2R:

tS2...Sec2...iL2.

The genetic distances between consecutive markers in chromosome 1R are compared to a physical map of this chromosome in Fig. 3. Our data agree with that from earlier reports (Lawrence and Appels 1986; Lukaszewski 1992; Alonso-Blanco et al. 1993), with the differences between physical and recombination-based relative distances indicating that recombination is mainly restricted to the distal regions of this chromosome.

The distances between *Sec3* and some of the markers studied in this work have been estimated by earlier authors in different materials. The relative position of locus *Sec3* on chromosome arm 1RL between the centromere and locus *Pgd2* is consistent with the observations of Lawrence and Appels (1986) and Benito et al. (1990), but the estimates of the distances between these two genes show important differences. Lawrence and Appels (1986), working with normal rye, observed a recombination frequency between *Sec3* and *Pgd2* (7.2 ± 1.8) that differed from that obtained by Benito et al. (1990), who were working with similar material (25.4 ± 2.6); the estimate obtained in the translocated 1R of the present work (17.3 ± 3.4; Table 2) is an intermediate value. The recombination frequency between *Sec3* and *T273W* can be compared to that between *Sec3* and the centromere because in this translocation there are no interstitial chiasmata (Sybenga 1975; Alonso-Blanco et al. 1993). The recombination frequency between *Sec3* and *T273W* obtained in the present work (25.8 ± 4.5; Table 2) is not very different from the estimate of 32.4 ± 3.28 between *Sec3* and the centromere made by Orellana et al. (1993) using telosomes, but it is greater than the recombination fractions between *Sec3* and the centromere obtained by Sybenga et al. (1990) using telosomes (ranging from 0% to 17.6% in different genotypes), the estimate of 11.3% obtained by Sybenga et al. (1990) between *Sec3* and *T273W*, and the distance between *Sec3* and the centromere (4.6 ± 1.0) obtained by Singh and Shepherd (1984) in a 1RL-1DS translocation chromosome. Orellana et al. (1993) found *Sec3* and the telomere of 1RL to be loosely linked (43.6 ± 3.5) in heterozygotes for telocentric chromosomes, but they segregated independently in the present work (Table 2). In conclusion, there are high discrepancies between the data of the different authors that could be accounted for in terms of genotypes differences concerning chiasma frequency and localization, but that does not seem to be directly related to the chromosomal rearrangements used. The

Table 2. Segregation and recombination frequencies between the most relevant pairs of markers of chromosomes 1R and 2R

Marker (*Aa, Bb*)	Parental genotype (*AB/ab*)	Phenotypes of progeny									Derived recombination frequency (%)
		AABB	*AABb*	*AAbb*	*AaBB*	*AaBb*	*Aabb*	*aaBB*	*aaBb*	*aabb*	
Chromosomes 1R and 5R[a]											
Gpi-R1, iS1	$Gpi^1\ iS1^+/Gpi^3\ iS1^-$	4	7	1	7	19	14	2	10	17	31.1 ± 5.4
Gpi-R1, iL1	$Gpi^1\ iL1^-/Gpi^3\ iL1^+$	4	5	2	6	16	13	1	10	13	33.1 ± 6.2
T273W, iS1	$273^N\ iS1^-/273^T\ iS1^+$	32	0	0	0	36	0	0	0	13	0.00
T273W, Mdh-R1	$273^N\ Mdh^3/273^T\ Mdh^1$	32	0	0	0	36	0	0	0	13	0.00
T273W, iL1	$273^N\ iL1^+/273^T\ iL1^-$	25	2	0	3	28	0	0	1	11	4.4 ± 1.8
T273W, Sec3	$273^N\ Sec3^2/273^T\ Sec3^1$	18	12	2	8	22	6	1	5	7	25.8 ± 4.5
T273W, Pgd2	$273^N\ Pgd^2/273^T\ Pgd^1$	18	11	3	13	18	5	0	6	7	28.3 ± 4.9
T273W, tS5	$273^N\ tS5^-/273^T\ tS5^+$	9	20	3	6	20	10	2	10	1	43.9 ± 10.7
Sec3, iL1	$Sec3^1\ iL1^-/Sec3^2\ iL1^+$	7	6	1	4	19	10	0	6	17	22.0 ± 4.3
Pgd2, iL1	$Pgd^1\ iL1^-/Pgd^2\ iL1^+$	7	4	2	4	15	10	0	12	16	26.8 ± 5.0
Sec3, Pgd2	$Sec3^1\ Pgd^1/Sec3^2\ Pgd^2$	12	2	1	3	25	11	0	8	19	17.3 ± 3.4
Sec3, tL1	$Sec3^1\ tL1^-/Sec3^2\ tL1^+$	4	6	5	10	17	12	7	15	5	50[b]
Pgd2, tL1	$Pgd^1\ tL1^-/Pgd^2\ tL1^+$	5	5	5	10	16	9	6	17	8	47.9 ± 19.1
Chromosome 2R[a]											
Sec2, tS2	$Sec2^1\ tS2^-/Sec2^2\ tS2^+$	20	3	0	0	37	0	0	1	9	2.9 ± 1.4
Sec2, iL2	$Sec2^1\ iL2^-/Sec2^2\ iL2^+$	4	12	7	7	19	11	3	4	3	50[c]

[a] Markers in chromosomes 1R and 5R segregated independently from markers in chromosome 2R
[b] The derived frequency of recombinant gametes was 53.2%
[c] The derived frequency of recombinant gametes was 54.6%

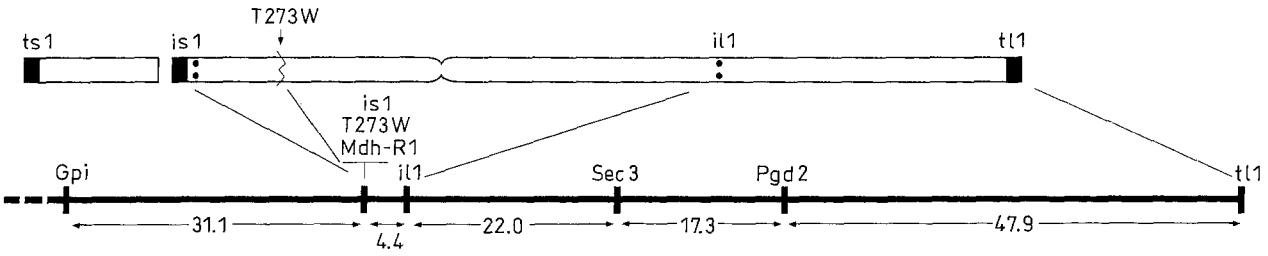

Fig. 3. Comparison of the physical map of rye chromosome 1R, based on synaptinemal complex and mitotic C-banding analyses, and the genetic map, based on recombination frequencies (%) between adjacent markers, in the progeny studied

reason for this loss of effect can be the long distance between such rearrangements (involving 1RS in translocation *T273W*, and the centromere in telosomics or the translocation 1RL-1DS) and locus *Sec3*. In fact, translocation *T273W* reduces the recombination frequency between markers located around the translocation breakpoint since *iS1* and *Mdh1* are not absolutely linked in non-translocated material (Alonso Blanco et al. 1993). The results obtained in the present work indicate that locus *Sec3* is physically located in the distal half of chromosome arm 1RL, probably closer to C-band *iL1* than to the telomere. The relative physical position of this locus within chromosome arm 1RL is comparable to that of the corresponding locus *Gli-B1* in chromosome 1B of wheat (Curtis and Lukaszewski 1991). It is also in agreement with the chromosome "in situ" hybridization results of Gustafson et al. (1990) who using a cDNA probe corresponding to the *Sec1* gene, observed a cross-hybridization near the middle of the long arm of chromosome 1R.

The data obtained in the present work indicate that the recombination frequency between locus *Sec2* and the telomere C-band of the short arm of chromosome 2R is 2.9 ± 1.4. This is the shortest distance among the few cases in rye in which telomeres have been found to be linked to another marker (Singh et al. 1990; Lukaszewski 1992; Orellana et al. 1993; Alonso-Blanco et al. 1993). In some of these cases the markers mapped within the relatively short satellite of 1R, and thus were physically located very close to the telomere. This suggests that the physical location of *Sec2* must equally be very close to the telomere of 2RS. On the other hand, earlier results (Goicoechea et al. 1987) indicate that chiasmata are practically absent from the interstitial region of 2RL between the C-band *iL2* and the centromere. Then, the independent segregation of *Sec2* and *iL2* (Table 2) must imply a similar independent segregation between *Sec2* and the centromere. This would explain why Sybenga et al. (1991) found no linkage between *Sec2* and translocations *T305W* and *T242W*, which have their breakpoints (confirmed by synaptinemal complex studies of heterozygotes) close to the centromere in 2RS and in the telomere of 2RL, respectively (unpublished results and Naranjo et al. 1989). Recently, Devos et al. (1993) reviewed the evolutionary translocations in the rye genome relative to that of hexaploid wheat and predicted a rather distal location of locus *Sec2* within chromosome arm 2RS. The results obtained in the present work confirm that prediction.

Acknowledgments. This work has been supported by grant AGR91-1305 of the CICYT, Spain. C. A.-B. and P. G. G are recipients of fellowships from the Ministerio de Educatión y Ciencia, Spain, and the FICYT, Principado de Asturias, Spain, respectively.

References

Alonso-Blanco C, Goicoechea PG, Roca A, Giraldez R (1993) A cytogenetic map on the entire length of rye chromosome 1R, including one translocation breakpoint, three isozyme loci and four C-bands. Theor Appl Genet 85:735–744

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

Carrillo JM, Vazquez JF, Orellana J (1990) Linkage relationships between the loci *Sec1* and *Sec3* in rye (*Secale cereale* L.). Heredity 64:125–130

Carrillo JM, Vazquez JF, Orellana J (1992) Identification and mapping of *Gli-R3* locus in chromosome 1R of rye (*Secale cereale* L.). Theor Appl Genet 84:237–241

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

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojc P, Xie DX, Gale MD (1993) Chromosomal rearrangements in the rye genome relative to that of wheat. Theor Appl Genet 85:673–680

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

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

Laemmli VK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:681–684

Lawrence GJ, Appels R (1986) Mapping the nucleolus organizer region, seed protein loci and isozyme loci on chromosome 1R

Shewry PR, Bradberry D, Franklin J, White RP (1984) The chromosomal locations and linkage relationships of the structural genes for the prolamin storage proteins (secalins) of rye. Theor Appl Genet 69:63–69

Shewry PR, Parmar S, Fulrath N, Kasarda D, Miller T (1986) Chromosomal locations of the structural genes for secalins in wild perennial (*Secale montanum* Guss.) and cultivated rye (*S.*