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Mapping of genes controlling seed storage-proteins and cytological markers on chromosome 1R of rye

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Summary. Rye secalins, telomeric C-bands, and telocentric chromosomes were used as markers in the progeny of a test-cross in order to determine the position of seed storage-protein genes *Glu-R1* and *Gli-R1* with respect to the centromere and both telomeres of chromosome 1R in rye. These genes were linked to the centromere ($32.35 \pm 3.28\%$ and $36.27 \pm 3.37\%$ recombination, respectively). *Glu-R1* was loosely linked to the telomere of the long arm ($43.63 \pm 3.47\%$ recombination), while *Gli-R1* was closely linked to the telomere of the short arm ($9.80 \pm 2.08\%$ recombination). This finding supports the possibility that rye ω- and γ-secalin genes may be located on the satellites, as has been described in wheat for genes that code similar proteins. The importance of metaphase-I pairing failure and its consequences for the estimation of the recombination fraction are also discussed.

Key words: Rye – Seed storage-proteins – Telomeric C-bands – Centromere – Linkage mapping

Introduction

The high-molecular-weight (HMW) secalins are coded by the *Glu-R1* (*Sec 3*) locus on the long arm of chromosome 1R (Lawrence and Shepherd 1981; Shewry et al. 1984, Singh and Shepherd 1984), and the ω- and γ-secalins by the *Gli-R1* (*Sec 1*) locus on the short arm of the same chromosome (Shepherd 1968; Bernard et al. 1977; Lawrence and Shepherd 1981). The linkage relationship between both loci are well known (Shewry et al. 1984; Lawrence and Appels 1986; Benito et al. 1990; Carrillo et al. 1990, 1992). There is some information on the genetic distance between the *Gli-R1* locus and the centromere, mainly obtained by translocation mapping procedures (Singh et al. 1990). However, there are still significant gaps in our knowledge of the position of these genes on chromosome 1R. Although the chromosomal locations of marker genes have been established by cytological techniques in bread wheat (see Law and Worland 1973) little progress has been made towards mapping rye genes with respect to cytological markers.

Chromosome 1R is becoming better characterized for biochemical markers since it carries three loci coding endosperm storage-proteins, one on the long arm and two on the short arm. These loci are highly polymorphic and suitable for mapping studies. Additionally, the short arm carries the nucleolar organizer region, which facilitates its recognition, and both telomeres possess a clear polymorphism for C-heterochromatin blocks which are easily distinguishable in mitotic as well as in meiotic cells.

This paper reports genetic distances for chromsome 1R by combining cytological and biochemical markers.

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Materials and methods

Genetic stocks

Two rye inbred lines, designated 8t and 6Ri, were crossed to obtain the F_1. Line 8t, derived from cv "Petkus Spring", was double ditelosomic for chromosomes 1R and 3R, while line 6Ri, derived from cv "La Raña", had complete chromosomes. One F_1 plant (8t × 6Ri) was test-crossed as male to the inbred line E(2666) and the progeny were analyzed electrophoretically and cytologically.

Cytological procedures

Pollen mother cells (PMCs) from an F_1 heterozygote were analyzed to estimate the degree of pairing in both arms of chromosome 1R. The anthers used in the cytological analysis, collected from the same F_1 plant used for producing the test-cross offspring, were fixed in acetic acid:ethanol, 1:3 and stored at 4 °C for several months.

Individual seeds obtained from the test-cross were cut in two halves. The embryo halves were germinated on wet filter paper in Petri dishes at 20 °C. When primary roots were 1 cm long they were excised and inmersed in tap water at 2 °C for 24 h to pretreat. After that they were fixed in acetic acid:ethanol 1:3 and stored at 4 °C for 2 months.

The fixed material was squashed and stained following the Giemsa C-banding technique described previously by Giraldez et al. (1979).

Seed protein extraction and electrophoresis separation

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). 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. Half grains of the inbred parental lines were analyzed in the same way.

Two different nomenclatures have been used for designating secalin loci: *Sec-*, or *Glu-* and *Gli-*. Given the similarities shown between prolamin storage-proteins in wheat and rye, we preferred the homoeologous nomenclature of wheat as was adopted by McIntosh (1988).

Apparent molecular weights of rye proteins were determined from the mobilities of the following proteins included in test mixture 4 (Serva): phosphorylase B (92,500), bovine albumin (67,000), egg albumin (45,000) and carbonic anhydrase (29,000).

Genetic analysis

Linkage was assessed using contingency χ^2 tests, and recombination fractions and their standard errors were estimated by the method of maximum likelihood according to Bailey (1961) for aberrant segregations.

Results and discussion

Lines 8t and 6Ri differed in several protein bands in both unreduced and reduced gels (Fig. 1, slots C and D), but only five of these bands were studied in the test-cross (Fig. 1, slots E-0). The bands were numbered from slower to higher mobility. Line 8t showed bands 2, 3 and 4 whereas line 6Ri had bands 1 and 5.

Bands 1 and 2 appeared only in reduced extracts because they are high-molecular-weight (HMW) secalins and could be separated only in the presence of a reducing agent such as 2-ME which breaks the disulphide bonds separating the aggregates formed (Shewry et al. 1983). The structural genes for these proteins are located on the long arm of chromosome 1R and have been assigned to the *Glu-R1* locus (Lawrence and Shepherd 1981).

Bands 3, 4, and 5 appeared in both reduced and unreduced extracts. Band 3 was assigned to the ω-secalin proteins located in the mobility zone of the 75 k γ-secalins described by several authors (see Carrillo et al. 1992). Bands 4 and 5 have been classified as 40 k γ-secalins. Both ω- and 40 k γ-secalins are controlled by a complex locus, named *Gli-R1*, located on the short arm of chromosome 1R.

Inbred lines 8t and 6Ri differed also in three cytological markers involving centromere and the C-heterochromatin of the telomeres. Chromosome 1R showed prominent C-heterochromatic blocks in the telomeres of both arms and was represented by its corresponding telosomes in inbred line 8t, whereas in 6Ri the homologous chromosome did not possess telomeric C-bands and was submetacentric. Figure 2 shows a cell from a progeny of the test-cross showing the parental karyotypes. Figure 3 shows several parental and recombinant chromosomes from different progeny.

The HMW subunits coded by *Glu-R1*, the ω- and γ-secalins coded by *Gli-R1*, the C-heterochromatin constitution of both telomeres, and the presence of telosomes or complete chromosomes, were classified without difficulty in all grains of the test-cross progeny. Altogether 204 test-cross seeds were analyzed and no aneuploids were detected among the progeny.

In the progeny of the test-cross a 1:1 ratio is expected for the two segregating alternatives of any cytological or genetic marker. However, three of the five markers showed an anomalous segregation; namely, *Gli-R1* and the two cytological markers. We have no explanation for this irregular segregation in our material.

The genes controlling ω- and γ-secalins segregated as a block showing complete linkage since no recombinants were found. Benito et al. (1990) and Carrillo et al. (1990, 1992) demonstrated linkage between these two types of secalin genes because they detected a very low level of recombination, indicating the existence of two tightly linked loci rather than one.

The number of the different phenotypic classes and the linkage values for the markers studied are given in Table 1.

Genetic mapping between Glu-R1 and Gli-R1

The recombination frequency between *Glu-R1* and *Gli-R1* was estimated as 44.12 ± 3.48 (Table 1) indicating no significant linkage. Therefore, they are widely separated on the two opposite arms of chromosome 1R. Similar behaviour between *Glu-1* and *Gli-1* has been reported in rye as well as in wheat (Lawrence and Shepherd 1981; Payne et al. 1982; Shewry et al. 1984; Benito et al. 1990; Carrillo et al. 1990, 1992).

Mapping the Glu-R1 locus on the long arm of chromosome 1R

The map distance between the *Glu-R1* locus and the centromere was estimated from both electrophoretic and cytological data, and the percentage of recombination was 32.25 ± 3.28. Comparable distances between *Glu-B1-*

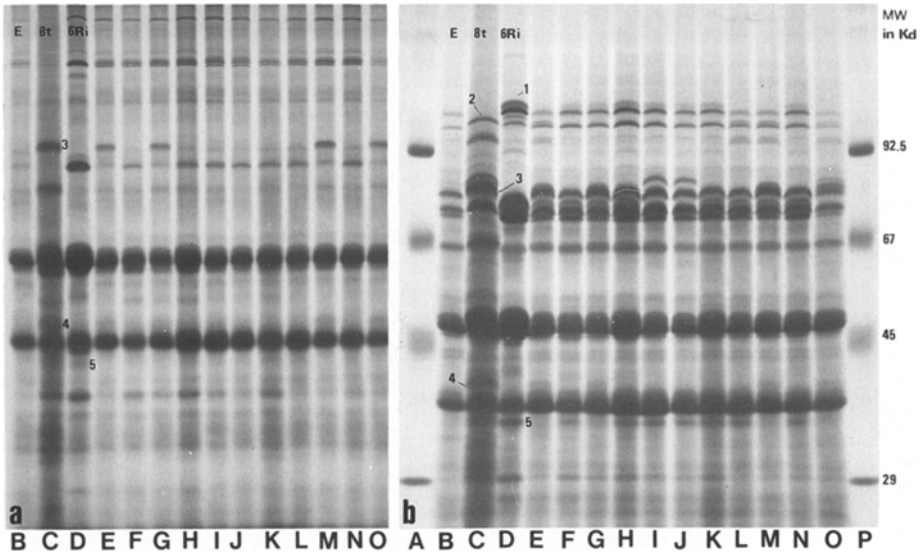

Fig. 1 a, b. SDS-PAGE patterns of reduced (**a**) and unreduced (**b**) secalin extracts of the same seeds. *A* and *P* molecular weight markers; *B* inbred line E(2666); *C* inbred line 8t; *D* inbred line 6Ri; *E–O* progeny of test-cross

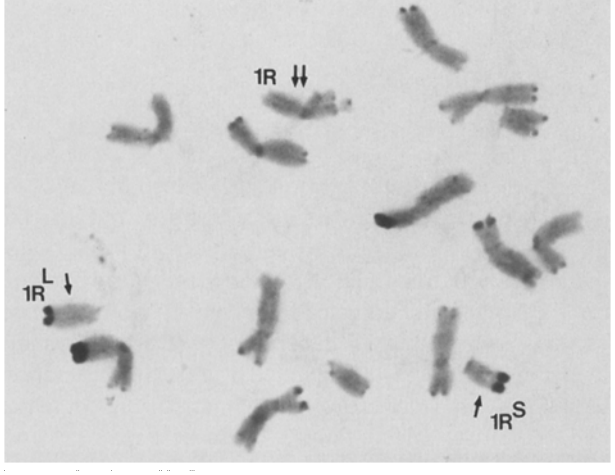

Fig. 2. Somatic metaphase from the seed of a progeny with parental chromosomes. *Arrows* indicate telosomes for chromosome 1R of line 8t; the *double arrow* indicates the complete chromosome 1R of line 6Ri

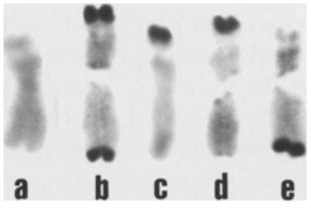

Fig. 3 a–e. Different chromosome types observed in the progeny of the test-cross. **a** Parental submetacentric chromosome; **b** two parental telosomes; **c** submetacentric recombinant chromosome; **d–e** recombinant telosomes

centromere and *Glu-D1*-centromere were obtained in wheat by Singh and Shepherd (1988 b). This similarity in the position of *Glu-R1* of rye and *Glu-B1* and *Glu-D1* in wheat supports the idea that these loci are actually homoeoallelic. However, the map distances are approximately three times higher than those reported by Payne et al. (1982), using a telocentric mapping procedure, for the position of the *Glu-A1* (7.6 ± 1.8), *Glu-B1* (9.2 ± 2.1), and *Glu-D1* (10.1 ± 2.3) loci with respect to the centromeres. It has been pointed out that recombination values obtained using telocentric mapping procedures may underestimate the true values as has been shown in cotton (Endrizzi and Kohel 1966) and wheat (Sears 1972).

The higher map distance for rye compared to wheat could be due to greater physical distance of the *Glu-R1* locus from the centromere or to differences between wheat and rye in chiasma localization in this region. Unfortunately there is no interstitial cytological marker in rye available for distinguishing between these two possibilities.

The linkage between the HMW secalin genes and the telomere was also estimated, the recombination percentage being 43.63 ± 3.47. This result indicates that the HMW secalin genes on chromosome 1R are loosely linked to the telomere on the long arm.

Mapping the Gli-R1 locus on the short arm of chromosome 1R

Analysis of the joint segregation indicated linkage between the centromere and the *Gli-R1* locus, the recombination frequency being 36.27 ± 3.37. This result is two or three times higher than that reported by Singh et al.

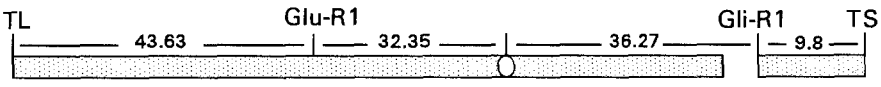

Fig. 4. Map of the different markers analyzed on chromosome 1R

Table 1. Phenotypic classes, contingency χ^2 tests and recombination percentages for the biochemical and cytological markers in chromosome 1R amongst progenies from the test-cross

Markers	Parentals	No. of progeny in phenotypic classes				Recombination percentage (±SE)	χ^2
		00	01	10	11		
TL/*Glu-R1*	00/11	53	29	60	62	43.63±3.47	4.74**
Glu-R1/centromere	00/11	80	33	33	58	32.35±3.28	24.32**
Centromere/*Gli-R1*	00/11	82	31	43	48	36.27±3.37	13.61***
Gli-R1/TS	00/11	116	9	11	68	9.80±2.08	128.15***
Glu-R1/*Gli-*R_1	00/11	74	39	51	40	44.12±3.48	1.89
Centromere/TS	00/11	82	31	45	46	37.25±3.39	10.86**

0, Submetacentric chromosome, euchromatin at the telomere, bands 1, 5
1, Telocentric chromosomes, heterochromatin at the telomere, bands 2, 3, 4
TL, Telomere of the long arm; TS, Telomere of the short arm
** and *** Significant at the 1% and 0.1% level, respectively

(1990) in rye using a translocation mapping procedure, but very similar to that of wheat for the same *Gli-B1*-centromere interval (Singh and Shepherd 1988a; Curtis and Lukaszewski 1991). Again, the differences could be explained by the existence of a differential chiasma frequency or a differential chiasma distribution in the short arm of chromosome 1R that could lead to differences in map distances.

In contrast to the *Gli-R1*-centromere segregation, many parental types were found when the *Gli-R1*-telomere segregation was analyzed. In the 204 progeny only 20 recombinants were detected, showing that the *Gli-R1* secalin genes on the short arm are closely linked to the telomere, the map distance being 9.8±2.08. This result agrees quite well with the results reported in hexaploid wheat by González et al. (1990) and in tetraploid wheat by Curtis and Lukaszewski (1991). Probably this locus is also located, as in wheat, on the satellited region (Payne et al. 1984).

The linkage maps for all markers analyzed on chromosome 1R are shown in Fig. 4.

Metaphase-I pairing and estimation of the recombination fraction

It has been claimed on several occasions that estimates of map distance can be significantly affected by lack of pairing (Payne et al. 1982; Singh and Shepherd 1984, 1988a). Payne et al. (1982) used metaphase-I pairing to correct the estimate of recombination as follows:

$$pc = p/x$$

where pc is the corrected frequency of recombination, p is the original frequency of recombination and x is the frequency of pairing. Obviously this correction calculates the recombination fraction under total pairing conditions. This gives an adjusted frequency of recombination which is hardly distinguishable from the original estimate if meiotic pairing is quite regular, but the differences between pc and p increase as meiotic pairing decreases and, therefore, pairing failures could be a major distortion factor in estimating map distances between loci. The modification introduced by Payne et al. (1982) is problematic for interstitial markers since it is impossible to ascertain whether or not that region is paired at metaphase-I, at least in cereals, and might only be used when the two markers are located at the telomere and at the centromere, respectively.

In our case, chromosome 1R could be identified easily in C-banded pollen mother cells at metaphase-I in the F_1 plants because line 8t has telosomes and prominent C-heterochromatin blocks at the telomeres. Hence, the frequencies of association at this stage could be determined for both arms. The long arm showed a very regular behaviour and no unpaired event was found. However, 16 of 50 PMCs showed the short arm unpaired. If meiotic pairing is a reflection of the cross-over frequency in a specific arm, we can assume that the frequency of at least one cross-over in the short arm of chromosome 1R

must be 0.68, leading to an estimate of 34% for the recombination percentage between the centromere and the telomere of such an arm. The original recombination fraction between the telomere and the centromere was 0.37 (very similar to that calculated from the metaphase-I data) and, as the pairing frequency was 0.68, the adjusted recombination fraction would be 0.55 and consequently, the two markers could be considered unlinked.

Nevertheless, the recombination fraction gives an idea of the position and distance of two or more linked loci although its value can vary among different genetic backgrounds and/or environmental conditions, depending on the cross-over frequency between the loci considered. Obviously any factor affecting the cross-over frequency will alter the recombination fraction.

Pairing failures should not be considered as a source of error in the estimate of the recombination fraction, since they are influential in determining the real level of recombination between two or more given markers. Consequently, any attempt to adjust the recombination fraction by modifying or altering the actual frequency of crossing-over also modifies the proper concept of recombination, and its use can lead to erroneous conclusions.

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