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Linkage relationships between the loci Sec 1 and Sec 3 in rye (*Secale cereale* L.)

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Rye secalins were analyzed in the progeny of three test-crosses by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using unreduced and reduced extracts of total seed proteins. Segregation for two ω-secalin bands and two 40K γ-secalin bands was studied in the three crosses. Both kinds of proteins showed a very tight linkage ($0{\cdot}25 \pm 0{\cdot}25$ per cent recombination), finding one recombinant in 394 seeds. Segregation for two high molecular weight (HMW) secalin bands was analyzed in one test-cross. The data indicated linkage ($36{\cdot}03 \pm 4{\cdot}12$ per cent recombination) between the structural genes for ω and 40K γ secalins (Sec 1) and for HMW secalins (Sec 3).

INTRODUCTION

In rye, four major groups of storage proteins (secalins) have been purified and characterized (Shewry *et al.*, 1982; Field *et al.*, 1982; Shewry *et al.*, 1983; Kasarda *et al.*, 1983). These four groups are called high molecular weight secalins, 75K γ-secalins, ω-secalins, and 40K γ-secalins. The structural genes for the group of high molecular weight (HMW) secalins were located on the long arm of chromosome 1R (Lawrence and Shepherd, 1981; Singh and Shepherd, 1984; Shewry *et al.*, 1984), and for the other two groups, the ω-secalins and the 40K γ-secalins, on the short arm of the same chromosome (Shepherd, 1968; Bernard *et al.*, 1977; Lawrence and Shepherd, 1981). The locus on the long arm has been designated Sec 3, and the locus on the short arm Sec 1 (Shewry *et al.*, 1984). The structural genes for the fourth group, the 75K γ-secalins, were located on chromosome 2R in *S. cereale* (Lawrence and Shepherd, 1981) and its locus has been called Sec 2 (Shewry *et al.*, 1984). In the ancient species *S. montanum* they were located on 6R (Shewry *et al.*, 1985).

The linkage relationships of the structural genes for some secalins have been determined. Shewry *et al.* (1984), Lawrence and Appels (1986) estimated the linkage between the loci on chromosome 1R responsible of HMW and ω-secalins, studying the segregation of one band of each kind of proteins. The locus Sec 3 which controls the HMW secalins was mapped close to the centromere (Singh and Shepherd, 1984).

This paper reports the segregation of some secalin bands among progeny of three test-crosses in rye. Segregation occurred at the Sec 1 locus for ω and 40K γ secalins and at Sec 3 for HMW secalins. Our results indicate that Sec 1 is a complex locus with families of genes tightly linked and also show that Sec 3 and Sec 1 loci are loosely linked.

MATERIAL AND METHODS

Crossing scheme

Some plants from a population of *Secale cereale* were pollinated with pollen from a 3R telocentric substitution line cv. "Petkus Spring". Three F_1 grains, coming from the same spike were sown and the F_1 plants were test-crossed to study segregation in secalins. One of them was pollinated by the inbred line A of *S. cereale* (cross 1), and the other two F_1 were crossed as females by the inbred line R of *S. cereale* (crosses 2 and 3). Both inbred lines have been maintained by selfing about thirty years.

Extraction and analysis of seed proteins

The grains obtained from the three crosses were divided in two halves. From one half total proteins were extracted into a buffered solvent containing

4 per cent (w/v) sodium dodecyl sulphate (SDS) and 5 per cent (v/v) 2-mercaptoethanol (2-ME), and from the other half the extraction was in the same solvent without 2-ME. The extracted proteins were fractionated by SDS-PAGE using 12 per cent gels in the discontinuous system of Laemmli (1970) as modified by Payne *et al.* (1980). Half grain of the three F_1 grains and some grains of the inbred lines were kept and analysed in the same way as the half grains of the crosses.

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

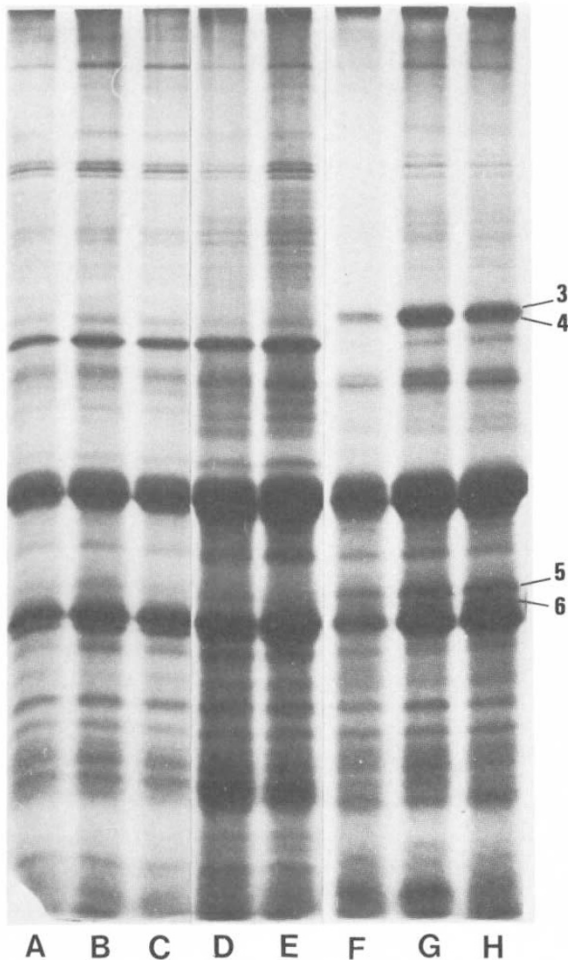

Figure 1 Fractionation by SDS-PAGE of unreduced total seed proteins. A-C, inbred line R; D-E; inbred line A; F-H, the three F_1 grains used in test-crosses.

Statistical analysis

Gene linkage was tested for by χ^2 tests, and recombination fractions and their standard errors were estimated by the method of maximum likelihood.

RESULTS AND DISCUSSION

The three F_1 grains showed the same patterns in each extraction procedure of total endosperm proteins, without 2-ME (fig. 1, slots F-H) and reducing the extracts (fig. 2, slots I-J). Slots A-E (fig. 1) and slots B-H (fig. 2) show separations of secalins from several grains of the inbred lines R and A used for the test-crosses. The pattern of all grains analysed within each line showed the homogeneity expected of inbred lines with reduced and unreduced proteins.

The segregation of seed protein differences was examined in the progeny of the three test-crosses. Secalin patterns without reduction are shown in fig. 3, where unreduced proteins of six grains from

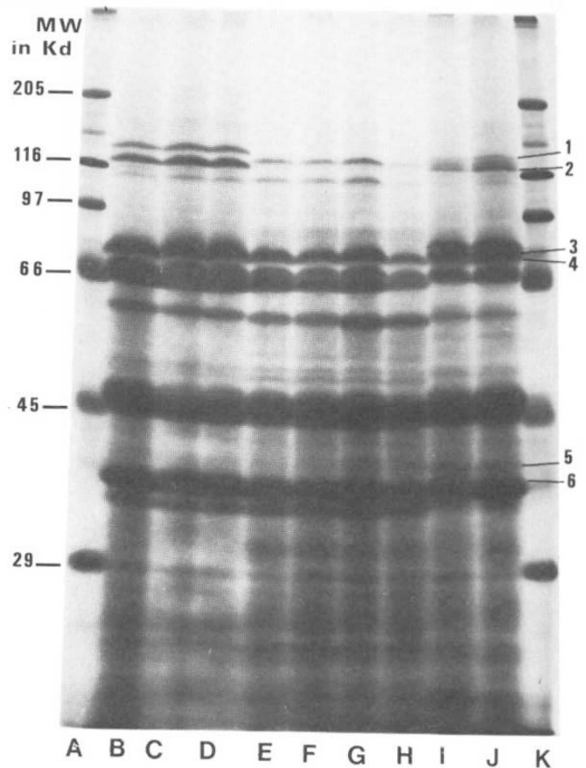

Figure 2 Fractionation by SDS-PAGE of reduced total seed proteins. A and K, molecular weight markers; B-D, inbred line R; E-H, inbred line A; I and J, the F_1 grains used in the test-crosses 1 and 2.

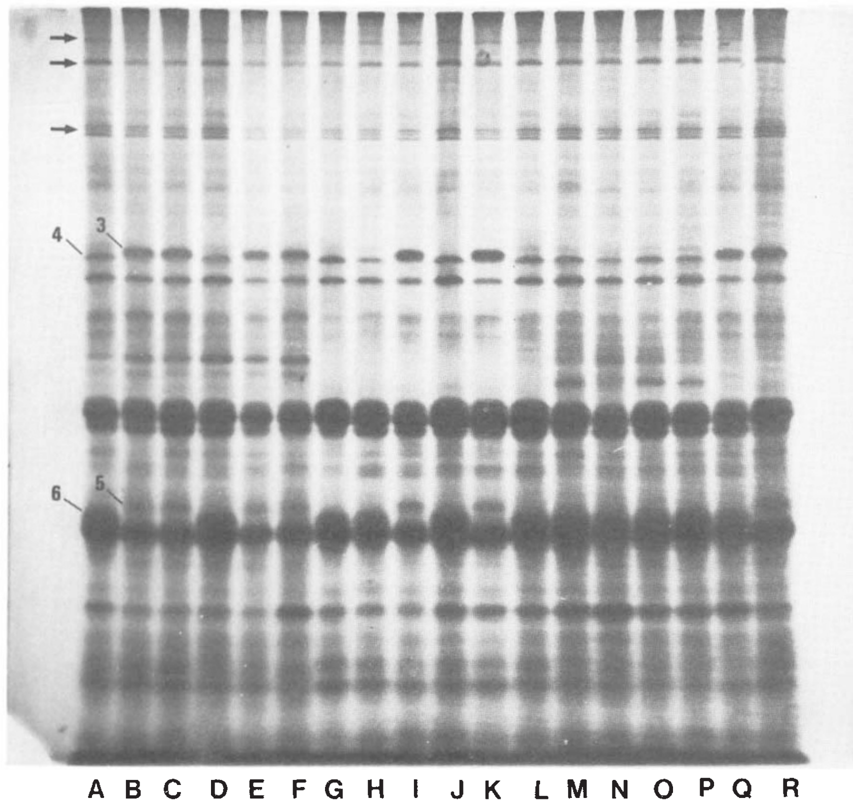

Figure 3 SDS-PAGE of unreduced total seed proteins. A–F, single seeds from progeny of test-cross 1; G–L, single seeds from progeny of test-cross 2; M–R, single seeds from progeny of test-cross 3.

each cross were separated by SDS-PAGE. In the upper (cathodal) part of the gel some uniform bands appeared (arrowed in fig. 3), and these bands were absent after reduction with ME (fig. 4). In wheat the only bands found, without reduction, in the high molecular weight region are the triplet protein bands (Singh and Shepherd, 1985). In rye more protein bands than in wheat were found. The reason could be that the aggregated proteins resulting from disulphide linkage could have smaller size in rye than in wheat and therefore they could penetrate easier the pores of the gel.

The other faster moving protein bands in unreduced extracts were thought to be monomeric secalins and soluble proteins (albumins and globulins). In the progeny of the three test-crosses band 3 was inherited as an alternative to band 4 (fig. 3). Band 5 was also an alternative to band 6 (fig. 3). The segregation in both situations and in the three crosses was statistically in agreement with a 1:1 ratio, consistent with the band 3 being allelic to band 4, and band 5 to band 6.

In fig. 4, showing reduced total proteins extracted from the other halves of the same grains, bands 3, 4, 5, and 6 are distinguishable in the three crosses. Their segregation in reduced extracts was exactly the same as in unreduced extracts of the other half grains.

Bands 3, 4, and 5, 6 were assigned to the ω-secalin and 40K γ-secalin classes of proteins respectively. Bands 3 and 4 were more clearly resolved without reduction. Their mobilities (fig. 4) indicated that bands 3 and 4 were located in the mobility zone of 75K γ-secalin proteins controlled by the Sec 2 locus on chromosome 2R (Lawrence and Shepherd, 1981; Shewry *et al.*, 1984). The molecular weight of these ω-secalins was considerably higher than the weight determined, about 52 Kd, for ω-secalins (Shewry *et al.*, 1983). This was to be expected. Shewry *et al.* (1984) mapped an ω-secalin band very close to 75K γ-secalin region and Lawrence and Appels (1986) mapped another ω-secalin band located among 75K γ-secalins, in a very similar location to that of bands 3 and 4. In barley and wheat the homologous proteins to ω-secalins have a wide range in their molecular weights (Faulks *et al.*, 1981; Kasarda *et al.*, 1983).

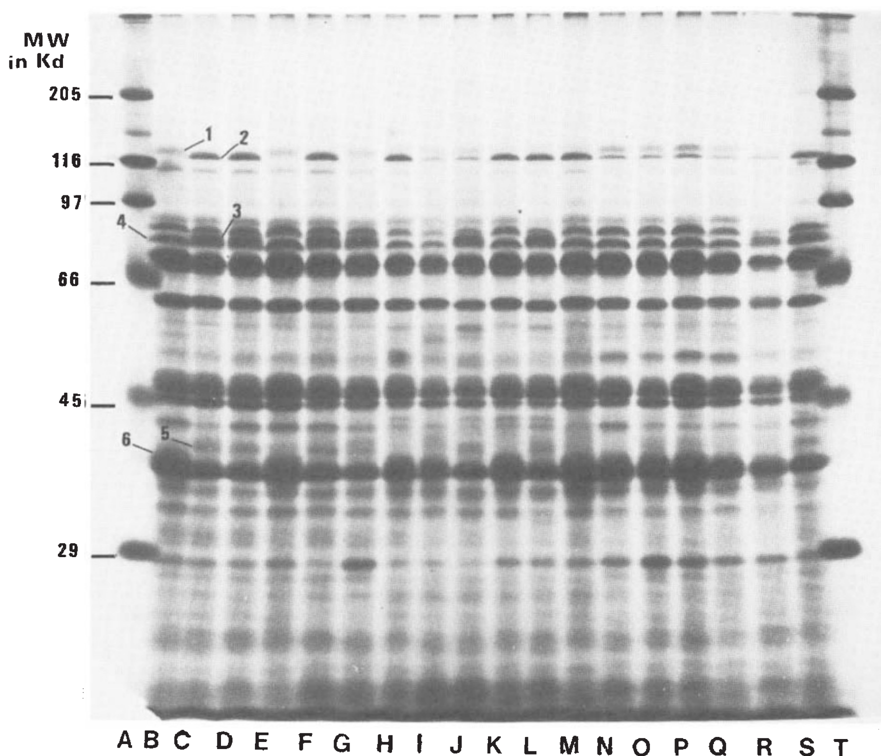

Figure 4 SDS-PAGE of reduced total seed proteins. A and T, molecular weight markers; B-G, single seeds from progeny of test-cross 1; H-M, single seeds from progeny of test-cross 2; N-S, single seeds from progeny of test-cross 3.

Bands 5 and 6 were distinguishable in reduced and unreduced total protein extracts and their mobilities suggested that they belonged to the group of 40K γ-secalins, being controlled, as the ω-secalins, by the Sec 1 locus located on the short arm of 1R (Shewry *et al.*, 1984). Both kinds of proteins, 40K γ-secalins and ω-secalins, showed complete linkage in crosses 1 and 2, and one recombinant with bands 3 and 6 was found in cross 3 (fig. 3, slot Q and fig. 4, slot R). The numbers of various phenotypic classes and the linkage values are given in table 1. The recombination percentage in cross 3 was $0{\cdot}91 \pm 0{\cdot}90$. Combining the results of the three crosses the recombination percentage was $0{\cdot}25 \pm 0{\cdot}25$. Shewry *et al.* (1984) suggested that the genes responsible for the 40K γ-secalins were linked to those for the ω-secalins, but a precise linkage relationship of the genes for the γ-secalins had not been established. Sec 1 seems to be a complex locus controlling ω and 40K γ secalins with at least two families of genes very tightly linked. It should be necessary to study more segregates to confirm that the situation in Sec 1 is similar to the homologous Gli-1 loci in wheat where the

Table 1 Phenotypic classes, linkage χ^2 and recombination percentages for the ω and 40K γ secalin bands segregating in Sec 1 locus, amongst progenies from three test-crosses

Cross	Nos. of progeny in phenotypic classes				Total No. of progeny	Linkage χ^2 df = 1	P	Recombination percentage (±S.E.)
	3/6	3/5	4/6	4/5				
1	0	64	72	0	136	136	<0·001	0·00
2	0	67	81	0	148	148	<0·001	0·00
3	1	51	58	0	110	106·3	<0·001	0·91 ± 0·90
1+2+3 pooled	1	182	211	0	394	390·0	<0·001	0·25 ± 0·25

Table 2 Phenotypic classes, linkage χ^2 and recombination percentages for the bands segregating in Sec 1 and Sec 3, amongst progeny from one test-cross

Secalin bands	Nos. of progeny in phenotypic classes				Total No. of progeny	Linkage χ^2 df = 1	*P*	Recombination percentage (±SE)
HMW-ω	1/3	1/4	2/3	2/4				
	42	27	22	45	136	10·62	<0·005	36·03 ± 4·12
HMW-40K γ	1/6	1/5	2/6	2/5				
	27	42	45	22	136	10·62	<0·005	36·03 ± 4·12

genes controlling ω and γ gliadins bands have been shown to segregate as a unit with nil or very rare recombination (Sozinov and Poperelya, 1980; Metakovsky *et al.*, 1984).

The mobility of the slower bands in fig. 4 indicated that they were HMW secalins. They were absent in unreduced extracts (fig. 3) and were separated only in the presence of a reducing agent, which breaks the disulphide bonds separating the aggregates formed by the HMW secalins (Shewry *et al.*, 1983). Their structural genes were assigned to the Sec 3 locus located on the long arm of 1R (Lawrence and Shepherd, 1981; Singh and Shepherd, 1984; Shewry *et al.*, 1984). It was possible to study segregation of bands 1 and 2 in test-cross 1 (fig. 4, slots B–G), but in the other two test-crosses the protein bands of inbred line R did not allow us to analyze clearly the segregation. Bands 1 and 2 were alternative and their segregation was in agreement with a 1:1 ratio. These HMW secalin bands were linked to 3,4 ω-secalin bands and to 5,6 40K γ-secalin bands (table 2). The recombination percentage between Sec 3 and Sec 1 was 36·03 ± 4·12, in complete agreement with the estimate of 36 ± 4·6 obtained by Lawrence and Appels (1986), and comparable to the recombination value of 40·8 ± 3·76 reported by the other mapping study of secalins in 1R of *Secale cereale* (Shewry *et al.*, 1984).

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