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# Identification and mapping of the *Gli-R3* locus on chromosome 1R of rye (*Secale cereale* L.)

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**Summary**. The progenies of two different rye test-crosses were analyzed for secalin proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using unreduced and reduced aqueous ethanol extracts. Segregation for two high-molecular-weight secalin bands (*Glu-R1* or *Sec3*), one $\omega$-secalin band (*Gli-R1* or *Sec-1*), two 40K $\gamma$-secalin bands (*Gli-R1* or *Sec1*) and two $\omega$-type secalin bands (new locus) were studied. One recombinant between $\omega$- and $\gamma$-secalins was found in one test-cross. The new locus, designated *Gli-R3* or *Sec-4*, was mapped between *Glu-R1* and *Gli-R1*, more displaced towards *Gli-R1*. In test-cross 1 recombination between *Glu-R1* and *Gli-R3* was $33.80 \pm 3.22\%$, and between *Gli-R3* and *Gli-R1*, $12.04 \pm 2.21\%$. In the other test-cross the map distances were relatively similar but smaller, likely due to less recombination within two different species of *Secale*. Genes coding for 40K $\gamma$-secalins at *Gli-R1* were likely proximal to the centromere with respect to genes coding for $\omega$-secalins at the same complex locus.

**Key words**: Secalin loci – Linkage mapping – Chromosome 1R – *Secale cereale*

## Introduction

In rye (*Secale cereale* L.) and wheat (*Triticum aestivum* L.) there are striking similarities in the locations of the major multigenic loci encoding prolamin storage proteins on the group 1 chromosomes. *Glu-1* is the major locus encoding the high-molecular-weight prolamin subunits (HMW subunits of glutenin in wheat and HMW secalins in rye), the structural genes of which have been located on the long arm of chromosomes 1A (Lawrence and Shepherd 1980), 1B (Bietz et al. 1975) and 1D (Orth and Bushuk 1974) for wheat and 1R for rye (Lawrence and Shepherd 1981 a). The *Glu-R1* locus has also been designated *Sec 3* (Shewry et al. 1984). The other major locus is *Gli-1* located on the short arm of the same chromosomes, which encodes a mixture of $\omega$-gliadins and $\gamma$-gliadins in wheat (Mecham et al. 1978) and $\omega$-secalins and the MW 40,000 (40K) $\gamma$-secalins in rye (Shepherd 1968). The *Gli-R1* locus has also been designated *Sec 1* (Singh and Shepherd 1984). In wheat there is a multigene family of low-molecular-weight (LMW) glutenins that is closely linked to the *Gli-1* loci (Jackson et al. 1983) and designated *Glu-3* (Singh 1985). In rye, a LMW glutelin band in *Secale montanum* was detected but its chromosomal location could not be determined (Gupta and Shepherd 1990 b).

The map distances of these loci for rye and wheat are comparable. Payne et al. (1982) found 8.9% recombination between the HMW locus on the long arm and the centromere for all three chromosomes 1A, 1B and 1D. Singh and Shepherd (1984) mapped the locus *Glu-R1* close to the centromere on chromosome 1R of rye. In wheat the *Glu-1* loci were seen to be weakly or not significantly linked to *Gli-1* (Lawrence and Shepherd 1981 b; Payne et al. 1982). In rye, a recombination between *Glu-R1* and *Gli-R1* of 40.8% was reported by Shewry et al. (1984), and values of 36% were reported by Lawrence and Appels (1986) and Carrillo et al. (1990). In the complex locus of *Gli-1* in wheat, no recombination between $\omega$- and $\gamma$-gliadins has been convincingly demonstrated (Payne 1987). In rye two recombinants between $\omega$- and 40K $\gamma$-secalin genes in *Gli-R1* have been reported (Carrillo et al. 1990; Benito et al. 1990).

In the two species the properties of the proteins coded by those genes are also similar. The circular dichroism

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(c.d.) spectra of $\omega$-gliadins and $\omega$-secalins are similar, indicating that they have similar $\beta$-turn-rich structures. Preliminary c.d. spectroscopy of HMW secalins and $\gamma$-secalins gave spectra that were almost identical to those of the HMW subunits of glutenin and $\gamma$-type gliadins respectively, indicating similar conformations (Shewry and Tatham 1990).

A second locus encoding $\omega$-type gliadins was located on the short arm of chromosomes 1A (Sobko 1984) and 1B (Galili and Feldman 1984; Jackson et al. 1985). These loci were designated *Gli-3* (Payne et al. 1988) and were mapped 22–31 cM proximal to *Gli-1*. A protein of MW 55,000 was located on the short arm of chromosome 1R and mapped 10 cM from *Gli-R1* (Benito et al. 1990).

In study presented here, a new locus *Gli-R3* or *Sec-4* was identified and mapped to the other secalin loci in chromosome 1R. The complexity of *Gli-R1* is also discussed. Two different nomenclatures have been used for designing secalin loci: *Sec-*, or *Glu-* and *Gli-*. Given the similarities shown between prolamin storage proteins in wheat and rye, we preferred the homoeologous nomenclature to wheat as it was adopted by McIntosh (1988).

## Materials and methods

A test-cross procedure utilizing several inbred lines differing in secalins was used to map the secalin genes of rye. Two test-crosses were used. The inbred lines R2 and J14 were crossed, and one $F_1$ plant as female was test-crossed with the inbred line E2666. This cross will be called test-cross 1. The second test-cross was produced by crossing the inbred line 8t and one plant of *Secale vavilovii* ssp. *iranicum*, a cleistogamous species, and one $F_1$ plant as female was test-crossed with the inbred line E2666. This cross will be called test-cross 2. The four inbred lines used have been maintained by self pollination for several years.

The secalins of the non-embryo halves of the seeds obtained from the two test-crosses were extracted in 70% aqueous ethanol following Gupta and Shepherd (1990a). The dried extract was redissolved in TRIS-HCl buffer containing 4% (w/v) sodium dodecyl sulphate (SDS).

Patterns of the secalins were determined by SDS-PAGE using 10% gels in the discontinous system of Laemmli (1970) as modified by Payne et al. (1980). The extracted secalins of each grain were run on two different gels. One gel contained part of the unreduced ethanol extract, and the other gel was loaded with the other part of the ethanol extract reduced with 5% (v/v) 2-mercaptoethanol. Some grains of the parental lines were analyzed in the same way as the half-grains of the crosses.

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

Gene linkage was tested using $\chi^2$ tests, and recombination fractions and their standard errors were estimated by the method of maximum likelihood.

## Results and discussion

In test-cross 1 the inbred lines R2 and J14 differed in seven protein bands (Fig. 1a, slots B and C). The bands

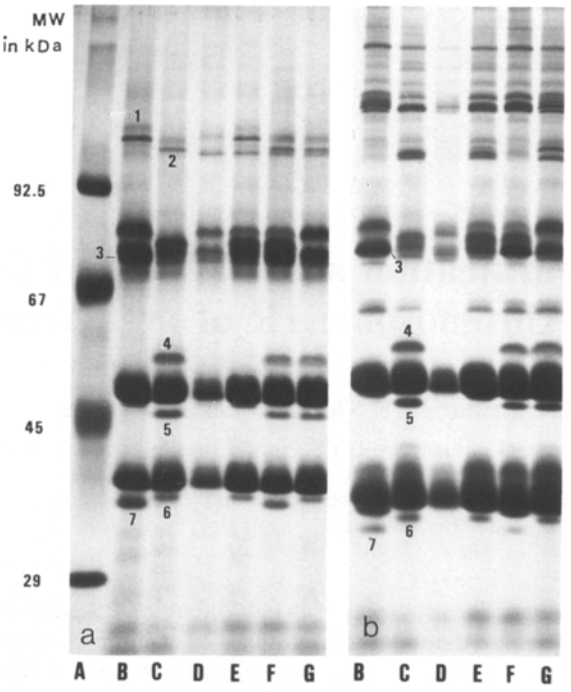


**Fig. 1 a and b.** SDS-PAGE patterns of reduced (**a**) and unreduced (**b**) secalin extracts of the same seeds. *A* Molecular weight markers, *B* inbred line R2, *C* inbred line J14, *D* inbred line E2666, *E–G* progeny of test cross 1

were numbered from slower to faster mobility. Bands 1, 3 and 7 belonged to R2 and bands 2, 4, 5 and 6 to J14. In the progeny of this test-cross band 1 was inherited as an alternative to band 2, band 3 to null band, bands 4 and 5 were inherited together as an alternative to null bands, and band 6 to 7. In test-cross 2 the inbred parental line 8t and the parental plant of *S. vavilovii* showed differences in six protein bands (Fig. 2a, slots B and C). Bands 2, 3, 4 and 5 were present in line 8t, and band 1 and 6 in *S. vavilovii*. The progeny of this test-cross showed as alternative bands 1 or 2, 5 or 6. Bands 3 and 4 were inherited as having a null band as alternatives. The segregation of all the alternatives was statistically in agreement with a 1:1 ratio, consistent with the expected ratio of being allelic forms.

The mobility of the slower bands 1 and 2 in both test-crosses indicated that they were high-molecular-weight (HMW) secalins. They were present in reduced extracts (Figs. 1a, 2a and 2c) and undistinguishable in unreduced extracts (Figs. 1b, 2b and 2d). They 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 *Glu-R1* locus (synonymous *Sec 3*) located on the long arm of chromosome 1R (Lawrence and Shepherd 1981a; Singh and Shepherd 1984; Shewry et al. 1984).

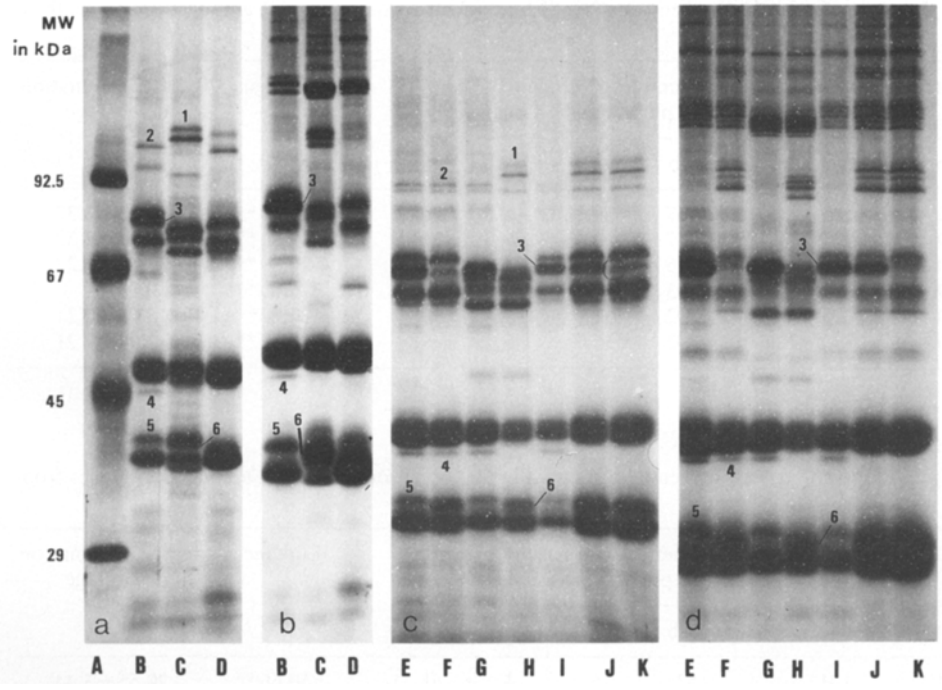


**Fig. 2a–d.** SDS-PAGE patterns of reduced (**a** and **c**) and unreduced (**b** and **c**) secalin extracts of the same seeds. *A* Molecular weight markers, *B* inbred line 8t, *C Secale vavilovii* ssp. *iranicum*, *D* inbred line E2666, *E–K* progeny of test-cross 2

All of the protein bands analyzed in this study, except HMW secalins, were distinguishable in reduced and unreduced ethanol protein extracts. Band 3 in both test-crosses was assigned to the $\omega$-secalin proteins. It was located in the mobility zone of the 75K $\gamma$-secalin proteins controlled by the *Gli-R2* locus (synonymous *Sec 2*) on chromosome 2R (Lawrence and Shepherd 1981a; Shewry et al. 1984), a zone with higher Mr value than the usual Mr values of about 48,000–53,000 reported for $\omega$-secalins (Shewry and Tatham 1990). But $\omega$-secalins with higher molecular weights have been described by several authors. Shewry et al. (1984) mapped one $\omega$-secalin band very close to the 75K $\gamma$-secalin region, Lawrence and Appels (1986), Benito et al. (1990) and Carrillo et al. (1990) mapped other $\omega$-secalin bands located among 75K $\gamma$-secalins as in these two test-crosses. In wheat the homologous proteins to $\omega$-secalins have a wide range in their molecular weights (Kasarda et al. 1983).

The mobility of bands 5 and 6 in test-cross 2 and bands 6 and 7 in test-cross 1 suggested that they belong to the group of 40K $\gamma$-secalins controlled, as the $\omega$-secalins, by the *Gli-R1* locus (synonomous *Sec 1*) located on the short arm of chromosome 1R (Shepherd 1968; Singh and Shepherd 1984; Shewry et al. 1984). Both kinds of proteins, $\omega$-secalins and 40K $\gamma$-secalins, showed complete linkage in the 216 progeny grains of test-cross 1 (Table 1), but in the 146 progeny of test-cross 2 one recombinant with bands 3 and 6 was found (Fig. 2c and d, slot J). There are two other studies where one recombinant has been described (Benito et al. 1990; Carrillo et al. 1990). The three recombinants were found with those $\omega$-secalins located in the mobility zone of about 75 kDa, not in the main group of $\omega$-secalins of about 50 kDa. In wheat, genes coding for $\omega$- and $\gamma$-gliadins are tightly clustered at a single, major locus, and no recombinants between $\omega$- and $\gamma$-gliadins have been reported in many thousands of segregating progeny (Payne 1987), although there is evidence that a few of the $\omega$-gliadin genes are separated from the main $\omega$-gliadin gene cluster (Metakovsky 1986). It might be that in rye two different gene families for $\omega$-secalins are present, one being the family of genes coding for the slower $\omega$-secalins, a little separated from the major locus where at least two families of genes, the $\omega$-main group and 40K $\gamma$-secalins, were very tightly linked.

In the progeny of both test-crosses two different segregating patterns of protein bands were observed in the mobility zone of $\omega$-secalins of MW about 50,000. In test-cross 1 there were seeds with band 4 (53 kDa) and band 5 (45 kDa) segregating together and seeds without bands 4 and 5 (Fig. 1a and b, slots E–G). In the progeny of test-cross 2 there were seeds with band 4 of MW about 45,000 and seeds without band 4 (Fig. 2c and d, slots E–K). These proteins were monomeric prolamins as they could be extracted with 70% aqueous ethanol and were distinguishable without any reducing agent. Due to their mobilities and the absence of any effect of reduction, they

**Table 1.** Phenotypic classes, linkage $\chi^2$ and recombination percentages for secalin loci on chromosome 1R amongst progenies from test-cross 1

| Loci | Parental genotypes | | Number of progeny in phenotypic classes | | | | Linkage $\chi^2$ | Recombination percentage (±SE) |
|---|---|---|---|---|---|---|---|---|
| | a/b | c/d | a/b | a/d | b/c | c/d | | |
| *Glu-R1*, *Gli-R1* (ω) | 1/5 | 2/0 | 67 | 49 | 36 | 64 | 9.80** | 39.35±3.32 |
| *Glu-R1*, *Gli-R1* (40K-γ) | 1/9 | 2/8 | 67 | 49 | 36 | 64 | 9.80** | 39.35±3.32 |
| *Glu-R1*, *Gli-R3* | 1/0+0 | 2/6+7 | 75 | 41 | 32 | 68 | 22.69** | 33.80±3.22 |
| *Gli-R1* (ω), *Gli-R1* (40K-γ) | 5/9 | 0/8 | 103 | 0 | 0 | 113 | 216.00** | 0.00 |
| *Gli-R1* (ω), *Gli-R3* | 5/0+0 | 0/6+7 | 92 | 11 | 15 | 98 | 124.52** | 12.04±2.21 |
| *Gli-R1* (40K-γ), *Gli-R3* | 9/0+0 | 8/6+7 | 92 | 11 | 15 | 98 | 124.52** | 12.04±2.21 |

** Significant at the 1% level

**Table 2.** Phenotypic classes, linkage $\chi^2$ and recombination percentages for secalin loci on chromosome 1R amongst progenies from test-cross 2

| Loci | Parental genotypes | | Number of progeny in phenotypic classes | | | | Linkage $\chi^2$ | Recombination percentage (±SE) |
|---|---|---|---|---|---|---|---|---|
| | a/b | c/d | a/b | a/d | b/c | c/d | | |
| *Glu-R1*, *Gli-R1* (ω) | 2/4 | 1/0 | 53 | 16 | 14 | 63 | 50.66** | 20.55±3.44 |
| *Glu-R1*, *Gli-R1* (40K-γ) | 2/7 | 1/8 | 53 | 16 | 13 | 64 | 53.04** | 19.86±3.30 |
| *Glu-R1*, *Gli-R3* | 2/6 | 1/0 | 59 | 10 | 10 | 67 | 76.96** | 13.70±2.85 |
| *Gli-R1* (ω), *Gli-R1* (40K-γ) | 4/7 | 0/8 | 66 | 1 | 0 | 79 | 142.03** | 0.68±0.68 |
| *Gli-R1* (ω), *Gli-R3* | 4/6 | 0/0 | 62 | 5 | 7 | 72 | 101.95** | 8.22±2.27 |
| *Gli-R1* (40K-γ), *Gli-R3* | 0/8 | 6/7 | 73 | 4 | 7 | 62 | 105.32** | 7.53±2.18 |

** Significant ata the 1% level

were tentatively identified as "putative ω-secalins". Additional work would be necessary to confirm their identity. Their linkage relationships to the other protein bands (Tables 1 and 2) showed that these ω-type secalins were located between *Glu-R1* and *Gli-R1*, closer to *Gli-R1*. In test-cross 1 the recombination distance to *Glu-R1* was 33.8% and to *Gli-R1*, 12.04%. In test-cross 2 the recombination with *Glu-R1* was 13.70%, showing 8.22% recombination with *Gli-R1* (ω) and 7.53% recombination with *Gli-R1* (40K γ). A protein band of MW 55,000 extracted with buffer containing SDS was described by Benito et al. (1990) to occupy a similar position on the 1R chromosome.

The locus controlling these ω-type secalins should be designated *Gli-R3* because it seems to be homoeologous to loci *Gli-A3* and *Gli-B3* in wheat, loci coding for ω-type gliadins (Payne et al. 1988). In wheat the *Gli-3* set of loci coding for ω-type gliadins has been located between *Glu-1* and *Gli-1*, a little more displaced towards *Glu-1* (Galili and Feldman 1984; Sobko 1984; Jackson et al. 1985). *Gli-R3* seems to be a complex locus coding for more than one protein as is indicated by the presence of two different protein bands segregating together in test-cross 1.

The order of loci controlling the secalins in chromosome 1R is represented in Fig. 3. The position of the loci on chromosome 1R was relatively similar in both test-crosses, although the values were smaller among loci in test-cross 2 than in test-cross 1, likely due to the presence of two different species of *Secale* in test-cross 2 giving less recombination between the chromosomes. The recombination percentage between *Glu-R1* and *Gli-R1* was 39.35±3.32% in test-cross 1, in complete agreement with other mapping studies of secalins on chromosome 1R of *Secale cereale* L. (Shewry et al. 1984; Lawrence and Appels 1986; Carrillo et al. 1990).

In *Gli-R1* there are two possible orders for the gene location of ω- and 40K γ-secalins. *Gli-R1* (ω) is either proximal or distal to the centromere with respect to *Gli-R1* (γ). The order was deduced from the position of the bands in the recombinant. The bands of the parent 8t were 3 (ω), 4 (ω-type) and 5 (γ), and the alternative bands of the parent *S. vavilovii* were null (ω), null (ω-type) and 6 (γ) (Fig. 2a and d, slots B and C). The two possible gene orders on the parental 1R chromosomes are 4 (*Gli-R3*), 3 (*Gli-R1*ω), 5 (*Gli-R1*γ)/null (*Gli-R3*), null (*Gli-R1*ω), 6 (*Gli-R1*γ) or 4, 5, 3/null, 6, null. The second order is the more likely to produce the recombinant null, 6, 3 because it implies only one crossing-over, whereas the first order would require a double crossing-over to produce the recombinant. Then, the genes coding for 40K γ-secalins at


**Fig. 3.** Map of secalin loci on chromosome 1R of rye

*Gli-R1* are likely proximal to the centromere with respect to the genes coding for ω-secalins at *Gli-R1* (Fig. 3). This loci order is in disagreement with the order reported by Pogna et al. (1990) for ω-gliadins and γ-gliadins genes at the *Gli-B1* locus.

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