Theor Appl Genet (1990) 79:347–352

Linkage and cytogenetic maps of genes controlling endosperm storage proteins and isozymes in rye (*Secale cereale* L.)

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Received August 16, 1989; Accepted October 3, 1989
Communicated by F. Salamini

Summary. An F_1 plant from *Secale cereale* ssp. *ancestrale* × telocentric substitution lines *3R* of the cultivated rye "Petkus spring" was used as female in a cross with the inbred line Riodeva (I28), which has the standard chromosome arrangement. Single plants from this backcross progeny were analyzed for chromosome constitution, storage protein, and isozymic patterns. The seed protein loci were identified as *Sec-1a* and *Sec-1b* loci controlling 40-K γ-secalins and ω-secalins, respectively. These loci are located on the short arm of chromosome *1R*. The *Sec-3* locus controlling high-molecular-weight secalins is located on the long arm of chromosome *1R*. A further seed protein locus, *Pr-3* (55-K protein), was located on the short arm of chromosome *1R*. A linkage was found between the *6Pgd-2* isozyme locus controlling 6-phosphogluconate dehydrogenase isozymes located on the long arm of chromosome *1R* and the four seed protein loci. The results favor the gene order: *6Pgd-2* ... *Sec-3* ... [centromere] ... *Pr-3* ... *Sec-1b* ... *Sec-1a*. Other linkages detected were *Per-3a* and *Per-3b* (0.33 ± 0.33 cM), *Est-8* and *Est-12* (0.33 ± 0.33 cM), and *Got-3* and centromere (20.57 ± 2.42 cM). The proxidase (*Per*), glutamate oxaloacetate transaminase (*Got*), and esterase (*Est*) loci were located on chromosome arms *2RS*, *3RL*, and *6RL*, respectively. The distances and the maps obtained are compared with data available in the literature.

Key words: Seed proteins – Isozymes – Cytogenetic mapping – *Secale cereale*

Introduction

Although loci condifying for isozyme markers have been located in rye chromosome arms (Bergman and Maan 1973; Tang and Hart 1975; Hart 1979; Rao and Rao 1980; Chojecki and Gale 1982; Salinas and Benito 1984a, b, 1985a, b; Lawrence and Appels 1986; Chenicek and Hart 1987), the corresponding cytogenetic maps are poorly developed. Linkage data are available for esterases (Wehling and Schmidt-Stohn 1984; Wehling et al. 1985), peroxidases (Garcia et al. 1982), 6-phosphogluconate dehydrogenase and glucose phosphate isomerase (Lawrence and Appels 1986), malate dehydrogenase and 6-phosphogluconate dehydrogenase, glutamate oxaloacetate transaminase and malate dehydrogenase, leaf peroxidases (Figueiras et al. 1985), endosperm alkaline phosphatases (Figueiras et al. 1987), and esterases and malate dehydrogenase and glutamate oxaloacetate transaminase (Figueiras et al. 1989, unpublished results).

Genetic and cytogenetic maps that consider both seed protein polymorphisms and isozyme loci have been reported in rye by Singh and Shepherd (1984, 1988a, b) and Lawrence and Appels (1986), including data on translocations and morphological markers (De Vries and Sybenga 1984) and on translocations and isozyme markers (Figueiras et al. 1985, 1989).

The genetic control, the monomeric or dimeric behavior, and the isozymic patterns of the different isozymes considered in this work have already been described by Pérez de la Vega and Allard (1984) and Figueiras et al. (1985). Here we present a set of data obtained from storage proteins and isozymic variants that define a detailed genetic map of chromosome *1R*, a cytogenetic map of chromosome *3R*, and a genetic map of chromosome *2R*.

Materials and methods

Materials

An F_1 plant obtained from *Secale cereale* ssp. *ancestrale* × telocentric substitution line *3R* of the cultivated rye

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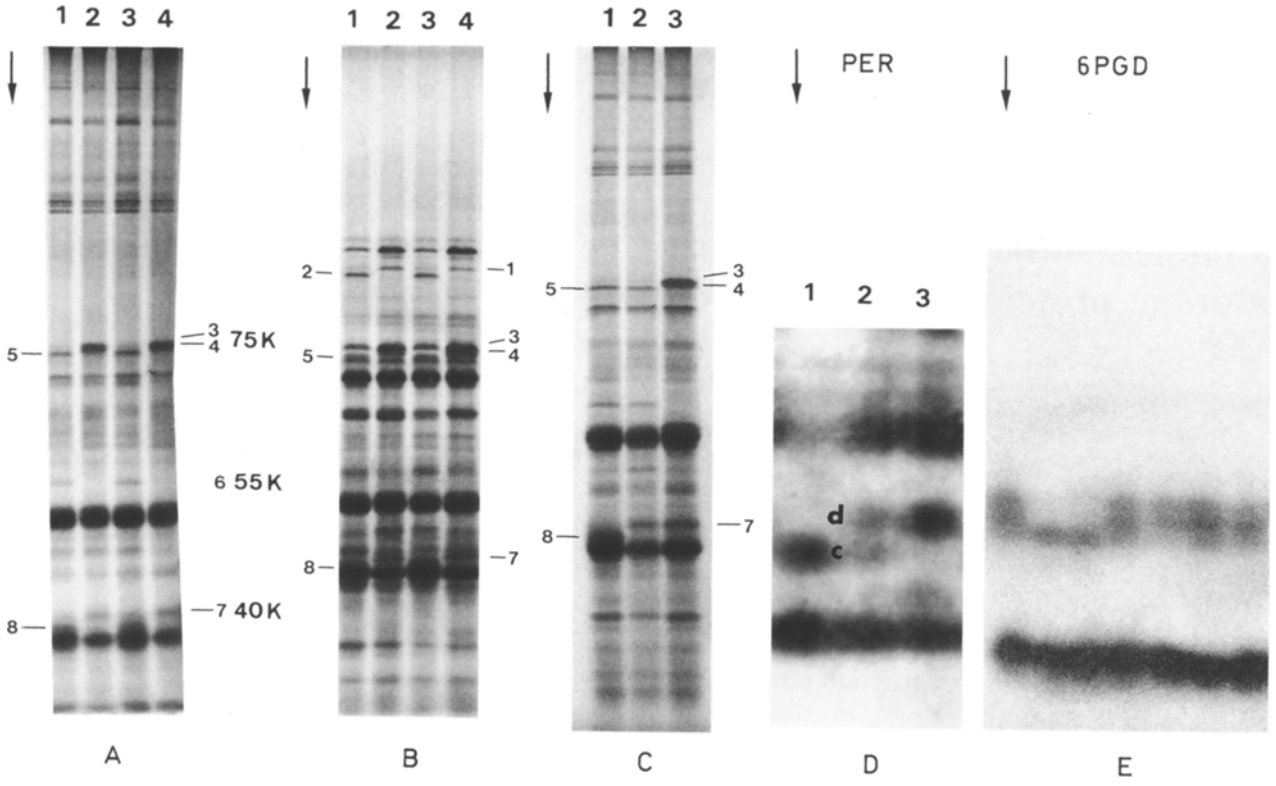

Fig. 1 A–E. Fractionation by SDS-PAGE of seed proteins. **A** and **B** – patterns without 2-ME and with 2-ME of the same seeds, respectively. **C** – patterns without 2-ME: *1* and *3* are the parental used in the backcross and *2* is the only recombinant plant found between *Sec-1b* and *Sec-1a*, respectively. **D** – zymogram showing leaf peroxidase isozymes: *1* and *3* are the parental of the backcross and *2* is the only recombinant found between *Per-3a* and *Per-3b* loci (this plant has bands c and d simultaneously). **E** – zymogram showing the 6-phosphogluconate dehydrogenase isozymes controlled by the *6-Pgd-2* locus. This gel shows five heterozygous plants (12) and two homozygous plants (11). The *arrow* shows direction of protein migration

"Petkus spring" was used as female in a cross with the inbred line Riodeva (I28), which has the standard chromosome arrangement. Single plants from this backcross progeny were analyzed for chromosome constitution, storage protein, and isozymic patterns.

The cytological study was made on root cells at mitotic metaphase following the aceto-carmine stain procedure. The plants of the progeny were classified as standard (s) with normal chromosome constitution, and telocentric (t) with telocentric chromosome *3R*.

Electrophoresis of seed proteins

The seed proteins were extracted from a half-grain with a solvent containing 4% (w/v) sodium dodecyl sulphate (SDS) without 2-mercaptoethanol (2-ME), and fractioned by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), as previously described by Payne et al. (1980). After running those gels, the remanent extracts were reduced, with a drop of 2-ME added, and fractioned by SDS-PAGE. The separating gels contained 10% acrylamide.

Isozyme assays

The half-grains with the embryo were allowed to germinate. The biochemical analyses were carried out on 12-day-old leaves. The isozymic patterns of glutamate oxaloacetate transaminase (GOT), esterases (EST), phosphoglucose mutase (PGM), glucosephosphate isomerase (GPI), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), and peroxidases (PER) were studied according to the protocols described in Figueiras et al. (1985).

The distances between the loci were estimated by means of the maximum likelihood method: distance = (recombinants/total progeny) × 100 (Allard 1956).

Results

Chromosome *1R* of rye carries genes controlling urea-soluble "secalins" on its short arm (Shepherd 1968; Shepherd and Jennings 1971) and genes controlling high-molecular-weight (HMW) secalins on the long arm (Lawrence and Shepherd 1981). The loci located on the short arm have been designated *Sec-1a* and *Sec-1b* and their products referred to as the 40-K γ- and ω-secalins, respectively (Shewry et al. 1984). The products of the locus on the long arm have been named either the HMW subunits of rye glutenin (and the corresponding locus *Glu-R1*) (Singh and Shepherd 1984) or the HMW secalins (locus *Sec-3*) (Shewry et al. 1984). The *Sec-3* designation has been adopted in this paper.

The banding patterns of seed proteins are shown in Fig. 1. The mobility of bands 1 and 2 in SDS gels indi-

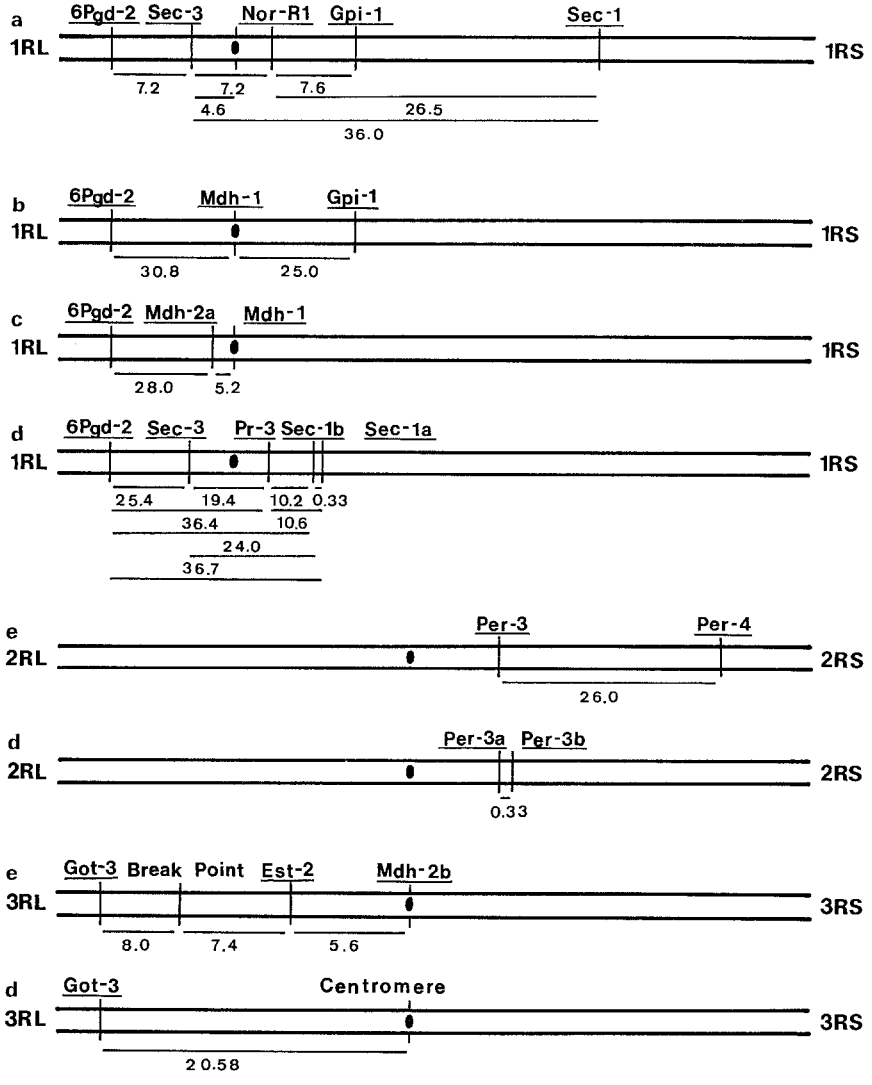

Fig. 2. Comparison of maps of chromosomes *1R*, *2R*, and *3R* of rye. *a* – Lawrence and Appels (1986) *b* – Figueiras et al. (1989) *c* – Figueiras et al. (1989). *d* – Maps of the present work. *e* – Figueiras et al. (1985). The order of the *Per* loci on *2RS* chromosome arm is tentative because we have not obtained direct estimates among these loci and the centromere

cates that they correspond to the high-molecular-weight secalins (=HMW glutenin subunits) and, thus, they were associated with the *Sec-3* locus located on the long arm of chromosome *1R*. In the backcross progeny, seeds were observed with band 1 and without band 2, as were seeds with band 2 and without band 1. The mobility of bands 3, 4, and 5 in SDS gels (Fig. 1) suggests that they are ω-secalins controlled by locus *Sec-1b*. Two different patterns were observed: seeds with band 3 and 4 and without band 5, and seeds with band 5 and without bands 3 and 4. The mobility of bands 7 and 8 identified them as 40 γ-secalins controlled by locus *Sec-1a*. Plants with band 7 and without band 8, and plants with band 8 and without band 7 were observed. Band 6 is a 55-K protein controlled by locus *Pr-3*. In the progeny, segregation for the presence or absence of band 6 was observed. The segregation of the different alleles of individual seed protein loci was an expected (1:1).

Banding patterns for the isozymes of glucosephosphate isomerase (GPI), glutamate oxaloacetate transaminase (GOT), phosphoglucose mutase (PGM), esterases (EST), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGD), and peroxidases (PER) were examined in the F_1 plants and in their progeny. The *Gpi-1*, *Mdh-1*, and *Mdh-2b* loci did not segregate. Segregation ratios of alleles of the individual loci – *Pgm-1*, *Got-3*, *Est-8*, *Est-12*, *6Pgd-2*, *Per-3a*, and *Per-3b* – were as expected (1:1). The locus *Pgm-1* was found independent of the other isozyme and seed protein loci examined.

In Table 1, linkages for the isozymic and seed protein loci are presented. The locus controlling the 6PDG-2 (zone of slower mobility) turned out to be linked to loci coding for seed protein. These loci have been located on chromosome arms *1RL* and *1RS*. The *Per-3a* and *Per-3b* loci were narrowly linked on the chromosome arm *2RS*, and the *Est-8* and *Est-12* loci were also linked (0.33 ± 0.33 cM). On the other hand, the locus *Got-3* was associated with the *3R* telocentric chromosome. The estimated distance between this locus (*Got-3*) and the centromere of chromosome *3R* was 20.57 ± 2.42 cM. *Sec-3*,

Table 1. Significant linkages observed among the loci considered in this study

Loci	Parental genotypes[a]	Distribution of progeny (phenotype)				X Linkage*	Distance cM
Chromosome *1R*		11 0	11 1	12 0	12 1		
6Pgd-2, Sec-1b	(11 1) × (12 0)	57	92	87	47	19.88	36.75 ± 2.87
6Pgd-2, Sec-1a	(11 0) × (12 1)	92	57	48	86	18.33	37.10 ± 2.87
6Pgd-2, Sec-3	(11 0) × (12 1)	107	42	30	104	68.27	25.44 ± 2.59
6Pgd-2, Pr-3	(11 0) × (12 1)	87	62	41	93	20.95	36.40 ± 2.86
		0 0	0 1	1 0	1 1		
Sec-1b, Sec-1a	(0 1) × (1 0)	1	143	139	0	279.01	0.35 ± 0.35
Sec-1b, Sec-3	(0 1) × (1 0)	33	111	104	35	76.36	24.03 ± 2.54
Sec-1b, Pr-3	(0 1) × (1 0)	9	135	119	20	178.89	10.25 ± 1.80
Sec-1a, Sec-3	(0 0) × (1 1)	104	33	36	110	74.29	24.38 ± 2.55
Sec-1a, Pr-3	(0 0) × (1 1)	119	21	9	134	175.72	10.60 ± 1.83
Chromosome *2R*		0 0	0 1	1 0	1 1		
Per-3a, Per-3b	(0 1) × (1 0)	0	163	132	1	292.01	0.33 ± 0.33
Chromosome *3R*		11 s	11 t	12 s	12 t		
Got-3, Centr	(11 t) × (12 s)[b]	29	126	94	28	95.92	20.57 ± 2.42
Chromosome *6R*		0 0	0 1	1 0	1 1		
Est-8, Est-12	(0 1) × (1 0)	1	141	156	0	294.22	0.33 ± 0.33

[a] Phenotypes (1) and (0) indicate isozyme of protein present and absent, respectively; (11) homozygote for allele 1; (12) heterozygote for alleles 1 and 2

[b] s – normal chromosome constitution, t – telocentric chromosome *3R*

* All values are significant ($P < 0.001$)

Sec-1a, Sec-1b, Pr-3, and *6Pgd-2* loci were linked on chromosome *1R*. Here the following order of loci was unambiguously determined:

6Pgd-2 ... Sec-3 ... Pr-3 ... Sec-1b ... Sec-1a.

This order of loci, together with distances in centimorgans between adjacent loci, is shown in Fig. 2. In this map the centromere has been located between the *Sec-3* and *Pr-3* on the basis of previous evidence that *Sec-3* is located on the long arm of *1R* (Lawrence and Shepherd 1981), while the *Nor-R1* locus corresponds to the secondary constriction observed on the short arm of *1R* (Lawrence and Appels 1986). Figure 2 also summarizes all linkage obtained in this study compared to other available data.

Discussion

Our data reveal that the *Got-3* locus is associated with the *3R* chromosome, and the estimated distance between the *Got-3* and the centromere is 20.57 ± 2.42 cM. These results fit with the chromosomal location data indicated by Figueiras et al. (1985). Other isozymic loci located on the *3R* chromosome are *Est-2* and *Mdh-2b*. The *Mdh-2b* locus is extremely closely linked to different rye translocations involving the *3R* chromosome (Figueiras et al. 1985, 1989). This suggests that *Mdh-2b* maps near the centromere. The estimated distance between *Got-3* and *Mdh-2b* in plants without translocations is 21.00 cM (Figueiras et al. 1985), again suggesting that *Mdh-2b* is quite near to centromere (*Got-3* ... 20.57 cM ... centromere. Data obtained in wheat (Hart 1983) indicated that the *Got-3* locus is located on the long arm of homoeologous group 3, thus the most probable location in rye for *Got-3* and the previous loci described by Figueiras et al. (1985, 1989, unpublished results) is chromosome arm *3RL*.

The *Per-3a* and *Per-3b* loci are closely linked (0.33 ± 0.33 cM) and have been located on the *2RS* chromosome arm (Salinas and Benito 1984b). Previous data suggested the existence of only one *Per-3* locus with two active alleles and one null allele (Figueiras et al. 1985). This paper presents evidence indicating the existence of two closely linked loci; each locus has an active and a null allele. Previous data (Figueiras et al. 1985) and the present results indicate that the short arm of the *2R* chromosome map, at least three linked peroxidase loci are present. The order shown in Fig. 2 for the *Per* loci on the *2RS* chromosome arm is tentative, because data are not available which map the three loci with respect to the centromere. The small distance found between *Per-3a* and *Per-3b* suggests that they may have originated by gene duplication.

The *Est-8* and *Est-12* loci are linked 0.33 ± 0.33 cM), and they segregate independently from the other loci considered. Genes controlling rye leaf esterases have been found on chromosomes *3R* and *6R* (Salinas and Benito 1985c). *Est-8* and *Est-12* have been previously

located on *6RL* (Salinas and Benito 1985c; Wehling et al. 1985). A remarkable series of tightly linked esterase loci have been found by Wehling and Schmidt-Stohn (1984), with a maximum recombination rate of 0.2% on chromosome *6R*. Probably the two mentioned esterases are included in this series.

The linkage map obtained for the five loci *1R* is consistent with the previous observations that have assigned *Sec-3* and *6Pgd-2* to the long arm of *1R*, and the *Sec-1* loci to the short arm (Lawrence and Appels 1986). Our map distance between *6Pdg-2* and *Sec-3* (25.44 ± 2.59 cM) is three times greater than the value found for equivalent regions (7.2 ± 1.8 cM) by Lawrence and Appels (1986). Also, the map distance between *6Pgd-2* and *Gpi-1* (55.8 cM) obtained by Figueiras et al. (1989) is about three times greater than the value found for the same region (20.7 cM) by Lawrence and Appels (1986). The map distance reported for the corresponding loci mapping in wheat on chromosome *1B* (Chojecki et al. 1983; Payne et al. 1984; Ainsworth et al. 1984; Snape et al. 1985) is, on the other hand, comparable to our results. Moreover, our value is similar to that found by Singh and Shepherd (1988a, b).

The distance between loci *Sec-3* and *Sec-1b* in our cross ($24.03 \pm 2\text{-}54$ cM) is shorter than the values found by Lawrence and Appels (1986) and Shewry et al. (1984) between *Sec-3* and *Sec-1*. The locus indicated in this paper as *Sec-1b* controls a ω-secalin evident in electrophoretic separations both in the presence and absence of 2ME. The mobility of this protein in SDS-PAGE gels is similar to that of *Sec-1* (Lawrence and Appels 1986), but the map distance obtained is different and is not comparable. The *Sec-1a* locus codes for a 40-K τ-secalin and is closely linked to *Sec-1b* (0.33 ± 0.33 cM). In wheat, it has been shown that the genes controlling monomeric gliadins (ω and γ) are also very closely linked on chromosome arms *1AS* and *1BS* (Payne et al. 1984), a finding which agrees with our observation that only one recombinant between *Sec-1a* and *Sec-1b* was found in the present analysis. On the other hand, the *Pr-3* locus of rye controls a 55-K protein and is located between *Sec-3* and *Sec-1b*. Two newly designated loci (*Gli-B3* and *Gli-A3*) coding for ω-type gliadins and D-subunits of glutenins have been located on chromosomes *1B* and *1A* of wheat, and mapped at the same distance between *Glu-B1* and *Gli-B1* loci by Payne et al. (1988). Also, Galili and Feldman (1984) found a gene controlling a particular protein band (B-30) mapping in wheat proximally to the *Gli-B1* locus, and showing 25.4% recombination with *Gli-B1* and 23.5% recombination with *Glu-B1* (in rye *Sec-3*). Considering that our results map *Pr-3* on the short arm near to centromere, this new locus could be homoeologous to *Gli-B3* and *Gli-A3*.

The distances obtained in previous works (Figueiras et al. 1985, 1989, unpublished results) for *6Pgd-2, Gpi-1, Mdh-1,* and *Mdh-2a* loci are comparable to those presented here, and suggest that *Mdh-1* and *Mdh-2a* are near the centromere of chromosome *1R*. The *Mdh-2* controls the isozymes of zone II that have been located on chromosomes *1R* and *3R* (Salinas and Benito 1985a). Recently it has been found that *Mdh-1* and *Mdh-2a* loci are linked (A.M. Figueiras, M.A. Elorrieta, C. Benito, unpublished results). In contrast, *Mdh-2a* and *Mdh-2b* map respectively near to the centromere of *1R* and *3R*.

Acknowledgements. This article was financed by two grants of the CICYT (PB87-0087 and AGR88-0051).

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