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RFLP-based genetic map of rye (*Secale cereale* L.) chromosome *1R*

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Summary. A map of chromosome *1R* of rye was constructed using 16 molecular and biochemical loci. From long arm to short arm, known-function loci were placed in the order: *XAdh – XLee – Glu-R1[Sec-3] – XPpdk-1R – XEm-1R-1 – XEm-1R-2 – Centromere – XNor-R1 – Gpi-R1 – XGli-R1[Sec-1a]* along with six anonymous genomic and cDNA clones from wheat. The map, which spans 106 c*M* with 12 loci clustered in a 15-c*M* region around the centromere, shows reasonably good agreement with previously published maps for the centromeric region, whereas the *XNor-R1 – Gpi-R1* region gives a much larger distance than previously reported.

Key words: Rye – Genetic mapping – RFLP – Storage protein – Isozyme

Introduction

Rye, *Secale cereale* L. (2n = 2x = 14), is of importance both as one of the major cereal crop species, and as the donor of a component genome of the synthetic cereal, triticale. In wheat breeding, rye is also important as a donor of chromosome segments carrying useful agronomic genes. Probably the most significant wheat-rye introgression to date is the *1BL.1RS* translocation that appears to have yield-enhancing properties (Rajaram et al. 1983), albeit with detrimental effects on bread-making quality (Zeller et al. 1982). In addition, *1RS* has been shown to carry genes for resistance to powdery mildew, *Erysiphe graminis* DC., *Pm8* (van Kints 1986), stem rust, *Puccinia graminis* Pers., *Sr31* (Hu and Roelfs 1986), leaf rust, *Puccinia recondita* Rob. ex Desm., *Lr26* (Bartos et al. 1984), yellow rust, *Puccinia striiformis* Westend., *Yr9* (Mettin et al. 1973; Zeller and Fuchs 1983) and greenbug, *Schizaphis graminum* Rond., *Gb* (Wood et al. 1974). Further manipulation of *1BL.1RS* by induced homoeologous recombination (Koebner and Shepherd 1986; Koebner et al. 1986), in order to maintain the yield advantage while avoiding the quality penalty, will require well-mapped short arms of *1R* and *1B*.

Our interest in the genetic map of rye is both to provide the means of manipulating the introgression of rye chromosome fragments into wheat and, since it is a simple diploid *Triticeae* species, as a prelude to mapping the more complex hexaploid bread wheat, *Triticum aestivum* L. (2n = 6x = 42).

Material and methods

Genotypes

Sixty F_2 plants or bulk F_3 progenies derived from individual F_2s from the cross between the experimental Polish inbred lines, DS2 and RxL, supplied by Dr. P. Masojć, Academy of Agriculture, Szczecin, were characterized and used for linkage analysis.

RFLP markers

Nine known-function and five anonymous wheat cDNAs, and four anonymous genomic wheat DNA clones were used as probes for RFLP analysis (Table 1). All these clones contain sequences which, when used as probes, cross-hybridize to rye genomic DNA. The anonymous cDNA and gDNA clones had previously been shown to be homologous to sequences on wheat group 1 chromosomes. The homoeologous arm locations in wheat had been ascertained by hybridization to the appropriate Chinese Spring ditelosomic lines (cDNAs: S. Chao, P. J. Sharp and M. D. Gale, unpublished results; gDNAs: R. L. Harcourt and M. D. Gale, unpublished results). The source references for information on known-function clones are given in Table 1.

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Table 1. DNA clones

cDNA			gDNA		
Clone	Location	Copy number[a]	Clone	Location	Copy number
PSR158	1L	1	PSR325	1L	1
PSR159	1L	1	PSR330	1L	1
PSR161	1S	1	PSR391	1L	1
PSR161	1L	1	PSR393	1S	1
PSR168	1S	1			

Known function clones:

Locus[b]	Clone	Function	Location	Copy number	Sources
XSec-3 (XGlu-R1)	pTag1290[c]	High-molecular-weight (HMW) glutenin	*1L*	2	R.D. Thompson
XSec-1a (XGli-R1-1)	pTag1436[d]	γ-gliadin	*1S*	3	R.D. Thompson
XAdh	p3NTR[e]	Untranslated region of *Adh1A*	*1L*[e]	1	E.S. Dennis
XNor-R1	pTA71[f]	Ribosomal DNA	*1RS*[l]	multiple	R.B. Flavell
XEm-1R	p1015[g]	Early-methionine-labelled polypeptide	*1L*[m]	3	A.C. Cuming
XPgk-1R	P7[h]	Chloroplast phosphoglycerate kinase	*1L*[n]	1	T.A. Dyer
XPpdk1-1R	PPDK4[i]	Pyruvate orthophosphate dikinase	*1L*[n]	1	P. Westhoff
XLec-R1	PNVRI[j]	Lectin	*1L*	4	T.A. Wilkins
XGlu-R3	pTag544[k]	Low-molecular-weight (LMW) glutenin	*1S*	1	R.D. Thompson

[a] Gene copy numbers were adjudged as the minimum number of hybridization fragments observed in the several restriction digests. [b] Molecular loci designations follow the guidelines for wheat (Hart and Gale 1988). [c] Thompson et al. (1983). [d] Bartels et al. (1986). [e] Mitchell et al. 1989. [f] Gerlach and Bedbrook (1979). [g] Williamson et al. (1985). [h] Longstaff et al. (1989). [i] Matsuoka et al. (1988). [j] Raikhel and Wilkins (1987). [k] Bartels and Thompson (1983). [l] Miller et al. (1980). [m] Futers et al. (1990). [n] Chao et al. (1989b)

RFLP analysis

DNA isolation, enzyme digestion, electrophoresis, Southern blotting, probe labelling and hybridization were as described by Sharp et al. (1988), except that Hybond-N plus (Amersham) filters were used. The restriction enzymes employed for most digestions were *Dra*I, *Xba*I, *Bgl*II, *Eco*RV and *Eco*RI (BRL). *Taq*I was used to detect variation at the *XNor-R1* locus using pTA71.

Protein and isozyme analyses

Storage proteins were analysed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), as described by Payne et al. (1980, 1982), under both nonreducing and reducing conditions. Glucose phosphate isomerase was analysed as described by Chojecki and Gale (1982).

Linkage analysis

The F_2 data were analysed using the program MAPMAKER (Version 2.0) supplied by E. S. Lander, Whitehead Institute of Biomedical Research, Cambridge/MA (Lander et al. 1987). The Kosambi transformation (Kosambi 1944) was used to convert recombination frequencies to centimorgans (cM).

Results

Polymorphism between parent lines DS2 and RxL

Of the 18 probes screened for RFLP between DS2 and RxL, 13 detected differences with at least one of the five restriction enzyme digests tested. The remaining probes, PSR159, PSR168, P7, PSR391 and pTag544 were monomorphic and were not investigated further.

Of the five restriction enzymes employed, *Dra*I was the least effective, exposing RFLP in only half the comparisons using low-copy probes. The other enzymes, all of which gave rise to larger hybridizing bands than *Dra*I on average (mean 6.2 kb), exposed variation in about three-quarters of the 16 comparisons.

The parents were also assayed for differences in storage proteins by SDS-PAGE, since two of the known-function probes showing polymorphism are derived from storage proteins in wheat: pTag1290 from a HMW subunit of glutenin encoded by *Glu-1*, and pTAG1436 from a γ-gliadin encoded by *Gli-1* (Table 1). Both nonreducing and reducing conditions were applied. Under non-reducing conditions, only proteins that do not form disulphide-bonded aggregates appear on the gel, including secalins encoded by *Sec-1* showing sequence homology with wheat γ-gliadins. Under reducing conditions, proteins that do form such aggregates also appear on the gel, including HMW secalins encoded by *Sec-3* showing sequence homology with wheat HMW glutenin subunits. Under both conditions, many components appeared on the gels and there were many differences between the parents (Fig. 1 A, B). Notably, the HMW secalins (*Sec-3*) present under reducing conditions showed a clear polymorphism between the parents. Some of the differences elsewhere in the gels would be expected to be the products of alleles at *Sec-1*.

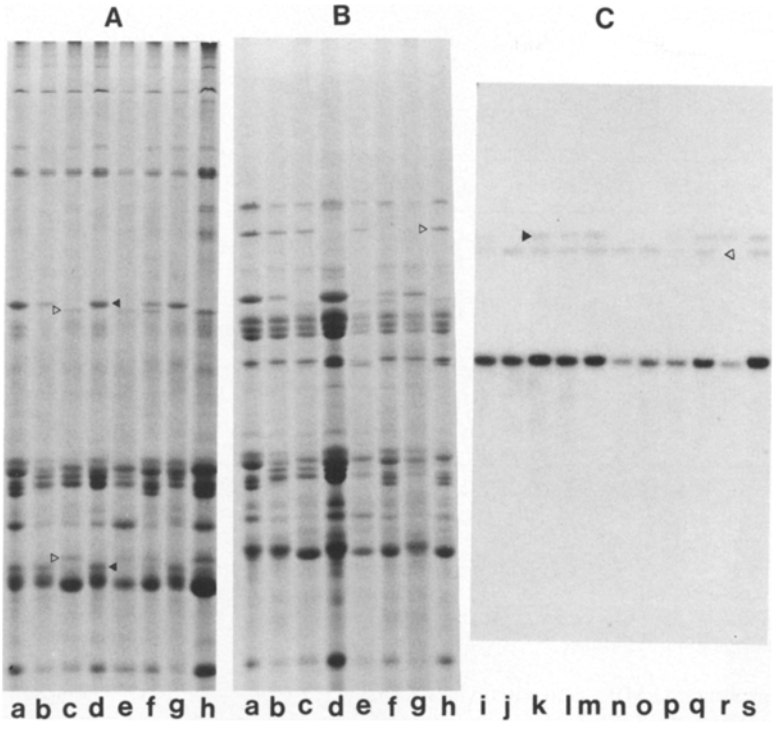

Fig. 1 A–C. Unreduced SDS-PAGE (**A**) and reduced SDS-PAGE (**B**) of total protein extracts. *a-f* are progenies of parents DS2 (*g*) and RxL (*h*). In **A**, closed triangles mark two of the proteins segregating as a block, presumably encoded by *Sec-1*, from parent DS2, and *open triangles* mark the corresponding proteins from parent RxL. In *B*, the *open triangle* marks a HMW secalin encoded by *Sec-3* from parent R*x*L, for which parent DS2 is null. **C** shows hybridization of pTag1290 to *Eco*RV-restricted genomic DNA from rye progenies. *j*, *n* and *o* show the RxL phenotype (*open triangle*), while *r* shows the DS2 phenotype (*closed triangle*); the remainder are heterozygotes

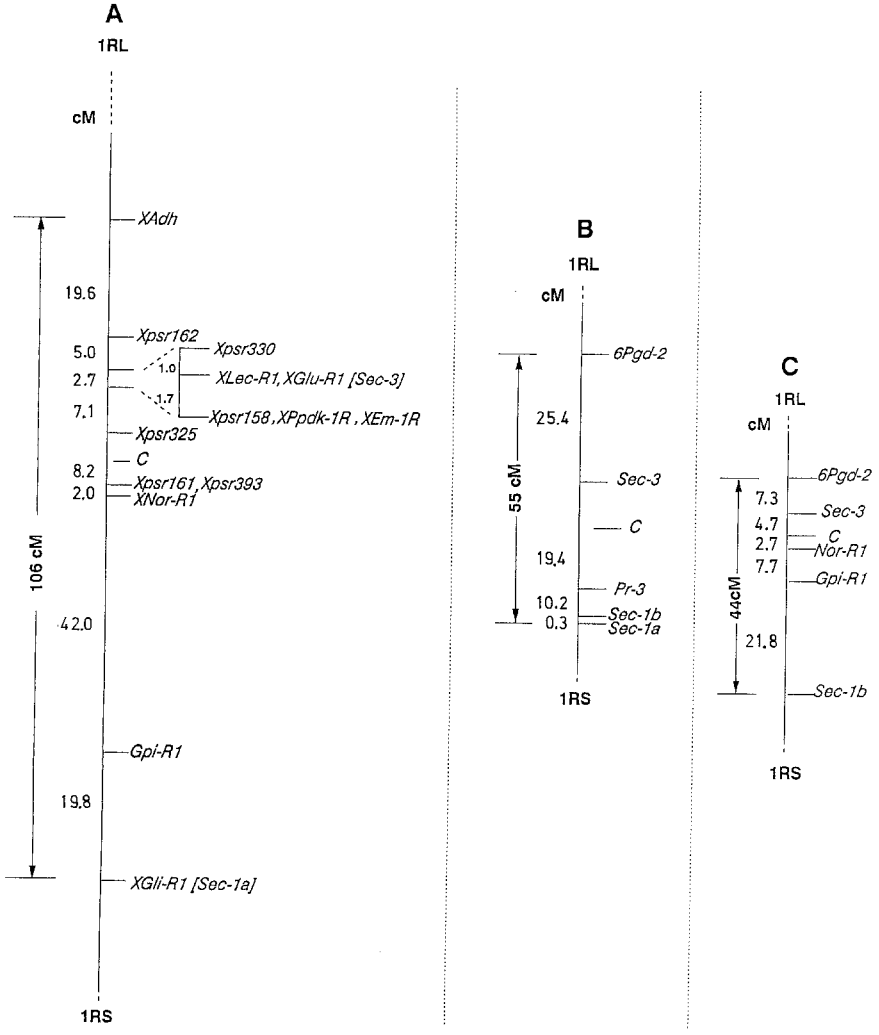

Fig. 2 A–C. Maps of chromosome *1R*. The centromere (*C*) locations are not precise, being placed only between flanking proximal markers on each arm. **A** This paper. **B** From Benito et al. (1990). **C** From Lawrence and Appels (1986)

Finally, the parents were assayed for glucose phosphate isomerase isozymes by isoelectric focusing, and a clear difference between the parents was found for *Gpi-R1*.

Analysis of F_2 progenies

The 60 F_2 DS2 × RxL progenies were analysed for RFLP with 13 probes, for storage protein variation and for isozyme variation at *Gpi-R1*. The analysis of HMW secalins encoded by *Sec-3* using SDS-PAGE under reducing conditions showed precise agreement with the RFLP segregation observed with pTAG1290 (Fig. 1 C). Under nonreducing conditions, several proteins segregated as a block in the F_2 (two of the most prominent in each parent are labelled in Fig. 1 A) and also showed precise agreement with the segregation observed for pTAG1436.

All 16 loci gave Mendelian segregation ratios. The best fit map is shown in Fig. 2 A. Three of these loci could be ordered with certainty (LOD difference between the two best solutions >3, $P<0.001$), using three-point mapping. These were *Xpsr162, Xpsr325* and *Xpsr161*. The order of the remaining loci could only be determined using a multipoint algorithm, leading to a number of alternative solutions separated by LOD differences ≈ 1.5, $P \approx 0.03$, of which the best is presented.

Discussion

The positions of all the loci shown on the map are consistent with the arm locations established in wheat by ditelosomic analysis. The precise location of the centromere was not determined. However, it may be confidently placed between *Xpsr325* and the point 8.2 cM away on the short arm, identified by *Xpsr393* and *Xpsr161*. Although close synteny between the A, B and D genomes of wheat and the R genome is expected, confirmation must await the transfer of the map to wheat.

An obvious feature of the *1R* map is the apparent clustering of loci around the centromere, where two-thirds of the points lie within a region corresponding to just 24% of the overall map length. This is a more extreme demonstration of a similar phenomenon noted by Chao et al. (1989a) in their maps of wheat homoeologous group 7 chromosomes. Although the map distance between the nucleolar organiser region and the centromere is less than 10.2 cM, C-banding shows that the physical distance is some two-thirds of *1R*S (Sybenga 1983). The 25-cM region around the centromere contains both randomly cloned genomic and cDNA sequences, which leads us to suspect that the clustering is a reflection of infrequent recombination in this region relative to the more distal chromosome region, rather than to nonrandom location of coding sequences.

The comparison with other available published maps is reasonably good in the centromeric region and would indicate that the *6Pgd-2* locus lies proximal to *XAdh* on the long arm. The reason for the obvious difference in the *Nor-R1 – Gpi-R1* region, where our map gives a much larger distance than either previous maps in rye (Fig. 2 B, C), or on *1D* in wheat (Chojecki et al. 1983) is unclear. There is no indication of any chromosomal rearrangement in *1R*S and the *Nor-R1 – Gpi-R1* linkage is significant at 42.0 cM (34.3% recombination).

As noted earlier, the SDS-PAGE storage protein analysis for HMW secalins, where the y-type secalin segregates in the F_2 population (Fig. 1 B), coincides precisely with the RFLP segregation observed with pTAG1290. Thus, the data provide no information of the order, relative to the centromere, of the two component genes of *Glu-R1* (*Sec-3*) encoding the x- and y-type secalins.

Maps of the homoeoloci in wheat and barley are being constructed and, as new probes become available, the rye map will be extended. It is clear that more probes are needed in the distal region of *1R*S so that recombination between *1B*S and *1R*S can be precisely manipulated. As noted above, this is necessary to test whether a chromosome can be constructed which maintains the beneficial effects, but which lacks the detrimental effects on quality, of the *1BL.1R*S translocations presently available to breeders.

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