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Comparative RFLP-based genetic maps of barley chromosome 5 (1H) and rye chromosome 1R

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Summary. A genetic map of barley chromosome 5 (1H) was constructed using DNA markers. Seventeen loci were mapped to 15 locations, and these included the known-function loci (in order from the most distal on the long arm) *XAdh* (alcohol dehydrogenase), *XLec* (homologous to wheat germ agglutinin), *XHor3* (D-hordein), *XPpdk* (pyruvate orthophosphate dikinase), centromere, *XIcal* (chymotrypsin inhibitor), and 6 loci in the B- and C-hordein cluster towards the end of the short arm. The gene order on the barley map agreed closely with that of chromosome 1 of rye. Intervarietal comparisons showed that single-copy cDNA and genomic DNA probes revealed about twice the level of RFLPs found in wheat.

Key words: Barley – Genetic mapping – RFLP – Storage proteins

Introduction

Barley (*Hordeum vulgare*) chromosome 5, also known as chromosome 1H, carries a number of genes of agricultural importance such as disease resistance and quality. The homoeologous chromosomes, 1A, 1B, 1D of wheat (*Triticum aestivum*) and 1R of rye (*Secale cereale*) carry similar, and probably homoeologous, genes. These chromosomes carry many of the structural genes for seed storage proteins. In barley, chromosome 5 carries loci for the D-hordeins (*Hor3*) on the long arm and a cluster of loci for B- and C-hordeins (*Hor2, Hor1*) and probably others on the short arm (Shewry et al. 1990). Other genes located on chromosome 5 include high lysine (*Lys4*), chymotrypsin inhibitor (*Ica1*) and genes conferring resistance to powdery mildew, *Erysiphe graminis* hordei (*Ml-a, Ml-at, Ml-d, Ml-nn* and *Ml-p*), stripe rust, *Puccinia striiformis* West (*Rps4*) and leaf rust, *P. hordei* Otth (*Rph4*) (see von Wettstein-Knowles 1990 for review).

Chymotrypsin inhibitors are of interest because they may protect the seed from fungal or bacterial attack (Ryan 1981) and because of their association with high lysine mutants. The hordeins are of obvious relevance to feed and malting quality. Also, because of the relatively close genetic linkages found between the *Hor1* and *Hor2* loci and the *Ml-a* locus (Jensen et al. 1980; Doll and Jensen 1986), this region of the short arm is being marked as a target for chromosome-walking to isolate *Ml-a*, which has many allelic forms and is one of the most important sites of mildew resistance in the barley genome. The mapping exercise described below was carried out not only to locate genes of known-function on the map of chromosome 5, but also to study the intergenomic relationship between barley and rye in terms of gene order and genetic map distance.

Materials and methods

Genotypes

The barley map was made using 120 F_2 plants from a cross *H. vulgare* cv 'Captain' × a *H. spontaneum* accession (IPSR no. 2370). In addition, the following varieties were also screened to assess the probes' potential to detect RFLP: 'Betzes', 'Golden Promise', 'Goldmarker', 'Triumph', 'FrankenIII', 'Sultan', 'Igri' and 'Magnum', obtained from the IPSR barley collection, and 1506C and E1388, old German breeding lines obtained from J. W. Snape, Cambridge Laboratory.

DNA probes

A total of 29 clones comprising 5 anonymous cDNAs from the library described by Chao et al. (1989), 12 anonymous *PstI* genomic clones from libraries prepared by R.L. Harcourt and

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Table 1. DNA clones

Anonymous clones

cDNA			gDNA			gDNA		
Clone	Location	Copy number[a]	Clone	Location	Copy number	Clone	Location	Copy number
PSR158	1L	1	PSR381	1S	1	PSR385	1L	1
PSR159	1L	1	PSR330	1L	1	PSR586	1L	1
PSR161	1S	1	PSR391	1L	1	PSR596	1S	1
PSR162	1L	1	PSR393	1S	1	PSR626	1L	3
PSR168	1S	1	PSR343	1L	1	PSR688	1S	3
			PSR361	–[b]	–	PSR653	1L	1

Storage protein clones

Locus[c]	Clone	Function	Location	Copy number	Sources
XHor3	pTag1290[d]	Wheat HMW glutenin	1L	2	R.D. Thompson
XHor	pTag1436[e]	Wheat γ-gliadin	1S	3	R.D. Thompson
XHor	pTag544[f]	Wheat LMW glutenin	1S	3	R.D. Thompson
XHor	pB7	Barley BIII hordein	1HS[m]	8	B.G. Forde
XHor	pB11	Barley BI/II hordein	1HS[m]	4	B.G. Forde
XHor1	pcP387	C-Hordein	1HS[m]	6	B.G. Forde

Other known function clones

XAdh	p3NTR[g]	Wheat untranslated region of Adh1A	1L[g]	1	E.S. Dennis
XIca1	pcI-1-4[h]	Barley chymotrypsin inhibitor	1HS[n]	3	P.R. Shewry
XEm	p1015[i]	Wheat early-methionine labelled polypeptide	1L[o]	3	A.C. Cuming
XPgk1	P7[j]	Wheat chloroplast phosphoglycerate kinase	1L[p]	1	T.A. Dyer
XPpdk	PPDK4[k]	Maize pyruvate orthophosphate dikinase	1L[p]	1	P. Westhoff
XLec	PNVRI[l]	Wheat germ agglutinin	1L	4	T.A. Wilkins

[a] Gene copy numbers were adjudged as the minimum number of hybridization fragments observed in several restriction digests
[b] The probe did not hybridize to barley
[c] Molecular loci designations follow the guidelines for wheat (Hart and Gale 1988)
[d] Thompson et al. (1983)
[e] Bartels et al. (1986)
[f] Bartels and Thompson (1983)
[g] Mitchell et al. (1989)
[h] Williamson et al. (1988)
[i] Williamson et al. (1985)
[j] Longstaff et al. (1989)
[k] Matsuoka et al. (1988)
[l] Raikhel and Wilkins (1987)
[m] Forde et al. (1985)
[n] This paper
[o] Futers et al. (1990)
[p] Chao et al. (1989)

K. M. Devos, Cambridge Laboratory and 12 known-function clones were used (Table 1). All the anonymous clones are from wheat but most hybridized adequately to barley DNA. Of the known-function clones, pcI-1-4, pB7, pB11 and pcP387 were from barley, PPDK4 was from maize and the remainder were from wheat. The homoeologous arm locations on wheat group 1 chromosomes had been previously ascertained by hybridization to the appropriate wheat cv 'Chinese Spring' nullisomic-tetrasomic and ditelosomic lines (S. Chao, P. J. Sharp, C. N. Chinoy, K. M. Devos, R. L. Harcourt and M. D. Gale, unpublished for all probe locations unreferenced in Table 1).

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^+ membranes (Amersham) were used. RFLP was identified by screening 4 of the 'Captain' × *H. spontaneum* F_2 lines with the restriction enzymes *DraI*, *Eco*RI, *Eco*RV, *Bgl*II, *Xba*I, *Sst*I, *Bam*HI and *Hin*dIII, all of which have a 6-base pair recognition site. The other 10 varieties were screened with *Eco*RI, *Eco*RV and *Hin*dIII digests.

Linkage analysis

The F_2 data were analysed using the programme MAPMAKER (Version 2.0) supplied by E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142. The Kosambi transformation (Kosambi 1944) was used to convert recombination frequencies to centimorgans (cM).

Results

Of the 25 non-barley DNA clones only 1, the wheat genomic clone PSR361, gave no detectable signal with barley genomic DNA. Most of the remaining 11 genomic clones gave poorer signals in barley than in wheat, but with adequate exposure times all could be assayed. Among the cDNAs, PPDK4 from maize gave the poorest signal in barley (see Fig. 1). All of the wheat multicopy probes gave the same copy numbers in the barley genome as in the A, B and D genomes of wheat, except PSR626 where the number of hybridizing bands was three in barley and five per genome in wheat.

RFLP between genotypes

In the mapping population RFLP was detected for 5 of the 17 single-copy probes and for all but 1 (p1015) of the multicopy probes. Over the 12 varieties, scored with just three restriction enzymes, only 4 probes (PSR159, PSR343, PSR393 and PSR653) detected no RFLP. Of the eight restriction enzymes used with the mapping population *Sst*I was most effective and *Hin*dIII the least effective in the detection of RFLP. Of the three enzymes used with the larger sample of genotypes, *Eco*RI was most effective in revealing RFLP. *Eco*RI gave a mean heterozygosity index, H, of 20% for single-copy probes, while *Eco*RV and *Hin*dIII gave H values of 17% and 11%, respectively.

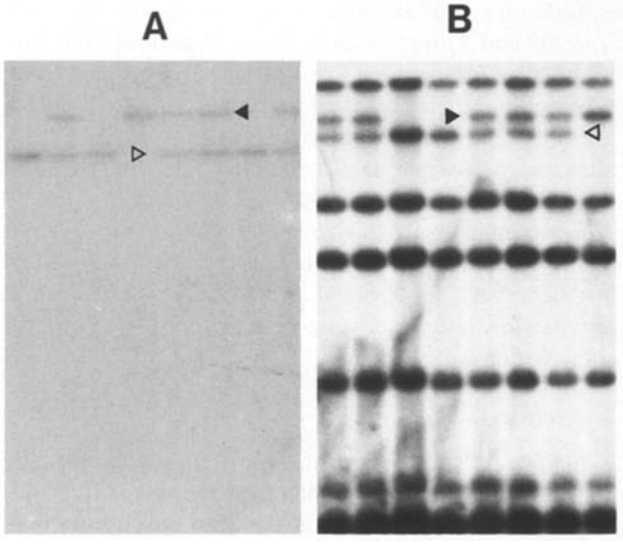

Fig. 1 A, B. Hybridization patterns obtained with eight F_2 plants of *H. vulgare* cv 'Captain' × *H. spontaneum*. ◂ *H. spontaneum* genotype; ▷ 'Captain' genotype. **A** A single copy number probe (PPDK4, cDNA) hybridized to F_2 progeny genomic DNA digested with *Dra*I; **B** A multiple copy number probe (pcP387, C-hordein) hybridized to F_2 genomic DNA digested with *Eco*RV

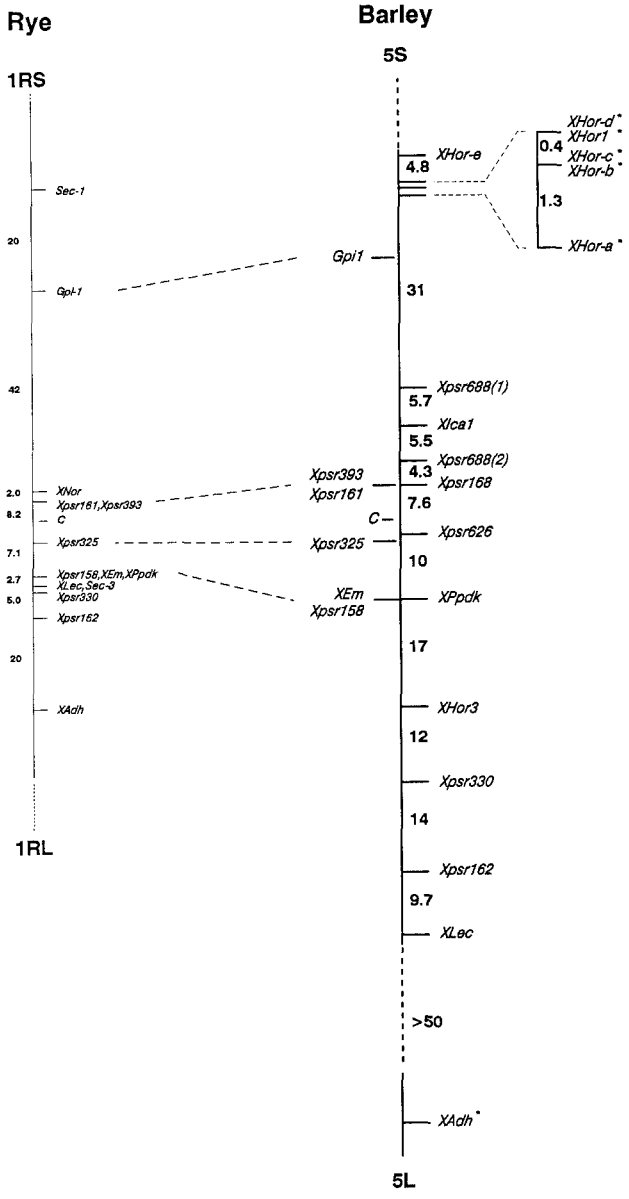

Fig. 2. A RFLP-based map of barley chromosome 5 and rye chromosome 1R. The distances between points are in cM, the loci marked on the RHS of the barley map were mapped in barley. The loci on the LHS are extrapolated locations from the chromosome 1R map, shown on a smaller scale to the LHS of the figure. For explanation see text

Analysis of F_2 progenies

The best fit map is shown in Fig. 2. All 17 loci segregated in a Mendelian manner, and most were completely classified as 1:2:1 segregations. However, a few of the multicopy probes revealed allelic hybridizing fragments that could not be scored readily because of interference from co-migrating bands, and these were scored as 3:1 segregations. Eleven points could be ordered with certainty, having a LOD difference between the two best orders greater than 3 ($P<0.001$). The remainder (marked with * in Fig. 2) could be ordered only by use of the multi-point algorithm, leading to a number of alternative orders separated by LOD differences as low as 1.5 ($P\sim0.03$), of which the best is presented. Our confidence in the map locations of all the points was enhanced by their agreement with the chromosome arm locations previously obtained in wheat and by the high degree of concurrence of locus order with a map previously made in rye (see below).

The linkage between *XAdh* and *XLec*, in the distal region of chromosome 5S was weak at 66 cM, LOD 0.3. Confidence in the chromosomal location of *XAdh* derives from comparison with the rye 1R and wheat group 1 locations. Two multicopy probes, PSR688 and pTag544, gave multiple allelic segregations at linked, but not identical locations. PSR688, with three copies, provided two locations flanking *XIca1*. The wheat low-molecular-weight glutenin probe, with three copies, mapped to two locations (identified here as *XHor-d* and *XHor-e*) 4.8 cM apart.

Discussion

The barley chromosome 5 (1H) map

With 29 DNA probes we have been able to map 15 points on barley chromosome 5. Excluding the multiple-copy clones, only five, i.e. about one-third of the probes, are polymorphic in this rather wide cross. Plainly, analysis of several, possibly many more, populations is required if all or most of the available molecular and biochemical markers are to be placed on the map. Since many of the RFLPs observed were detected with several restriction enzyme digests they were probably deletion-insertion events, and it is doubtful whether analysis with more enzymes than the eight used here would have allowed the mapping of many more points.

The 15 mapped points leave two large recombinational intervals, between *XAdh* and *XLec* (>50 cM) and between Xpsr688(1) and the hordein complex on the short arm (~31 cM). The glucose phosphate isomerase (*Gpi1*) locus can, however, be confidently expected to bridge the latter 'gap' by extrapolation from the rye map. Nevertheless, to reduce even the present span to a 10-cM maximum interval map it is probable that as many markers again will be needed.

Correspondence with the genetic map of 1R

Of the markers investigated in the two genomes the only major difference involves the nucleolar organizer region (*Nor*), which is present on the short arm of 1R but which is located on barley chromosomes 7 (5H) and 6 (6H) (Gerlach and Bedbrook 1979). Otherwise, with one exception, the genetic map of barley chromosome 5 (1H) corresponds closely to that of 1R (Wang et al. 1991) with respect to gene order (Fig. 2). The exception concerns the location of *XLec*, which in rye lies very close to *Sec-3* (equivalent to *Hor3*), but which maps in a more distal location in barley. This may be the result of different members of the four-copy lectin multigene family showing RFLP in the two mapping populations, and, therefore, is not necessarily a reflection of non-colinearity between the maps.

The correspondence in gene order between the two maps justifies the placing of a further 6 loci on the map of barley chromosome 5 by extrapolation. *Xpsr158* and *XEm* may be placed close to *XPpdk*, although because *XEm* has three copies it should be noted that they may not all map at the one point. *Xpsr325* may be placed close to *Xpsr626*. *Xpsr161* and *Xpsr393* may be placed close to *Xpsr168*. Glucose phosphate isomerase, *Gpi-1*, may be placed with less precision in the interval between *Xpsr688* and the hordein complex on the short arm.

Although the order of loci in the rye and barley maps corresponds well, the distances between markers vary considerably. For example, in rye the distance between *Xpsr162* and *Xpsr161* is only about 23 cM, while in barley the distance between *Xpsr162* and *Xpsr168*, which by extrapolation is expected to map very close to *Xpsr161*, is more than twice the rye distance at 60.6 cM. The overall distance between the distal points, *XAdh* and the storage protein loci, *Sec-1* in rye and its equivalent *Hor1/Hor2* complex locus, is also longer with at least 172 cM in barley compared to 110 cM in rye. This may reflect both a higher overall rate of recombination in barley and less localization of crossing-over in the distal chromosome regions.

Known-function loci

XAdh and XPpdk. Both of these loci are found on single restriction fragments, and their locations may be considered fixed. More markers on the long arm are needed, however, to provide a more precise estimate of the recombinational distance of *XAdh* from the proximal marker loci *XLec* and *XEm*. The barley lectin (wheat germ agglutinin) genes are present in four copies on barley chromosome 5, all on the long arm but possibly not closely clustered, as discussed above. Confirmation that

the four copies are dispersed will devalue the use of PNVRI as an RFLP probe.

A similar situation may be found with *XEm*, identified with p1015, which is present in three copies on 1HL.

XIcal. The location of *XIcal* (chymotrypsin inhibitor 1) on the short arm of 1H contradicts the findings of Hejgaard et al. (1984a) who inferred a long arm location from the absence of the inhibitor protein in a supposed 2AS.1HS wheat/barley translocation. It is possible, however, that the translocation is not centromeric and that it carries only the distal part of 1HS, as suggested by Koebner (1990). The short arm location is consistent with Koebner's (1990) 1S location of the genes controlling subtilisin inhibitors in wheat and rye, and the immunological relationship between *Ica1* protein and rye *Si-R2* protein (Hejgaard et al. 1984a, b) suggests that they are homoeoallelic.

The hordein complex on 1HS

As with the results obtained with other multicopy probes, the B- and C-hordein region of the map is unlikely to transfer precisely to other mapping populations because RFLPs have been mapped at only some of the approximately 20 hordein gene copies. In addition, because of the extensive cross-hybridization between the 4 clones that detect B-hordein-like gene sequences and the lack of corroborative evidence from storage protein analysis, the RFLP loci have been identified simply as *XHor*. The locus *XHor-a* was identified with the pB11 (BI-hordein) probe, *XHor-b* with pTag1436 (wheat γ-gliadin), *XHor-c* with pB7 (BIII-hordein) and *XHor-d* and *-e* with pTag544 (wheat low-molecular-weight glutenin). Barley cDNA clone pcP387 (C-hordein, *XHor1*) is a multiple copy probe (see Fig. 1). The B-hordein-like sequences span this single mapped C-hordein sequence, *XHor1* (see Fig. 2), and show some similarity to the map made by protein analysis reported by Shewry et al. (1990).

The difficulties posed by the use of multicopy probes for the extrapolation of maps to other genotypes also extend to the use of the map for long-range mapping leading to the identification and eventual isolation of the *Ml-a* locus, which has been reported to lie between the B- and C-hordein genes (Jensen et al. 1980; Doll and Jensen 1986; Shewry et al. 1980, 1988).

RFLP levels

Over the 12 varieties tested, the mean level of variation varied from H = 0% to 48% for single-copy probes. The cDNAs gave a higher mean level, 20%, than the genomic clones, 13%, although the variability between individual probes probably renders this difference non-significant. These values are different from those obtained in wheat where cDNAs, at about 10%, give lower values than gDNAs at about 19% (Devos et al. 1992). Whether this difference is meaningful should await results of equivalent numbers of barley clones used in wheat. It is possible that the higher levels of RFLP seen with gDNAs in wheat derive from their use as probes of wheat sequences that are not conserved over genomes. Other studies in barley show similar levels of RFLP (Graner et al. 1990; Liao and Niks 1991). Although these levels of RFLP are low they have been assessed in quite diverse varieties, thus even lower levels may be expected in the more closely related germplasm used in commercial breeding programmes. Plainly, more probes like PSR168 (H = 48%) are needed.

Apart from the detailed information obtained concerning the locations of a number of known-function and anonymous DNA sequences on barley chromosome 5, a few generalizations may also be made. Firstly, the close homoeology between Triticeae genomes may be employed to extrapolate between genomes in much the same way as we would extrapolate between different mapping populations within a species to provide approximate gene location and gene orders. Thus, the information gained from the various ongoing initiatives to map wheat, barley and rye will provide information that can be pooled, providing an adequate number of common 'landmark' loci are used. Secondly, the results obtained with multicopy clones, at least for those which have all copies located on a single chromosome arm, may provide ambiguous locations that may not be transferrable between genotypes. Thirdly, cDNA probes are likely to provide better markers for use across species as adjudged by the finding that the wheat genomic clones mostly gave reduced hybridization signals in barley.

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