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Production of wheat-barley recombinant chromosomes through induced homoeologous pairing

1. Isolation of recombinants involving barley arms 3HL and 6HL

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Summary. Sears' *phlb* mutant was used successfully for the first time to induce pairing and recombination between specific barley chromosomes and their wheat homoeologues. Pairing was induced in specially constructed genetic stocks having 19 pairs of wheat chromosomes and triply monosomic for either barley chromosome arm 6HL or 3HL, a related wheat chromosome, and chromosome 5B of wheat carrying the *phlb* mutation. Wheat-barley recombinant chromosomes were isolated from among the progeny obtained from self-fertilization of the triple monosomic stocks, by screening for dissociation of biochemical markers on the barley arms. Glutamic oxaloacetic transaminase (GOT), aconitase hydratase (ACO), and dipeptidase (DIP) isozymes were used to select recombinants involving the 6HL arm, and esterase (EST) and malate dehydrogenase (MDH) were used for the 3HL arm. Altogether, six recombinants involving 6HL (1.4%) and six involving 3HL (1.1%) were isolated. These wheat-barley recombinant chromosomes are being used to construct a detailed gene order map of barley based on biochemical and molecular markers.

Key words: Isozyme markers – Homoeologous pairing – *phlb* mutant – Wheat-barley recombination

Introduction

The isolation of six of the seven possible addition lines having individual pairs of barley chromosomes added to wheat (Islam et al. 1981) has made it possible to carry out further manipulations of barley chromosomes in a wheat background. Besides providing an opportunity to produce substitution lines using conventional cytogenetic procedures (Islam and Shepherd 1981), a scheme has been outlined showing that, provided the barley chromosomes could be induced to pair with related wheat chromosomes, it should be possible to isolate wheat-barley recombinant chromosomes (Shepherd and Islam 1987). Such lines would be the vehicles for transferring barley characters into wheat and, furthermore, since these lines would have different lengths of barley chromatin in a wheat background, they could be used to establish gene order along the barley chromosome, when mapping genes controlling barley isozymes and restriction fragment length polymorphisms (RFLPs).

By utilizing cytologically distinguishable barley telocentric chromosomes, we have now obtained direct evidence that barley chromosomes can be induced to pair with wheat chromosomes (Islam and Shepherd 1988), provided that the inhibiting effect of the *Phl* gene located on wheat chromosome arm 5BL is either suppressed by adding the *Triticum speltoides* genome (Riley et al. 1968) or removed by using the *phlb* mutant (Sears 1977). The level of pairing observed between the barley telocentrics and wheat chromosomes was higher in the presence of the *T. speltoides* genome (1.2–4.5%) than when *phlb* was used (0.3–0.7%), but these results are not directly comparable, because there were four homoeologous univalent chromosomes available as potential pairing partners in the hybrids with *T. speltoides*, but only one univalent with the *phlb* studies.

The observed pairing between wheat and barley chromosomes was expected to generate wheat-barley recombinant chromosomes, and the purpose of the present study was to detect and isolate such recombinants involving barley telocentrics 6HL and 3HL. The methods used were similar to those proposed earlier (Shepherd and Islam 1987; Islam and Shepherd 1988), and in outline consisted first in producing triple monosomic plants containing 19 pairs of wheat chromosomes, a single dose of

the relevant barley telocentric, a related wheat chromosome, and chromosome 5B of wheat carrying the *phlb* mutant gene. The progeny from these triple monosomics were then screened for two (or three) isozyme marker genes known to be located on the barley telocentrics, to search for possible recombinants showing dissociation of the barley markers. The *Est-1,2,4* (nomenclature of-Brown and Munday 1982) (abbr. *Est-1*) and *Mdh-H2* loci were used as selective markers for the 3HL arm, and *Got-H2*, *Aco-H1*, and *Dip-H1* markers were used for the 6HL arm.

The results of these isozyme screenings are presented in this paper, together with a brief discussion of the value of the recombinant lines in breeding and genetics.

Materials and methods

Plant materials

The starting materials included: (a) ditelosomic wheat-barley substitution lines 6HL(6A) and 3HL(3A) in cv Chinese Spring (CS) wheat background (A.K.M.R. Islam and K.W. Shepherd, unpublished results); (b) CS monosomic 5B and double monosomics involving wheat chromosome 5B in combination with 3A, 6A, or 6D as the other univalent, all produced by Dr. E. R. Sears; (c) the *phlb* mutant gene in CS (Sears 1977).

Production of triple monosomic stocks. Triple monosomic stocks with constitution 19″+t′6HL+5B′*phlb*+6A′, 19″+t′3HL+5B′*phlb*+3A′, and 19″+t′6HS+5B′*phlb*+6A′(or 6B′ or 6D′) were produced by the methods described earlier (Shepherd and Islam 1987). The 3HL and 6HS stocks had been produced earlier and used in chromosome pairing studies (Islam and Shepherd 1988), whereas the 6HL stocks were new to this study. The putative 6HL triple monosomic stocks were selected on the basis of root-tip chromosome counts, and were grown in the glasshouse and allowed to self-fertilize for maximum seed production. At meiosis their chromosome constitution and pairing behavior was examined in pollen mother cells (PMCs) to confirm that they had been correctly chosen.

A homozygous *phlb phlb* plant (19″+t′3HL+5B″*phlb*+3A′) was selected in the progeny obtained by, backcrossing a triple monosomic hemizygous for *phlb*, obtained earlier, with the *phlb* mutant.

Electrophoresis

Seeds obtained from self-fertilization of the triple monosomic plants were germinated on filter papers in germination trays placed in an incubator maintained at 20 °C. Extracts from 7- to 10-day-old green leaf tissue were electrophoresed in polyacrylamide slabs to determine GOT-2 and EST-1 zymogram phenotypes, using procedures similar to that described by Hart et al. (1980) for GOT and EST isozymes with disc electrophoresis. Assays for ACO-1, DIP-1, and MDH-2 phenotypes were made on extracts from 7- to 10-day-old green leaf tissue electrophoresed in horizontal starch gels, following the methods of Brown (1983).

Cytology

Root tips and anthers for meiotic studies were fixed in a 3:1 mixture of ethanol and glacial acetic acid. Standard Feulgen staining techniques were used for all cytological preparations. The presence of two doses of wheat 5B chromosome in the plant supposedly homozygous for *phlb* has been confirmed with N-banding of chromosomes following the procedure of Islam (1980).

Markers used for selection of recombinants

Biochemical markers specific to the barley chromosomes were used for the isolation of wheat-barley recombinants. Since barley telocentrics instead of entire barley chromosomes were present in the triple monosomic plants, any dissociation of barley marker genes in the progeny of these plants was taken as evidence of recombination involving that arm. Progeny plants showing dissociation of *Got-H2*, *Aco-H1*, and *Dip-H1* were selected as putative recombinants involving barley arm 6HL. Similarly, recombinants involving the 3HL arm were selected from observed dissociation of markers *Est-1* and *Mdh-H2* known to be located on that arm.

The putative recombinant plants were planted in the glasshouse for further isozyme testing and seed production for progeny testing. A second and sometimes third round of isozyme analysis was performed on leaf samples from these plants to confirm their classification. The meiotic chromosome pairing behavior in PMCs from these plants was examined and the spikes were pollinated with CS wheat to transfer the recombinant chromosomes to a normal wheat (*Phl*) background.

Results

Induced pairing between wheat and barley chromosomes

In the present study, meiotic pairing data were obtained from triple monosomic plants carrying barley chromosome arm 6HS with wheat univalents 6A and 6D in different plants and barley arm 6HL with wheat 6A (Table 1). Barley chromosome arm 6HS was observed to pair with frequencies of 1/236 (0.4%) and 5/426 (1.1%) in PMCs from triple monosomics involving 6A and 6D, respectively. The level of pairing was similar to that observed earlier between 6HS and wheat 6B and between 3HL and 3A (Table 1). In contrast, barley chromosome arm 6HL showed a much higher level (2.6%) of pairing in the PMCs examined from a triple monosomic carrying wheat 6A as the univalent (Fig. 1).

Table 1. Frequency of pairing of barley telocentries in triple monosomic plants hemizygous for *phlb*

Barley telocentric	Wheat univalent	PMCs at metaphase 1		Frequency of pairing (%)
		No. examined	No. with telocentrics paired	
6HS	6A	236	1	0.4
6HS	6B	130	1	0.7[a]
6HS	6D	426	5	1.1
6HL	6A	154	4	2.6
3HL	3A	682	2	0.3[a]

[a] From Islam and Shepherd (1988)

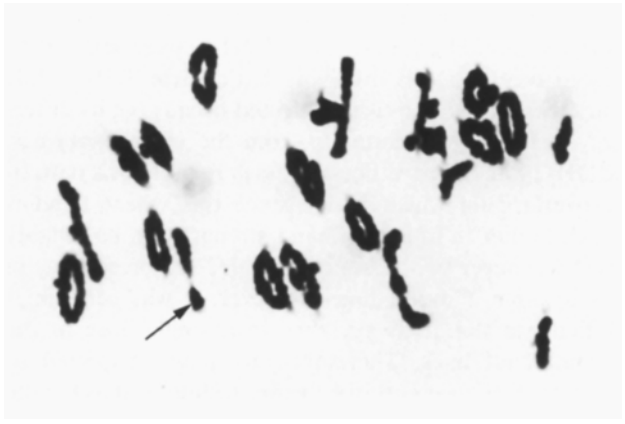

Fig. 1. Pairing between barley telocentric 6HL (*arrowed*) and wheat chromosome 6A in a PMC from triple monosomic stock

Isolation of recombinants

Since the markers used to screen for homoeologous recombination are codominant, it is equally efficient to use F_2 progeny instead of testcross progeny to screen for rare recombination (Mather 1938). Furthermore, it is much simpler to generate large populations of progeny by self-fertilization in wheat compared to controlled testcrosses; hence F_2 progeny were analyzed in the present study. The only recombinant gametes that will escape detection with this procedure are those where the recombinant chromosome is present with the barley telocentric.

Recombinants involving barley arm 6HL. A total of 472 progeny from two 6HL, 6A triple monosomic plants was screened for recombinant chromosomes using GOT-2, ACO-1, and DIP-1 isozyme bands as the selective markers. Barley GOT-2 can be easily detected because of its slower electrophoretic mobility compared to the corresponding group of CS wheat isozymes, and the two hybrid isozymes formed also migrate to positions cathodal to the wheat isozymes. Similarly, barley ACO-1 can be easily detected by its different mobility to the wheat isozymes. Although the barley DIP-1 band partially overlaps that of the most anodal wheat band, its mobility is sufficiently different to allow it to be easily detected in the disomic 6H addition line, due to the extra width of the band. However, in the putative recombinant lines having only one dose of the *Dip-H1* gene, the presence of the DIP-1 band cannot always be reliably scored, because of the reduced band staining intensity due to hemizygosity of this gene and the overall lesser activity of this enzyme in the leaf tissue. Therefore, all plants having phenotypes GOT-2^- ACO-1^- and GOT-2^+ ACO-1^+ were tested for the presence or absence of the DIP-1 band, and any plants suspected to be DIP-1^+ in the former case and DIP-1^- in the latter case were retained for later progeny testing.

Table 2. Barley isozyme phenotypes of progeny from triple monosomics

No. of plants	6HL phenotype		
	GOT-2	ACO-1	DIP-1
Parentals			
155	+	+	+
311	−	−	−
Recombinants			
2	−	+	+
2	+	−	−
1	−	+	−
1	−	−	+
	3HL phenotype		
	EST-1	MDH-2	
Parentals			
145	+	+	
408	−	−	
Recombinants			
1	+	−	
5	−	+	

All plants showing an apparent dissociation of these barley markers were kept as presumptive recombinants and planted in pots in the glasshouse. The results are summarized in Table 2. Because the seedlings had been weakened by the long period in germination trays while assaying for the three enzymes, some of the suspected recombinants died after transferral to pots in the glasshouse. Furthermore, due to the problem in scoring for *Dip-H1* mentioned earlier, some of the progeny with break-points between the *Aco-H1* and *Dip-H1* loci may have escaped detection during screening. Five of the six survivors appear to have break-points in the interval between the *Got-H2* and *Aco-H1* loci and two between *Aco-H1* and *Dip-H1*.

The phenotype of these putative recombinant plants was confirmed in repeated isozyme tests on the growing plants and in progeny tests. Reciprocal types of recombinants appear to be represented among the products of crossing-over between the *Got-H2* and *Aco-H1* loci. Thus, the two plants with phenotype GOT-2^+ ACO-1^-DIP-1^- were observed to possess a telocentric chromosome that frequently pairs with a wheat chromosome at meiosis, and it can be deduced that this telocentric has the centromere and proximal region of barley 6HL and a distal wheat segment that pairs with a homologous wheat chromosome. Therefore, *Got-H2* is proximal to *Aco-H1* and *Dip-H1*.

Two recombinants with phenotype GOT-2^-ACO-1^+ DIP-1^+ were obtained, and five progeny from each were

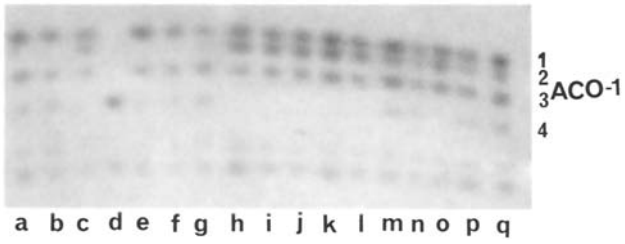

Fig. 2. Aconitase zymogram phenotypes produced by CS wheat, Betzes barley, and some recombinant lines involving barley chromosome arm 6HL. ACO-1 bands 1, 2, 3, and 4 are controlled by wheat chromosomes 6D, 6A, 6B, and barley chromosome 6H, respectively. *a–b*, *e–g* progeny of GOT-2$^-$ ACO-1$^+$ DIP-1$^+$ recombinant no. 1; *c* CS; *d* Betzes; *h–l* progeny of a GOT-2$^+$ ACO-1$^-$ DIP-1$^-$ recombinant; *m–q* progeny of GOT-2$^-$ ACO-1$^+$ DIP-1$^+$ recombinant no. 2

tested for ACO-1. While all of the progeny from one recombinant possessed barley ACO-1 and lacked the ACO-1 band controlled by wheat 6A, four out of the five progeny from the other recombinant possessed both the wheat 6A and barley ACO-1 bands (Fig. 2). The first recombinant line probably lacks a normal wheat 6A chromosome, and thus the recombinant chromosome is frequently transmitted through the pollen, due to the selective advantage for pollen carrying this chromosome over nulli haploid wheat pollen. It also appears that the barley chromosome segment carrying *Aco-H1* in this line replaced the corresponding wheat 6A segment carrying *Aco-A1*. The high transmission rate of *Aco-A1* in the progeny of the other recombinant line could be due to either a recombinant chromosome carrying both *Aco-A1* and *Aco-H1* loci, or simply to the presence of a normal 6A chromosome in the recombinant line. The limited data available do not distinguish between these two possibilities.

One recombinant each was obtained having the phenotype GOT-2$^-$ ACO-1$^-$ DIP-1$^+$ and GOT-2$^-$ ACO-.1$^+$ DIP-1$^-$. Since no recombinants with phenotypes GOT-2$^+$ ACO-1$^+$ DIP-1$^-$ or GOT-2$^+$ DIP-1$^+$ ACO-1$^-$ were recovered, it is not possible with the current information ot establish the gene order of *Aco-H1* and *Dip-H1* on the 6HL arm. Assuming the gene order *Got-H2-Aco-H1-Dip-H1* determined by Brown et al. (1989) from intercrosses between *Hordeum spontaneum* lines and *H. vulgare* × *H. spontaneum* crosses, the recombinant with phenotype GOT-2$^-$ ACO-1$^+$ DIP-1$^-$ would represent the product of a double-crossover between *Got-H2* and *Dip-H1*. However, if *Aco-H1* is distal to *Dip-H1*, then the other recombinant GOT-2$^-$ ACO-1$^-$ DIP-1$^+$ would require a double-crossover.

Since more DIP-1 activity is observed in embryonic tissue than in leaf tissue, embryo halves of dried seeds were used in the DIP-1 assay of progeny, making it much easier to detect the presence or absence of this isozyme.

Recombinants involving barley arm 3HL. Two barley isozyme markers, EST-1 and MDH-2, were selected to screen recombinants involving barley arm 3HL. While barley EST-1 can be easily detected because of its different electrophoretic mobility from the wheat isozymes, MDH-2 was rather difficult to assay because of a partially overlapping wheat band. Since this wheat band is much fainter in intensity, band strength can be reliably used whenever two doses of *Mdh-H2* are present, as in the disomic addition line. However, it was sometimes difficult to detect its presence in a single dose in the recombinant lines. Therefore, any plant suspected to have *Mdh-H2* was kept for further testing, just as for the screening of *Dip-H1* mentioned earlier. Progeny from two different stocks were screened for recombinants: one was a triple monosomic line carrying a single dose each of barley 3HL, wheat 3A, and wheat 5B with a single dose of the *phlb* mutation, and the other stock differed in having two doses of 5B carrying the *phlb* mutation. A total of 222 progeny screened from the triple monosomic plant yielded one presumptive recombinant, whereas five possible recombinants were obtained from 337 progeny of the plant having two doses of *phlb* (Table 2), however this difference is not significant ($P=0.41$). Reciprocal types of recombinants appear to have been recovered again as shown in Table 2.

The recombinant status of these plants was checked in isozyme tests at a later growth stage, and five out of the six recombinants were tested and confirmed in progeny tests. However, the other presumptive recombinant remains to be progeny tested. Surprisingly, none of the recombinants of either reciprocal group (Table 2) possess a telocentric chromosome, and thus the single recombinant *Est-1*$^+$ *Mdh-H2*$^-$ must result from two crossovers with a homoeologous wheat chromosome, one proximal and the other distal to the *Est-1* locus on barley 3HL arm. Thus we conclude that *Est-1* is proximal to *Mdh-H2*.

The recombinant plant with genotype *Est-1*$^+$ *Mdh-H2*$^-$ exhibits $20''+1'$ at meiosis. Since most of the progeny from this plant possess barley 3HL esterases, the recombinant chromosome is more likely to be the univalent, and hence transmitted in a much higher frequency through the pollen, due to the competitive advantage of pollen carrying this chromosome over 20-chromosome nullihaploid pollen.

Discussion

Barley chromsome arm 6HS showed very low pairing (0.4–1.1%) with the wheat chromosomes of homeologous group 6, whereas barley arm 6HL paired with wheat 6A at a much higher frequency (2.6%). The lower pairing observed with the 6HS arm may result from fewer

pairing recognition sites due to its smaller size or, alternatively, this arm may have a different affinity for its wheat homoeologues than 6HL. However, all of these values are likely to be underestimates of the true pairing frequency because of the likely occurrence of desynapsis, as discussed by Islam and Shepherd (1988). It is of interest to note that the level of pairing observed between barley 6HL and wheat 6A is comparable to the 2.5% pairing observed between wheat 6D and *Agropyron elongatum* 6Ag in the equivalent triple monosomic stock (Yasumuro et al. 1981). These authors reported a two fold increase in pairing (4.9%) between these chromosomes in a nulli-5B background. However, comparable values are not available with barley 6H.

The frequency of recombinants detected from barley 3HL arm was higher in the presence of two doses of *phlb* (1.48%) than with one dose (0.45%). Although this difference is not statistically significant ($P=0.41$), the observation is similar to the findings of Wang et al. (1977), who observed a four fold increase in pairing between wheat chromosome 4B and *A. intermedium* chromosome Ai when the dosage of *phlb* was increased from one to two. However, they did not report on comparative recombination frequencies in their work.

Because biochemical marker genes have not been located near the centromere of each barley chromosome arm and because recombination occurs very infrequently between wheat and barley chromosomes, it will probably be more efficient to select for recombinants by using complete barley chromosomes in the triple monosomic and selecting for the dissociation of two markers located distally on the opposite arms of the barley chromosome. This procedure will make it possible for recombinants to be selected from crossovers occurring over the whole barley chromosome in a single progeny population. However, double-crossovers would escape detection with this method, and telocentrics resulting from misdivision of the centromere would be incorrectly selected as putative recombinants. The latter group could be identified by cytological screening for the presence of telocentrics. Lines with an entire barley chromosome and a homoeologous wheat univalent in a homozygous *phlb* mutant background are now being constructed to enhance the frequency of recombination as well as the efficiency of selection.

The aim of this work was to produce a set of wheat-barley recombinant lines for each barley chromosome arm. These lines will be stable genetic stocks and could be used as reference material in gene mapping studies around the world, like at wheat-barley addition lines produced earlier (Islam et al. 1981). The principal use of these lines will be in developing a gene order map of barley based on RFLPs between wheat and barley and other biochemical characters. Although no information on map distance between the RFLP markers can be acquired from this analysis, the gene order obtained will supplement and complement the information coming from conventional segregation analysis in F_2 populations from barley × barley crosses. An advantage with the analysis of recombinant lines over analysis of F_2 is that a greater level of polymorphism for biochemical and RFLP markers is expected between wheat and barley than between any two barley lines, thus adding more markers for analysis with this approach. There is also an intrinsic interest in the phenomenon of pairing and recombination between chromosomes of different species or genera. Thus, it is expected that studying the frequency and position of chiasmata resulting from pairing between wheat and barley chromosomes will contribute fundamental and important information regarding the genome organization and evolution of these two important cereals.

Some of the wheat-barley recombinant lines generated in the present study might be useful for incorporating the barley resistance to barley yellow dwarf virus (BYDV) controlled by gene *Yd2* into wheat. McGuire and Oualset (1990) have isolated the critical addition line and located the gene on barley arm 3HL. In a joint project we are attempting to utilize our existing 3HL recombinant lines and their addition line, carrying the resistance gene and the closely linked protein marker detected by Holloway (personal communication), to transfer the *Yd2* gene to a wheat chromosome through pairing between the homologous barley segments (Fig. 3). Other useful characters known to occur in barley, such as resistance to cereal cyst nematode (CCN), could possibly be transferred to wheat in a similar manner in the future.

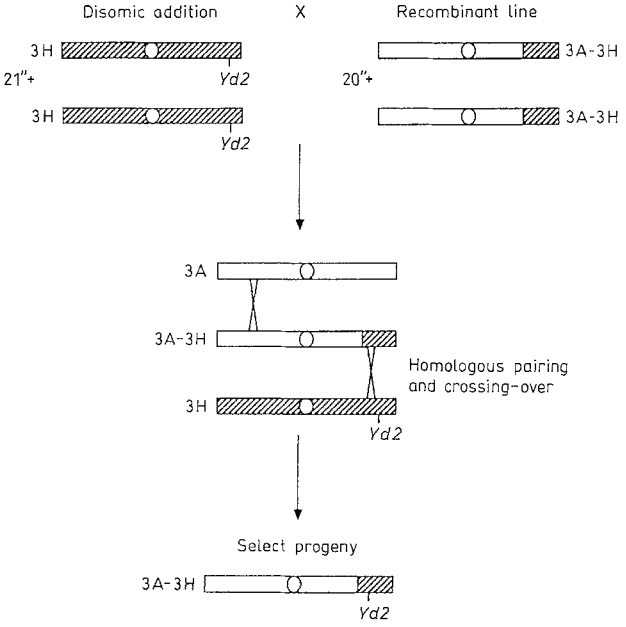

Fig. 3. Scheme for transferring the *Yd2* gene into wheat using a wheat-barley recombinant line

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