Theor Appl Genet (1995) 91:681–690

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A barley RFLP map: alignment of three barley maps and comparisons to Gramineae species

Received: 19 December 1994 / Accepted: 17 Febuary 1995

Abstract Several gene linkage maps have been produced for cultivated barley. We have produced a new linkage map for barley, based on a cross between *Hordeum vulgare* subsp. *spontaneum* and *Hordeum vulgare* subsp. *vulgare* (Hvs × Hvv), having a higher level of polymorphism than most of the previous barley crosses used for RFLP mapping. Of 133 markers mapped in the Hvs × Hvv F_2 population, 69 were previously mapped on other barley maps, and 26 were mapped in rice, maize, or wheat. Two known gene clones were mapped as well as two morphological markers. The distributions of previously mapped markers were compared with their respective barley maps to align the different maps into one consensus map. The distributions of common markers among barley, wheat, rice and maize were also compared, indicating colinear linkage groups among these species.

Key words RFLP · Barley · *Hordeum vulgare* ssp. *spontaneum* · Gramineae · Comparative mapping

Introduction

Cultivated barley (*Hordeum vulgare* L.) is an important cereal crop, ranking fifth in world crop production (Nevo 1992). Due to its agronomic importance and its suitability as a model for grass species, barley has been the subject of many genetic studies. In the last 4 years, several restriction fragment length polymorphism (RFLP) maps for the barley genome have been published. In 1990, Shin et al. produced a preliminary map with 17 RFLP markers. A map containing 155 RFLP and two morphological markers was published by Heun et al. in 1991. In the same year, Graner et al. (1991) constructed RFLP maps made from two different crosses: one between barley cultivars 'Igri' and 'Franka' (114 markers) and another between barley cultivar 'Vada' and the wild barley species *Hordeum spontaneum* C. Koch (160 markers). In that study, 27 out of 226 loci were common between the two maps and allowed their alignment. In 1993, the North American Barley Genome Mapping Project (NABGMP) published two maps with 295 markers (Kleinhofs et al. 1993a) in the first, followed by an updated map with 127 additional markers (Kleinhofs et al. 1993b). Presently, there are more than 600 markers among all the maps. Alignment of the various maps would provide researchers with a greater number of markers for specific chromosome regions.

Mapping a common set of markers in a single population is needed in order to align the different barley maps. A mapping population produced from a cross between genetically divergent, but sexually compatible, species or accessions would be expected to have higher levels of polymorphism than a population derived from a cross between cultivars. At the present time, the widest cross in barley used for mapping is between *H. vulgare* and *H. spontaneum* (Graner et al. 1991). This population was found to have a higher level of polymorphism than a cross between two barley cultivars. In addition to increased polymorphism, a *H. spontaneum* × *H. vulgare* mapping population is of particular interest since *H. spontaneum* provides a wide range of genetic diversity for the improvement of agronomic traits such as disease and pest resistance (Nevo 1986).

Most investigators accept that *H. spontaneum* is the progenitor of *H. vulgare* (Nevo 1986). There is evidence to suggest that *H. spontaneum* and *H. vulgare* are more closely related than initially thought. For example, *H. spontaneum* and *H. vulgare* are cross compatible. The

Communicated by K. Tsunewaki

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Published with the approval of the Director of the Colorado State University/Agricultural Experiment Station.

hybrids produced from such a cross are fertile with complete chromosome synapsis (Harlan and Zohary 1966). Therefore, *H. spontaneum* will be designated in our study with the systematically correct epithet: *H. vulgare* subsp. *spontaneum* (Zohary and Hopf 1988).

An objective of the present study was to construct a barley RFLP map that incorporates markers from previously published barley maps to enable alignment of the different maps. In this paper, we report the construction of an RFLP map in barley made from a cross between *H. vulgare* subsp. *spontaneum* (Hvs) and *H. vulgare* subsp. *vulgare* (Hvv). This map contains 133 markers. Sixty-nine of these markers were previously mapped on other barley maps. Map locations of a heat-shock-protein gene, a hordein gene, and loci for two morphological traits, pericarp color and growth habit, are also reported. Thirty-four RFLP markers that have not been previously mapped are also included. Twenty-six markers in this map were previously mapped in rice, allowing a preliminary comparison between the maps of barley and rice, as well as other Gramineae species.

Materials and methods

Plant materials

The RFLP mapping population consisted of 58 individual F_2 plants derived from a cross between *H. vulgare* subsp. *spontaneum* (var. *transcapsicum*) and *H. vulgare* 'Shin Ebisu 16' (SE16). The parental seed stocks which had been maintained for the development of cytogenetic lines were obtained from Dr. T. Tsuchiya (deceased, Fort Collins, Colo.).

For determining chromosomal assignments of linkage groups certain RFLP markers were hybridized to either of two sets of wheat-barley addition lines. Wheat-barley chromosome or ditelosomic addition lines contain the complete chromosome complement of wheat ($2n = 6x = 42$) plus an additional pair of barley chromosomes or ditelosomes, respectively. Wheat-barley chromosome addition lines 1,2,3,4,6 and 7, wheat-barley ditelosomic addition lines (1S, 1L, 2S, 2L, 3S, 3L, 4S, 4L, 6S, 6L, 7S and 7L) (Islam et al. 1981), the hexaploid wheat parent 'Chinese Spring', and the barley parent 'Betzes', were all obtained from the Wheat Genetics Resources Center (Manhattan, Kan.). The addition line for barley chromosome 5 was not available at the time we began our study.

DNA extraction, restriction enzyme digestion, Southern blotting and hybridization

DNA was extracted from young leaves of the parents and F_2 plants according to McCouch et al. (1988). If F_2 DNA was insufficient, 9–12 F_3 plants were grown to reconstitute an individual F_2. This sample size gives a 99% probability of correctly establishing an F_2 genotype (Hanson 1959). For wheat-barley addition lines, only plants that have been cytologically verified to contain the additional chromosome or ditelosome were used for DNA extraction. Four restriction enzymes (*Eco*RI, *Eco*RV, *Dra*I, *Xba*I) were used to evaluate the parents (Hvs and Hvv) for polymorphisms. Two barley cultivars, 'Steptoe' and 'Morex', were similarly surveyed for polymorphism. Restriction digestions were done on 10 µg of DNA using 30 units of enzyme, incubated at 37 °C for a minimum of 6 h. The digested DNA was run on a 0.9% agarose gel as described in Bernatzky and Tanksley (1986), and alkaline blotted onto Hybond-N+nylon membranes (Amersham) for 3–6 h. Probes were labelled with ^{32}P by random priming (Feinberg and Vogelstein 1993). Hybridization and washing were according to the procedures described in Bernatzky and Tanksley (1986). Homologous hybridizations (i.e., barley clones to barley DNA) were washed at 0.2 × SSC and 0.001% SDS, while heterologous probes (wheat, oat, or rice clones to barley DNA) were washed at a lower stringency of 0.5 × SSC and 0.025% SDS for the final wash.

Fig. 1 The *H. vulgare* subsp. *spontaneum* × *H. vulgare* subsp. *vulgare* map that includes 133 markers. Each linkage group has the clone name to the right and the distance between markers to the left. The previously mapped markers are indicated by a *capital letter* after the clone name. Each letter indicates the population in which the marker had been previously mapped: *S* × 'Steptoe'/'Morex'; *N* × 'Nudinka'/'Proctor'; *I* × 'Igri'/'Franka'; *V* × 'Vada'/*H. spontaneum*; *R* × rice (see text for references). The *superscripts* indicate the previously mapped markers that were not colinear: [1] - indicates non-colinear markers that are on the same chromosomes as those previously mapped but are not in the same order; [2] - indicates non-colinear markers that are on different chromosomes than those previously mapped; ■ - indicates that the orientation of markers is not definitive; * - indicates that the marker was positively assigned to a chromosome based on wheat-barley addition line hybrdization; () - indicates that specific marker to be mapped at LOD 2.0; ---- - indicates map distances greater than 32.7 cM yet with LOD 3.0 linkages

DNA probes

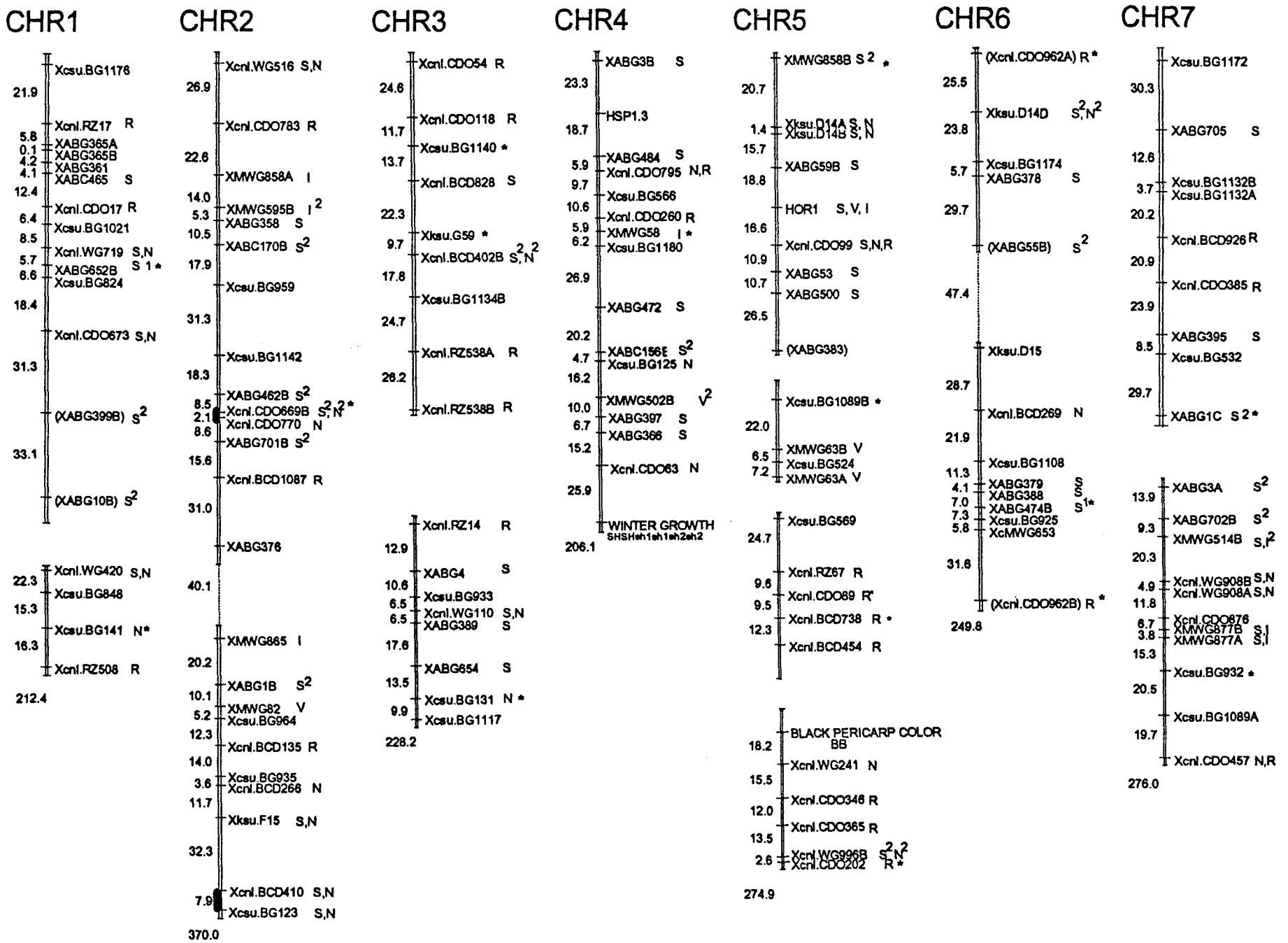
The cDNA clones used as probes were from oat (CDO) (Heun et al. 1991), barley (ABC and BCD) (Heun et al. 1991; Kleinhofs et al. 1993a) and rice (RZ) (Ahn et al. 1993). Genomic clones were from barley (MWG, ABG and BG) (Graner et al. 1991; Kleinhofs et al. 1993a; Lapitan unpublished), wheat (WG) (Heun et al. 1991) and *Triticum tauschii* (DG) (Gill et al. 1991). The clones pHor1 (Forde et al. 1985) and pHsp 1.3 (Wu et al. 1988), which contain a portion of the barley hordein gene and a heat-shock-protein gene from *Arabidopsis thaliana*, respectively, were also used.

Morphological markers

H. vulgare 'SE16', is a Japanese cultivar that is a 2-rowed, white-seeded, spring barley, while *H. vulgare* subsp. *spontaneum* is a 2-rowed, black-seeded, winter barley. Two morphological traits segregating in the mapping population were pericarp color (*B*) and winter-growth habit (*Sh*). Black pericarp (*B*) has been characterized as a simply inherited trait with black (*B_*) dominant over white (*bb*) seed color (Haus and Tsuchiya 1970). The winter-habit trait (*Sh*) is multigenic, but the epistatic interactions are such that the winter habit is only expressed with a single genotype (*ShShsh2sh3sh3*) (Takahashi and Yasuda 1970). Each trait was evaluated in the greenhouse on 58 F_2 individuals used for genetic mapping.

Genetic mapping and marker nomenclature

To ensure accurate scoring, all RFLP markers were independently scored at least twice. Ambiguous genotypes were jointly resolved by assigning a blank score to the individual score for map construction. Genetic linkages were determined using MAPMAKER version 3.0 (Lander et al. 1987). Genetic distances were calculated using the Kosambi function. Linkage groups were determined and verified using two progressions of Mapmaker commands: near, try, compare and map; group, order, try, ripple, and map. The linkage groups were verified with 128 markers linked at LOD 3.0 and a maximum recombination distance of 37.2 cM. Unlinked markers were assessed at or near LOD 2.0 and a maximum recombination distance of 50 cM. Five additional markers were successfully mapped within the 50-cM mapping distance (enclosed in parentheses in Fig. 1). The 14


CHR1
Xcsu.BG1176
21.9
Xcnl.RZ17 R
5.8
XABG365A
0.1
XABG365B
4.2
XABG361
4.1
XABC465 S
12.4
Xcnl.CDO17 R
6.4
Xcsu.BG1021
8.5
Xcnl.WG719 S,N
5.7
XABG652B S 1 *
6.6
Xcsu.BG824
18.4
Xcnl.CDO673 S,N
31.3
(XABG399B) S2
33.1
(XABG10B) S2
22.3
Xcnl.WG420 S,N
15.3
Xcsu.BG848
Xcsu.BG141 N*
16.3
Xcnl.RZ508 R
212.4
CHR2
Xcnl.WG516 S,N
26.9
Xcnl.CDO783 R
22.6
XMWG858A I
14.0
XMWG595B I 2
5.3
XABG358 S
10.5
XABC170B S2
17.9
Xcsu.BG959
31.3
Xcsu.BG1142
18.3
XABG462B S2
8.5
Xcnl.CDO669B S,N 2,2*
2.1
Xcnl.CDO770 N
8.6
XABG701B S2
15.6
Xcnl.BCD1087 R
31.0
XABG376
40.1
XMWG865 I
20.2
XABG1B S2
10.1
XMWG82 V
5.2
Xcsu.BG964
12.3
Xcnl.BCD135 R
14.0
Xcsu.BG935
3.6
Xcnl.BCD266 N
11.7
Xksu.F15 S,N
32.3
Xcnl.BCD410 S,N
7.9
Xcsu.BG123 S,N
370.0
CHR3
Xcnl.CDO54 R
24.6
Xcnl.CDO118 R
11.7
Xcsu.BG1140 *
13.7
Xcnl.BCD828 S
22.3
Xksu.G59 *
9.7
Xcnl.BCD402B S,N 2,2
17.8
Xcsu.BG1134B
24.7
Xcnl.RZ538A R
26.2
Xcnl.RZ538B R
Xcnl.RZ14 R
12.9
XABG4 S
10.6
Xcsu.BG933
6.5
Xcnl.WG110 S,N
6.5
XABG389 S
17.6
XABG654 S
13.5
Xcsu.BG131 N *
9.9
Xcsu.BG1117
228.2
CHR4
XABG3B S
23.3
HSP1.3
18.7
XABG484 S
5.9
Xcnl.CDO795 N,R
9.7
Xcsu.BG566
10.6
Xcnl.CDO260 R
5.9
XMWG58 I *
6.2
Xcsu.BG1180
26.9
XABG472 S
20.2
XABC156E S2
4.7
Xcsu.BG125 N
16.2
XMWG502B V2
10.0
XABG397 S
6.7
XABG366 S
15.2
Xcnl.CDO63 N
25.9
WINTER GROWTH
SHSHsh1sh1sh2sh2
206.1
CHR5
XMWG858B S 2 *
20.7
Xksu.D14A S, N
1.4
Xksu.D14B S, N
15.7
XABG59B S
18.8
HOR1 S, V, I
16.6
Xcnl.CDO99 S,N,R
10.9
XABG53 S
10.7
XABG500 S
26.5
(XABG383)
Xcsu.BG1089B *
22.0
XMWG63B V
6.5
Xcsu.BG524
7.2
XMWG63A V
Xcsu.BG569
24.7
Xcnl.RZ67 R
9.6
Xcnl.CDO89 R*
9.5
Xcnl.BCD738 R *
12.3
Xcnl.BCD454 R
BLACK PERICARP COLOR
BB
18.2
Xcnl.WG241 N
15.5
Xcnl.CDO346 R
12.0
Xcnl.CDO365 R
13.5
Xcnl.WG996B S,N 2,2
2.6
Xcnl.CDO202 R*
274.9
CHR6
(Xcnl.CDO962A) R *
25.5
Xksu.D14D S,N 2,2
23.8
Xcsu.BG1174
5.7
XABG378 S
29.7
(XABG55B) S2
47.4
Xksu.D15
28.7
Xcnl.BCD269 N
21.9
Xcsu.BG1108
11.3
XABG379 S
4.1
XABG388 S
7.0
XABG474B S1*
7.3
Xcsu.BG925
5.8
XcMWG653
31.6
(Xcnl.CDO962B) R *
249.8
CHR7
Xcsu.BG1172
30.3
XABG705 S
12.6
Xcsu.BG1132B
3.7
Xcsu.BG1132A
20.2
Xcnl.BCD926 R
20.9
Xcnl.CDO385 R
23.9
XABG395 S
8.5
Xcsu.BG532
29.7
XABG1C S 2 *
XABG3A S2
13.9
XABG702B S2
9.3
XMWG514B S,I2
20.3
Xcnl.WG908B S,N
4.9
Xcnl.WG908A S,N
11.8
Xcnl.CDO876
6.7
XMWG877B S,I
3.8
XMWG877A S,I
15.3
Xcsu.BG932 *
20.5
Xcsu.BG1089A
19.7
Xcnl.CDO457 N,R
276.0

linkage groups generated were assigned to chromosomes by hybridization of selected probes from each linkage group (designated with an asterisk in Fig. 1) to wheat-barley addition lines. These data, along with information from previous maps, were used to determine the correct orientation of linkage groups. In the case of chromosome 1, 3, 5 and 7, with more than one linkage group, fragments were checked for linkage to each other using three-point and multipoint analyses. In one case, the fragment containing pHor1 through MWG858b was successfully linked to the fragment containing CDO99, thus reducing the number of individual linkage groups to 13.

Markers were named according to the nomenclature recommended by the 'Guidelines for Nomenclature of Biochemical and Molecular Loci in Wheat and Related Species' (McIntosh et al. 1994). Clones segregating for more than one locus were designated with a letter following the marker name (i.e., Xcnl.BCD266A). Careful attention was given to naming loci detected by multicopy clones previously mapped in other barley maps. For example, XABG399 mapped to chromosome 3 in Kleinhofs et al. (1993a), but was found to map to chromosome 1 in this study (see Fig. 1). Therefore it was designated as XABG399B.

Results and discussion

Polymorphism

A high level of polymorphism between parents facilitates the construction of a high-density consensus map. In our study, 56% was the overall level of polymorphism observed between *H. vulgare* subsp. *spontaneum* (Hvs) × *H. vulgare* 'SE16' (Hvv), based on four restriction enzymes (*Eco*RI, *Eco*RV, *Dra*I, and *Xba*I) and 375 probes (240 genomic and 135 cDNA). The genomic probes exhibited a greater polymorphism level of 60% compared to 48% for the cDNA probes. A comparison of polymorphism levels between barley cultivars 'Steptoe' × 'Morex' (i.e., Hvv × Hvv) and Hvs × Hvv was accomplished with a set of 123 common markers (57 genomic and 66 cDNA) and the same four restriction enzymes. A lower overall polymorphism rate of 40% (22/57 genomic, 27/66 cDNA) between 'Steptoe' × 'Morex' (Hvv × Hvv) was observed compared to 48% between the Hvs × Hvv cross (33/57 genomic, 25/66 cDNA). It is interesting to note that genomic clones were more polymorphic than cDNA clones between Hvs and Hvv (58% vs 38%), whereas genomic and cDNA clones were similarly polymorphic between 'Steptoe' × 'Morex' (38% vs 41%). 'Nudinka' × 'Proctor' also exhibited greater levels of polymorphism for genomic probes compared to cDNA probes (Heun et al. 1991). In tomato, Zamir and Tanksley (1988) observed a similar trend with genomic probes showing higher levels of polymorphism when compared to cDNA probes.

Graner et al. (1991) observed 76% polymorphism between parents of an intersubspecies cross (Hvv × Hvs) compared to 56% for the same subspecies used in our study. The greater level of polymorphism in the Graner et al. (1991) cross could indicate a greater genetic divergence between their lines. The lines used in our cross were the same lines used by Dr. T. Tsuchiya to develop cytogenetic barley stocks and have been inbred for many years, possibly reducing detectable polymorphisms. The differences in levels of polymorphisms may also be due to differences in the clones screened.

Mapping

The map generated from the F_2 population derived from our intersubspecies cross is shown in Fig. 1. This map has a total length of 1817.4 cM, containing two morphological markers and 131 RFLP markers (90 genomic and 41 cDNA). Of the RFLP markers, the following were previously mapped (Fig. 1, denoted by capital letters after probe name): 55 in 'Steptoe'/'Morex' (Kleinhofs et al. 1993a), 25 in 'Nudinka'/'Proctor' (Heun et al. 1991), seven in 'Igri'/'Franka' (Graner et al. 1991), five in the 'Vada'/*H. vulgare* subsp. *spontaneum* cross (Graner et al. 1991), and 26 in rice (Ahn and Tanksley 1993; Ahn et al. 1993). The 131 RFLP markers are distributed among 13 linkage groups (Fig. 1). Chromosomes 1, 3, and 7 consist of two unlinked groups, while chromosome 5 consists of four unlinked groups. The remaining chromosomes consist of a single linkage group. Each linkage group was assigned to a chromosome by hybridizing at least one marker within the group to a Southern blot containing DNA from wheat-barley addition lines. The clones used to confirm chromosome association are indicated with an asterisk following the probe name (Fig. 1). A locus is assigned to a barley chromosome based on the presence of bands in barley and a specific wheat-barley addition line (Fig. 2). In the two examples of chromosome assignment shown in Fig. 2, ksu.G59 was assigned to chromosome 3 (Fig. 2 A), and CDO202 was assumed to be located in chromosome 5 (Fig. 2 B). Following the assignment of linkage groups to chromosomes, the orientation of long and short arms was determined based on wheat-barley ditelosomic addition lines and the positions of previously mapped markers from the 'Steptoe'/'Morex' map (Kleinhofs et al. 1993a), where centromere positions had been determined using wheat-barley ditelosomic lines.

A putative locus for a heat-shock-protein gene in barley was mapped using a cloned *Hsp* gene from *A. thaliana*. This locus was mapped to the short arm of chromosome 4. The chromosomal locations of genes controlling heat-shock proteins in wheat were previously determined to be on chromosomes 3, 4, and 7, using wheat ditelosomic lines and isoelectric focusing gels (Porter et al. 1989). Since wheat chromosome 4 is homoeologous to barley chromosome 4, the locus we mapped may correspond to one of the heat-shock-protein genes in wheat. Also, a locus for a hordein gene (*Hor1*) was mapped to chromosome 5 using a clone from barley (Forde et al. 1985). The hordein gene has been previously mapped on chromosome 5 (Jensen et al. 1980; Kleinhofs et al. 1993a).

The two morphological markers, pericarp color (*B*) and winter-growth habit (*Sh*), were mapped to the same chromosomes as in the classical map (Tsuchiya 1987).

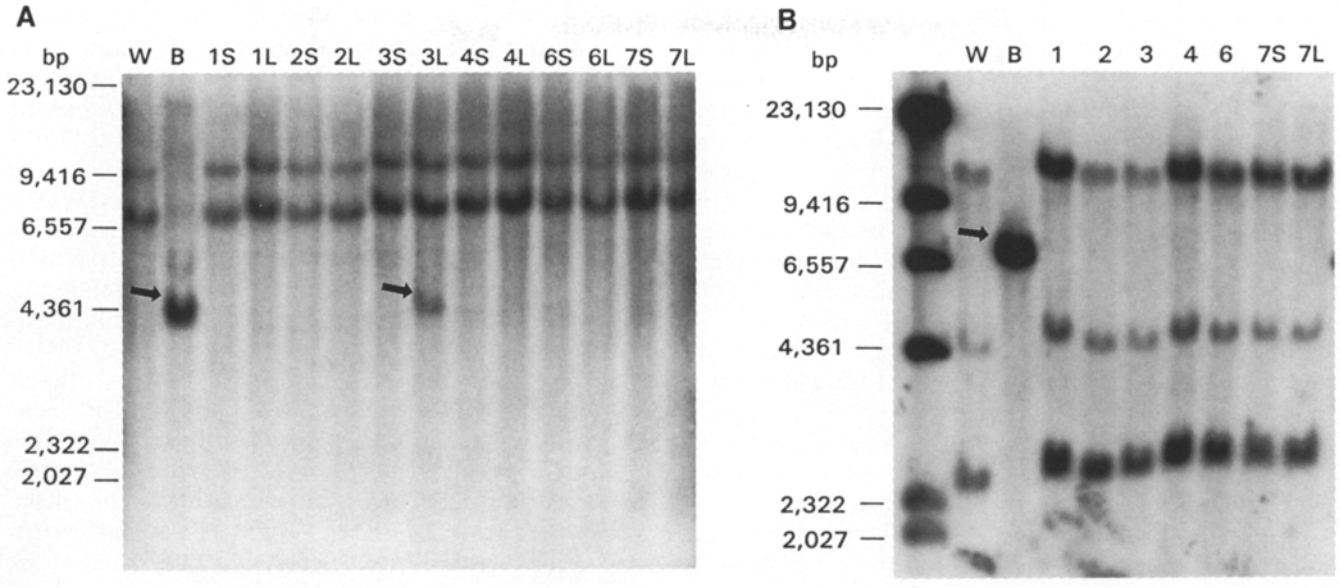


Fig. 2A, B Autoradiograms of wheat barley addition line DNA used to associate linkage groups with specific chromosomes. The genomic DNA was digested with *Eco*RV. **A** Autoradiogram showing hybridization of wheat-barley ditelosomic lines to ksuG59. *Lane 1*, parental wheat DNA ('Chinese Spring'); *lane 2*, parental barley DNA ('Betzes'); *lanes 3–14* contain DNA from different wheat-barley ditelosomic lines. The barley ditelosomic chromosomes are indicated on the top of the lanes. Note the same band in *lane 3* ('Betzes') and *lane 9* (3L) as indicated by the *arrow*, confirming the assignment of the polymorphic marker ksuG59 to the long arm of barley chromosome 3. **B** Autoradiogram showing hybridization of wheat-barley addition lines to CDO202. *Lane 1*, Lambda *Hin*dIII; *lane 2*, parental wheat ('Chinese Spring'); *lane 3*, parental barley ('Betzes'); *lanes 4–9*, different wheat-barley addition lines. The additional barley chromosomes are indicated on top of the lanes. A strong band is seen in *lane 3* ('Betzes'). No such band was observed in the lanes containing the wheat-barley addition lines1, 2, 2, 4, 6 and 7. Therefore, Xcnl.CDO202 was assumed to be located on chromosome 5

Utilizing a chi-square goodness of fit ratio, ($P = 0.05$) it was confirmed that the two morphological traits were to be treated as simply inherited dominant loci. The gene for pericarp color (*B*) was classically placed on chromosome 5 and Fig. 1 confirms the placement of this trait to linkage group 5. While there are three genes controlling winter-growth habit, *Sh*, *Sh2* and *Sh3* on chromosomes 4, 7 and 5 respectively, the only combination which will express the winter-growth habit is *ShShsh2sh2sh3sh3*. All other combinations of genotypes express a spring-growth habit. In our material the winter-growth habit segregated in a 3:1 ratio. The *Sh* gene was mapped to chromosome 4 (Fig. 1) in the Hvs/Hvv map, which confirms the classical placement of this gene.

The number of markers and the genetic lengths that compose each of the seven barley chromosomes in the Hvs/Hvv map are shown in Table 1. The cytological lengths as measured from N-banded chromosomes (Singh and Tsuchiya 1982) are also shown. There was no correlation between the cytogenetic lengths and the number of markers or the genetic lengths ($r = 0.2252$ and $r = 0.2983$ respectively). As expected, the longest chromosome (2) contained the greatest number of markers and the two longest chromosomes (2 and 7) had the greatest genetic lengths. However, the smallest chromosome (5) contained one of the largest number of markers and the third largest genetic length. In fact, if chromosome 5 is removed from the comparison of the number of markers and cytological length, then the correlation is improved and becomes significant

Table 1 A comparison of the number and types of markers mapped and the cytological lengths of each of the seven barley chromosomes

Chromosome	Marker type			Total no. markers	Genetic length (cM)	Physical length (μm)[a]
	Morphological	Genomic	cDNA			
1	0	13	5	18	212	15.21
2	0	16	8	24	370	16.22
3	0	10	7	17	228	15.76
4	1	10	5	16	206	14.24
5	1	12	11	24	275	12.62
6	0	11	3	14	250	13.89
7	0	16	4	20	276	16.04

[a] The physical lengths of barley chromosomes were taken from Table 2 of Singh and Tsuchiya (1982). The measurements of chromosomes 6 and 7 included the satellites

($r = 0.8367$). Graner et al. (1991) also observed that chromosome 5 contained a greater number of markers than several physically larger chromosomes (i.e., Chr. 1 and 7). One explanation for the lack of correlation between marker numbers or genetic lengths and the physical lengths of chromosomes is that the map is not saturated.

Although a relatively large number of markers mapped to chromosome 5, it consists of four linkage groups that will not link to each other. Chromosome 5 has the greatest proportion of mapped cDNA clones (42%) which may indicate that this chromosome also contains many expressed sequences (Table 1). Cytogenetic evidence supports the suggestion that chromosome 5 might have an unusually high proportion of expressed sequences, since it has the smallest proportion of heterochromatin of all the chromosomes as shown by C-banding (Noda and Kasha 1978) and N-banding (Singh and Tsuchiya 1982). If chromosome 5 contains a disproportionate number of expressed sequences the four groups might be unlinked because the majority of the markers used for screening polymorphisms were genomic clones. 'Steptoe'/'Morex' also exhibited a higher proportion of cDNA probes mapped to chromosome 5. Another possible reason why we were unable to link these groups might be an increased amount of recombination on chromosome 5 (see discussion below).

Comparison of the Hvs × Hvv map with other barley maps

Despite a relatively high level of polymorphism (56%) in the Hvs × Hvv F_2 population compared to other barley mapping populations, only 25% of the total number of clones surveyed were polymorphic in both Hvs × Hvv and 'Steptoe' × Morex'. Thus, generating a consensus map required extensive work. The Hvs/Hvv barley map presented contains 69 markers that were previously mapped in existing barley maps. The 'Steptoe'/'Morex' map (Kleinhofs et al. 1993a) contains 48 markers in common with Nudinka/Proctor and seven markers in common with 'Vada'/*H. spontaneum*. The updated 'Steptoe'/'Morex' map (Kleinhofs et al. 1993b) contains 127 additional markers, with 60 of those from Graner's library [barley genomic (MWG) and cDNA (cMWG)], but only six additional markers in common with 'Vada'/*H. spontaneum*. There are currently 105 total common markers between the 'lgri'/'Franka' map and that of 'Steptoe'/'Morex' (Graner, personal communication).

A comparison of common markers between Hvs/Hvv and other barley maps shows a similar linear order for the majority of markers (Fig. 1). These markers are designated with the letters 'N', 'S', 'I', and 'V' after the clone names in Fig. 1 to indicate that they have been previously mapped in 'Nudinka'/'Proctor', 'Steptoe'/'Morex', 'lgri'/'Franka', and 'Vada'/*H. spontaneum*, respectively. Mapping of previously mapped markers in the Hvs/Hvv map enables the alignment of different barley maps. In chromosome 4, for example, markers XABG3B, XABG484, XABG472, XABG397 and XABG366 were mapped only in 'Steptoe'/'Morex' but not in 'Nudinka'/'Proctor', while Xcnl.CDO795, Xcsu.BG125 and Xcnl.CDO63 were mapped in 'Nudinka'/'Proctor' but not in 'Steptoe'/'Morex'. Mapping of these markers together in one map shows their linear relationships to each other and other markers included on the chromosome-4 linkage maps of 'Nudinka'/'Proctor' and 'Steptoe'/'Morex'.

The map distances of common intervals between the Hvs/Hvv, 'Steptoe'/'Morex', and 'Nudinka'/'Proctor' maps are compared in Table 2. In general, map distances of the same intervals are greater in the Hvs/Hvv map relative to the other maps. Common intervals in Hvs/Hvv were on average 2.37 ± 0.77 times greater than 'Nudinka'/'Proctor' and 2.75 ± 1.746 times greater than 'Steptoe'/'Morex'. The increase in genetic distance may reflect higher recombination frequencies between Hvs

Table 2 Common marker intervals (distances from published maps)

Chromosome	Marker interval	Genetic distances (cM)		
		HvsHvv	'Nudinka'/'Proctor'	'Steptoe'/'Morex'
1	WG420–BG141	37.6	31.2	–
	ABC465–CDO673	58.0	–	31.1
2	KSUF15–BG123	40.2	52.4	83.6
	WG516–BG123	370.0	188.3	166.4
	WG516–CDO770	157.4	95.3	–
	CDO770–BG123	212.6	93.0	–
3	ABG4–ABG654	41.2	–	18.8
	WG110–BG131	37.6	52.0	–
4	BG125–CDO63	48.1	12.0	–
	ABG484–ABG366	123.0	–	67.1
5	CDO99–ABG500	25.1	–	18.0
	CDO99–HorI	16.6	–	16.9
6	ABG378–ABG388	143.1	–	66.0
7	ABG705–ABG395	82.3	–	8.9
	MWG514–MWG877	43.7	–	18.8
	WG908–CDO457	77.8	33.1	–

and Hvv than between 'Steptoe' and 'Morex' or 'Nudinka' and 'Proctor'. Similarly, Graner et al. (1991) reported greater recombination frequencies in 'Vada'/*H. spontaneum* compared to the cross between two barley cultivars ('Igri'/'Franka'). These observations might seem unexpected since the greater genetic divergence between these parents could result in less recombination between homologous chromosomes. However, Hvs and Hvv are homologous enough to have complete synapsis (Harlan and Zohary 1966). Thus, Hvs and Hvv may not be genetically divergent enough for recombination to be reduced. The increased level of recombination could be caused by many factors. One obvious difference is the manner in which the populations were produced. The mapping populations obtained from the crosses between 'Steptoe' × 'Morex', 'Nudinka' × 'Proctor', and 'Igri' × 'Franka' were all doubled haploid populations, while the Hvs × Hvv populations in Graner et al. (1991) and our study utilized F_2 populations. Even the doubled haploid populations were produced in two different ways: microspore cultures ('Nudinka' × 'Proctor' and 'Igri' × 'Franka') and embryo rescue after hybridization with *H. bulbosum* ('Steptoe' × 'Morex'). Powell et al. (1986) showed that there were different amounts of linkage between the same markers in populations produced by microspore culture and the *H. bulbosum* method.

The greater recombination frequencies in the present cross may explain the difficulty in connecting linkage groups known to belong to the same chromosome. On the other hand, the advantage of greater recombination frequencies is that it enabled the ordering of markers that co-segregated in other mapping populations. For example, the markers Xcnl.BCD 266 and Xksu.F15 are both linked to each other on chromosome 2 in the Hvs/Hvv and 'Nudinka'/'Proctor' maps. Although the two markers are 0 cM apart and their relative orders are unknown in 'Nudinka'/'Proctor', crossovers between the two markers were found among the Hvs × Hvv progeny, resulting in a genetic distance between them of 12 cM. The Hvs/Hvv map showed that Xcnl.BCD266 is proximal to Xksu.F15. The ability to determine the orders of tightly linked markers will be valuable for future chromosome-walking experiments.

There were also cases where some common markers between the Hvs/Hvv map and other barley maps were not colinear (depicted in Fig. 1 by superscript 1). For example, XABG652 (chromosome 1) and XABG474 (chromosome 6) each mapped to the same chromosomes but not to the same positions as previously reported (Kleinhofs et al. 1993a). Twenty-three previously mapped markers that were assigned to different chromosomes in our population are shown by superscript 2 in Fig. 1. Table 3 lists these 23 clones and their chromosome location in the Hvs/Hvv and other maps. All of the clones that detected markers on different chromosomes in the different mapping populations were multicopy. It is possible that the non-colinearity of these markers was due to the mapping of different segregating loci from multiple fragments in the different mapping populations. These loci were therefore designated with letters after the probe names in the Hvs/Hvv map to distinguish them from those found in previous maps. Similarly, 15 of the 48 common markers between 'Steptoe'/'Morex' and 'Nudinka'/'Proctor' mapped to different linkage groups. All 15 of these probes were multicopy. Of the 13 probes that were common between 'Vada'/*H. spontaneum* and 'Steptoe'/'Morex', five mapped to different chromosomes and were also multicopy.

Comparison of barley, rice, wheat, and maize maps

Twenty-six markers included in the barley map were previously mapped in rice allowing comparisons between the two species (Table 4 and Fig. 3). Although the number of markers being compared is small, synteny between barley and rice chromosomes is apparent. As Table 4 illustrates, when there are more than two rice markers that mapped to the same barley chromosome, the majority of the markers also mapped to a single rice chromosome. Furthermore, the order of the common markers is similar between barley and rice. This conservation is particularly evident for barley chromosomes 3 and 5. All four markers from rice chromosome 1 mapped to barley chromosome 3 in the same linear order (Ahn et al. 1993). Five markers from rice chromosome 5 mapped to barley chromosome 5 in a similar order relative to rice, with the exception of a switch in the position of BCD454 in the two maps (Fig. 3). This indicates that these linkage groups have been highly conserved in evolution. In contrast, syntenic relationships of barley chromosomes 6 and 7 with rice chromosomes is not evident from the results of this study. Only one marker

Table 3 Clones from Hvs/Hvv that mapped to different chromosomes in previous maps

Clone	Chromosome location in			
	HvsHvv	NP	SM	VHs
XABG10B	1		3	
XABG399B	1		3	
XABG1B	2		6	
XABC170B	2		6	
XABG462B	2		3	
XABG701B	2		1	
XMWG595B	2			3
Xcnl.CDO669B	2	4	4,7	
Xcnl.BCD402B	3	4	4	
XABC156E	4		1,2,3	
XMWG502	4			5
Xcnl.WG996B	5	2	2	
XMWG858B	5		2	
XABG55B	6		5	
Xksu.D14D	6	5	1	
XABG3A	7		4	
XABG702B	7		5	
XMWG514B	7		7	6
XABGIC	7		6	

Table 4 Common markers between barley (this study) and rice (Ahn and Tanksley 1993; Ahn et al. 1993) and their chromosome locations in the different species

Common marker	Chromosome location		Common marker	Chromosome location	
	Barley	Rice		Barley	Rice
RZ17	1	10	CDO99	5	8
CDO17	1	6	RZ67	5	5
RZ508	1	6	CDO89	5	5
CDO783	2	4	BCD738	5	5
BCD1087	2	9	BCD454	5	5
BCD135	2	4	CDO346	5	5
CDO54	3	1	CDO365	5	11
CDO118	3	1	CDO202	5	5
RZ538	3	1	CDO962	6	1
RZ14	3	1	BCD926	7	9
CDO795	4	3	CDO385	7	7
CDO260	4	3	CDO457	7	3

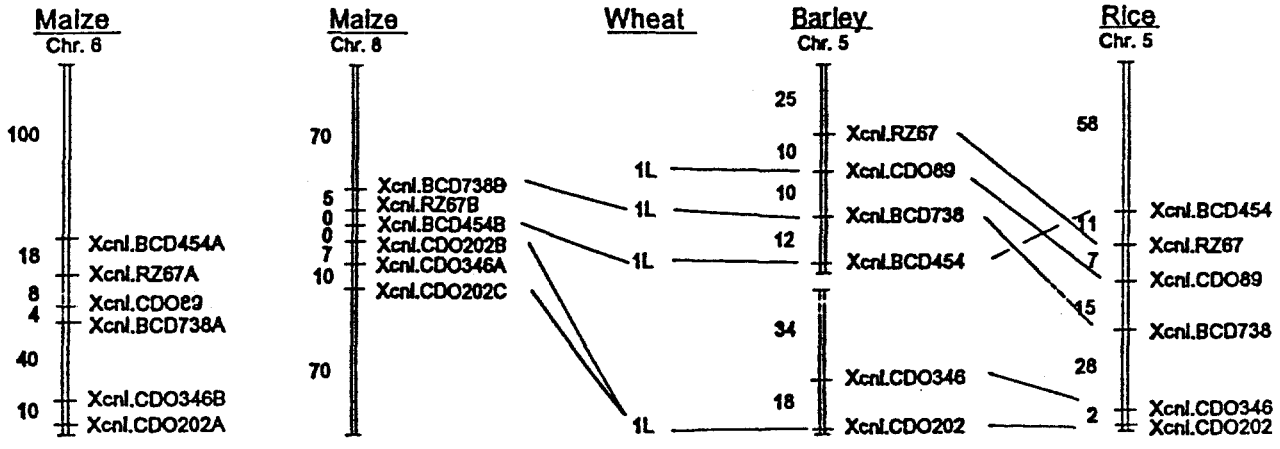


Fig. 3 A comparison of a conserved linkage group between maize, wheat, barley and rice. Maize contains two similar linkage groups on two different chromosomes to the single barley linkage group. The markers on maize chromosome 6 are colinear to barley 5 except for a duplication of markers CDO202 in maize. The order of these markers is unknown in wheat. However, they all occur in the same linkage group in the ling arm of wheat chromosome 1. The markers are colinear in barley and rice except for marker BCD454. Linkage groups for maize and rice are taken from Ahn and Tanksley (1993). Assignment to wheat chromosomes is from Ahn et al. (1993)

from rice mapped to barley chromosome 6. Three markers that mapped to barley chromosome 7 previously mapped to three different rice chromosomes. These results suggest that barley chromosomes 1, 2, 3, 4 and 5 are syntenic to at least parts of rice chromosomes 6, 4, 1, 3, and 5, respectively.

Comparative mapping studies have been done between wheat and rice (Ahn et al. 1993; Kurata et al. 1994) and rice and maize (Ahn and Tanksley 1993). These studies illustrated the presence of conserved linkage groups among these species. The rice and maize chromosomes shown to have synteny with wheat chromosomes are summarized in Table 5. An example of a conserved linkage group among rice, maize, and wheat is also shown in Fig. 3. Since homoeologous relationships between barley and wheat chromosomes have been defined (Benito et al. 1985; Hart 1987; Kam-Morgan et al. 1989; Namuth et al. 1995, see Table 5), it is possible to predict the chromosomes of rice and maize that share conserved linkage groups with barley. As Table 5 and Fig. 3 illustrate, the results of this study agree with expectations based on previous comparative mapping studies. Table 5 also indicates the rice chromosomes expected to show synteny with barley chromosomes 6 and 7. Our inability to determine these latter relationships may be due to the small number of common markers used for comparisons. A more extensive comparison based on a larger number of common markers is needed to verify the predicted synteny between barley chromosome 6 and rice chromosome 2, and barley chromosome 7 and rice chromosome 9. Further study might also determine the chromosomal changes that have taken place during evolution, explaining the differences in chromosome numbers between barley, rice and maize.

Finding conserved linkage groups among barley, wheat, rice and maize has important implications and practical applications. First, it indicates that these species share a common ancestral origin. The colinearity of (cDNA) markers as well as isozymes (Ahn et al. 1993; Milne and McIntosh 1990) indicates that the location of genes of interest are most likely also conserved

Table 5 Chromosomes sharing markers that are syntenous or colinear among barley, wheat, rice and maize

Barley	Wheat[a]	Rice[b]	Maize[c]
1	7	6	6, 9
2	2	4	2, 10
3	3	1	3, 8
4	4	3	1
5	1	5	3, 8
6	6	2	4, 5
7	5	9	2, 7

[a] Wheat-barley comparisons based on Namuth et al. (1994) and Hart (1987)
[b] Wheat-rice comparisons based on Ahn et al. (1993) and Kurata et al. (1994)
[c] Rice-maize comparisons based on Ahn and Tanksley (1993)

among these species. Therefore, a species with a small genome like rice (4.5×10^8 bp/haploid genome; Arumuganathan and Earle 1991) can be exploited for isolating the corresponding genes that have not been mapped in the other species with large genomes (Bennett and Smith 1976), such as barley (5×10^9 bp/haploid genome) and wheat (1.5×10^{10} bp/haploid genome). Finally, by determining conserved linkage groups among the different species, the number of potential markers for any one species in increased.

Acknowledgements The authors are grateful to the late Dr. T. Tsuchiya and Dr. S. Wang for seed samples and cross-pollination expertise and to Leslie Richter for technical assistance throughout this project. The heat-shock-protein gene polymorphism was identified by Dr. Scott Haley. Dr. Wayne Kennard, Dr. Lee Panella, and Dr. Tracy Halward contributed many helpful comments and suggestions. Probes for this work were kindly provided by Drs. Steve Tanksley, Mark Sorrells, Andreas Graner, Andy Kleinhofs, Bikram Gill and Chris Sommerville. Work was partially supported by USDA/CSRS Contracts No. 92-34205-6953 and No. 58-5366-1-171, the North American Barley Genome Mapping Project, and Hatch Funds 644.

References

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Ahn S, Anderson JA, Sorrells, MA, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483–490

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Benito C, Figuerias AM, Gonzales-Jaen MT, Salinas J (1985) Biochemical evidence of homoeology between wheat and barley chromosomes. Z Planzenzuchtg 94:307–320

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Phil Trans Roy Soc Lond 274:227–274

Bernatzky R, Tanksley SD (1986) Methods for detection of single- or low-copy sequences in tomato on Southern Blots. Plant Mol Biol Rep 4:37–41

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to a high specific activity. Anal Biochem 132:6–13

Forde BG, Kreis M, Williamson MS, Fry RB, Pywell J, Shewry PR, Bunce N, Miflin BJ (1985) Short tandem repeats shared by B- and C-hordein cDNAs suggest a common evolutionary origin for two groups of cereal storage-protein genes. EMBO 4:9–15

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Hermann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Hanson, WD (1959) Minimum family size for the planning of genetic experiments. Agron J 51:711–715

Harlan JR, Zohary D (1966) Distribution of wild wheats and barley. Science 153:1074–1080

Hart GE (1987) Genetic and biochemical studies of enzymes. In: Heyne E (ed) Wheat and wheat improvement. American Society of Agronomy, Madison, Wisconsin., pp 119–214

Haus TE, Tsuchiya T (1970) Description of genetic stocks in the barley genetic stock center at Fort Collins, Colorado. Barley Genet Newslett 1:103–193

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley(*Hordeum vulgare*). Genome 43:437–447

Islam AKMR, Shephred KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46:161–174

Jensen J, Jorgensen JH, Jensen HP, Giese H, Doll H (1980) Linkage of the hordein loci *hor1* and *hor2* with powdery mildew resistance with MI-k and MI-a on barley chromosome 5. Theor Appl Genet 58:27–31

Kam-Morgan LNW, Gill BS, Muthukrishanan S (1989) DNA restriction fragment length polymorphism: a strategy for genetic mapping of the D genome of wheat. Genome 32:724–732

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan NLV, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp S, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993 a) A molecular, isozyme and morphological map of the barley genome. Theor Appl Genet 86:705–712

Kleinhofs A, Kilian A, Kudrna D, NABGMP (1993 b) The NABGMP Steptoe × Morex mapping progress report. Barley Genet Newslett 23:79–83

Kurata N, Moore C, Nagamura Y, Foote T, Yano M, Minobe Y, Ceale M (1994) Conservation of genome structure between rice and wheat. Bio/Technal 12:276–278

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181

McCouch SR, Kochert B, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

McIntosh RA, Hart GE, Gale MD (1994) Catalogue of gene symbols for wheat In: Quick J (ed) Annu Wheat Genet Newslett 40:362–375

Milne DL, McIntosh RA (1990) *Triticum aestivum* (common wheat) In: O'Brien SJ (ed) Genetic Maps. Cold Spring Harbor Press, Cold Spring Harbor, New York

Namuth DM, Lapitan NLV, Gill KS, Gill BS (1994) Comparative RFLP mapping of *Hordeum vulgare* and *Triticum tauschii*. Theor Appl Genet 89:865–872

Nevo E (1986) Genetic resources of wild cereals and crop improvement: Israel a natural laboratory. Israel J Bot 35:255–278

Nevo E (1992) Origin, evolution, population genetics and resources for breeding of wild barley *Hordeum spontaneum* in the fertile crescent. In: Shewry PR (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International Oxon, UK, pp 19–43

Noda K, Kasha KJ (1978) A proposed karyotype revision based on C-band chromosome identification. Crop Sci 18:925–930

Porter Dr, Nguyen HT, Burke JJ (1989) Chromosomal location of genes controlling heat-shock proteins hexaploid wheat. Theor Appl Genet 78:873–878

Powell W, Borrino EM, Allison MJ, Griffiths DW, Asher MJC, Dunwell JM (1986) Genetical analysis of microspore-derived plants of barley (*Hordeum vulgare*). Theor Appl Genet 72: 619–626

Shin JS, Chao S, Corpuz L, Blake, T (1990) A partial map of the barley genome incorporating restriction fragment length polymorphism, polymerase chain reaction, isozyme and morphological marker loci. Genome 33:803–810

Singh RJ, Tsuchiya T (1982) Identification and designation of telocentric chromosomes in barley by means of Giemsa N-banding technique. Theor Appl Genet 64:13–24

Takahashi R, Yasuda S (1970) Genetics of earliness and growth habit in barley. In: Nilan RA (ed) Barley genetics II. Washington State University Press, pp 388–408

Tsuchiya T (1987) Linkage maps of barley. In: O'Brien S (ed) Genetic maps, vol 4. Cold Spring Harbor Press, New York, pp 708–731

Wu CH, Caspar T, Browse J, Lindquist S, Sommerville C (1988) Characterization of an HSP70 cognate gene family in *Arabidopsis*. Plant Physiol 88:731–740

Zamir D, Tanksley SD (1988) Tomato genome is comprised largely of fast-evolving, low-copy number sequences. Mol Gen Genet 213:254–261

Zohary D, Hopf M (1988) Domestication of plants in the old world. Clarendon Press, Oxford, England