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A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome

A. Kleinhofs[1,2], A. Kilian[1,*], M. A. Saghai Maroof[3], R. M. Biyashev[3], P. Hayes[4], F. Q. Chen[4], N. Lapitan[5], A. Fenwick[5], T. K. Blake[6], V. Kanazin[6], E. Ananiev[6,], L. Dahleen[7], D. Kudrna[1], J. Bollinger[1], S. J. Knapp[4], B. Liu[4], M. Sorrells[8], M. Heun[8,***], J. D. Franckowiak[9], D. Hoffman[10], R. Skadsen[11], B. J. Steffenson[12]**

[1] Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420, USA
[2] Department of Genetics and Cell Biology, Washington State University, Pullman, WA 99164-6420, USA
[3] Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[4] Crop Sciences Department, Oregon State University, Corvalis, OR 97331, USA
[5] Department of Agronomy, Colorado State University, Fort Collins CO 80523, USA
[6] Department of Plant and Soil Sciences, Montana State University, Bozeman, MT 59715, USA
[7] USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105, USA
[8] Department of Plant Breeding, Cornell University, Ithaca, NY 14853-1902, USA
[9] Department of Crop and Weed Sciences, North Dakota State University, Fargo, ND 58105-5051, USA
[10] USDA-ARS, National Small Grains Germplasm Research Facility, Aberdeen, ID 83210, USA
[11] Cereal Crops Research Unit, Madison, WI 53705-2334, USA
[12] Department of Plant Pathology, North Dakota State University, Fargo, ND 58105, USA

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Abstract. A map of the barley genome consisting of 295 loci was constructed. These loci include 152 cDNA restriction fragment length polymorphism (RFLP), 114 genomic DNA RFLP, 14 random amplified polymorphic DNA (RAPD), five isozyme, two morphological, one disease resistance and seven specific amplicon polymorphism (SAP) markers. The RFLP-identified loci include 63 that were detected using cloned known function genes as probes. The map covers 1,250 centi-Morgans (cM) with a 4.2 cM average distance between markers. The genetic lengths of the chromosomes range from 124 to 223 cM and are in approximate agreement with their physical lengths. The centromeres were localized to within a few markers on all of the barley chromosomes except chromosome 5. Telomeric regions were mapped for the short (plus) arms of chromosomes 1, 2 and 3 and the long (minus) arm of chromosomes 7.

Key words: RFLP – Mapping – Barley – Genome – Centromeres

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* *Permanent address:* Department of Genetics, Silesian University, Katowice, Poland

** *Permanent address:* Department of Plant Genetics, NI Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow

*** *Permanent address:* Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616, USA

Correspondence to: A. Kleinhofs

Introduction

Barley (*Hordeum vulgare* L.) is a diploid organism with seven large, cytologically distinct chromosomes containing approximately 5.5×10^9 bp DNA (Bennett and Smith 1976). The barley genome is well characterized with respect to classical genetics and cytogenetics. Over 1,000 genes and 500 translocation stocks are

known, but only a small number of these have been mapped to specific locations on the barley chromosomes (von Wettstein-Knowles 1992; Sogaard and von Wettstein-Knowles 1987).

Partial restriction fragment length polymorphism (RFLP) maps of the barley genome have been published (Kleinhofs et al. 1988; Shin et al. 1990; Hinze et al. 1991). Recently, more extensive RFLP linkage maps have been constructed (Heun et al. 1991; Graner et al. 1991).

The use of RFLPs as markers to construct genetic maps was first proposed by Botstein et al. (1980), and since then they have been extensively used to construct genetic maps in many organism. RAPD (random amplified polymorphic DNA) genetic marker technology (Williams et al. 1990) for developing genetic maps and for plant breeding has yet to be proven. SAP (specific amplicon polymorphism) is a term we have applied to specific primer amplified genomic DNA regions (amplicons) whose polymorphism can then be analyzed by numerous techniques. These include gel electrophoresis, with or without digestion with restriction enzymes, denaturing gradient gel electrophoresis, heteroduplex mobility shift and single-strand conformational polymorphism.

For utility in solving practical barley breeding problems, four elite barley cultivars were chosen as parents, two six-rowed cultivars 'Steptoe' and 'Morex' and two two-rowed cultivars 'Harrington' and 'TR306'. At least 150 doubled haploid lines (DHL) were developed from each cross. Doubled haploids simplify analyses and provide an immortal reference population for mapping and phenotype assessment work.

This paper presents a molecular, isozyme and morphological marker map of the barley genome developed in the 'Steptoe'/'Morex' population. The molecular markers include RFLP, RAPD and SAP. This map is unique since for the first time the centromere regions are identified for most chromosomes and some of the telomeres have been mapped. In addition, most of the cloned barley and wheat known function genes were mapped. This map provides coverage of the barley genome to an average distance of 4.2 cM with only a few gaps exceeding 20 cM.

Materials and methods

Plant materials

A population of 150 DHLs developed from the 'Steptoe' × 'Morex' cross by the *Hordeum bulbosum* method (Chen and Hayes 1989) were chosen at random from 310 DHLs and used to construct the map. A single plant from each cultivar was chosen and selfed to provide the plants used for the cross. This ensured that the plant material was homozygous and homogeneous. The parents, 'Steptoe' and 'Morex', were selected for their diversity of agronomic traits and good DNA polymorphism. 'Steptoe' is a high yielding, broadly adapted six-rowed feed-type barley (Muir and Nilan 1973). 'Morex', a midwestern six-rowed cultivar used as the American malting industry standard, was developed at the University of Minnesota, St. Paul Minn. (Rasmusson and Wilcoxson 1979). 'Steptoe', selected from a 'WA3564'/'Unitan' cross, is a Coast-type barley originating in North Africa. 'Morex', selected from a 'Cree'/'Bonanza' cross, is derived from the Manchuria type of barley that originated in Manchuria.

Seed of the parents and DHLs are available from P. Hayes, Oregon State University, Corvallis, Ore. North American barley Genome Mapping Project (NABGMP) probes are available from A. Kleinhofs, Washington State University, Pullman, Wash.

Seed of wheat-barley telosomic addition lines (Islam 1983) were obtained form Dr. B. Gill, Kansas State University, Manhattan Kan.

Anonymous function clones

Anonymous barley cDNA probes were isolated from cDNA libraries constructed from 'Steptoe' seedling leaf mRNA in pUC13 or malted seedling polysomal RNA in Lambda ZAP II. These are designated ABC (American Barley cDNA) plus an arbitrary number. When a probe identified more than 1 locus, a capital letteer was added following the arbitrary number to differentiate them. Lambda ZAP II clones were converted to pBluescript according to the manufacturer's protocol (Stratagene, La Jolla Calif.). Plasmid miniprep DNA was prepared by the boiling CTAB method (Del Sal et al. 1989). The DNA was digested with the appropriate restriction enzyme and separated on 1% low-melting-point agarose; the insert was then cut out and stored at −20 °C. Alternatively, the inset was amplified by the polymerase chain reaction (PCR) using M13 forward (GTA AAA CGA CGG CCA GT) and M13 reverse (AAC AGC TAT GAC CAT G) primers.

Anonymous barley genomic clones were isolated from a cv 'Shin Ebisu 16' *Pst*I genomic library. These are designated with ABG (American Barley Genomic) numbers. BG123, previously mapped by Heun et al. (1991), also came from this library. The genomic DNA was digested with *Pst*I and separated on a 10–40% sucrose density gradient; the 0.5- to 2-kb fragments were collected and cloned into pGEM-4 vector (Promega). Inserts were prepared as described above except that GF (GAT CCT CTA GAG TCG AC) and GR (GAG ACA AGC TTG CAT GC) primers were used (Heun et al. 1991).

Barley cDNA (BCD), oat cDNA (CDO) and wheat genomic (WG) clones are described in Heun et al. (1991). *Triticum tauschii* genomic clones, designated ksu, were obtained from Dr. B. Gill (Gill et al. 1991). Wheat cv 'Chinese Spring' cDNA clones, designated PSR, were obtained from Dr. M. Gale (Chao et al. 1988).

Known function clones

Known function clones from barley, wheat and maize were obtained from individual investigators. Previously used or proposed gene symbols were used as much as possible. If previous gene designations were not available, new symbols were proposed in accordance with barley gene designation rules (Barley Genetics Committee 1981). When it was not possible to determine which previously used locus designation corresponded to the locus or loci uncovered by an RFLP probe, a new locus number was temporarily assigned. For example, we mapped 5 alcohol dehydrogenase loci using an alcohol dehydrogenase cDNA probe (Good et al. 1988), but could not be sure which of

the RFLP loci corresponded to *Adh1, 2* and *3* loci identified by isozyme analyses (Sogaard and von Wettstein-knowles 1987).

Primers

Primers for RAPDs were purchased from Operon Technologies, Inc (Alameda, Calif.) or Genosys Biotechnologies, Inc (The Woodlands, Tex.). These are designated ABR (American Barley RAPD) plus an arbitrary number. Primers for amplification of specific genomic regions were synthesized by standard phosphoramidite chemistry on an Applied Biosystems 391 DNA synthesizer. Some primers were custom made by The Laboratory of Biotechnology and Bioanalyses at Washington State University, Pullman.

Molecular markers

RFLPs

The plant DNA isolation, prehybridization, hybridization and washing of membranes were modified from Sharp et al. (1988). Barley DNA was extracted from freeze-dried, 3- to 4-week-old seedling leaves by a combination of the proteinase K and CTAB methods. Reactions were stopped with a bromophenol blue, glycerol and EDTA dye mix and separated on 0.8% agarose in TAE buffer (Sambrook et al. 1989). Runs were terminated when the blue dye had migrated approximately 10 cm.

DNA was transferred from the gels to charged nylon membranes (generally GeneScreen Plus) according to Reed and Mann (1985) except that a 0.4 *M* NaOH plus 0.6 *M* NaCl solution was used. The membrane was air dried at room temperature for at least 3 h before further use.

The dried membranes were rinsed twice in 2 × SSC for 1–2 min and prehybridized for at least 6 h for new membranes and 3 h for previously used membranes. The membranes were exposed to X-ray film (Kodak X-OMAT AR) with one intensifying screen (DuPont Cronex Lightning Plus) for 4–5 days or as needed.

RAPDs

RAPDs were analyzed by several different methods. The method yielding the best results to date is described. Reactions were performed in 12.5 ul containing 50 ng DNA, 12.5 pmoles (1 μ*M*) primer, 100 μ*M* dNTPs, 0.5 U Taq DNA polymerase (Promega) and 1 × buffer (Promega 10 × = 100 m*M* TRIS pH 9.0, 500 m*M* KCl, 15 m*M* $MgCl_2$, 0.1% gelatin, 1% Triton X-100). The reactions were covered with two drops of mineral oil. Several thermal cyclers were used, but most commonly a MJ Research, Inc microtiter plate model was used with Falcon 3911 microtiter plates covered with Saran Wrap.

Reactions were incubated for 40 cycles of 1 s at 94 °C, 30 s at 92 °C, 2 min at 36 °C, a 3-min ramp to 72 °C, and 2 min at 72 °C. The 40 cycles were followed by 7 min at 72 °C, and the reaction was then held at 10 °C until used.

To recover the samples, 25 μl of stop mix (7.5% Ficoll, 0.75 × TBE and bromophenol blue) was added, and the samples were loaded into 4.5 by 1.5 mm wells before the running buffer (TBE) was added. Electrophoresis was conducted in 1% agarose plus 1% NuSieve GTG (FMC) agarose in TBE at 80 V for 3 h. PCR products were visualized by ethidium bromide staining and recorded for analyses by photography.

SAPs

Specific amplicon polymorphisms were designated ABA for American Barley Amplicon plus an arbitrary number. Primers were designed based on either published or determined sequences of clones used in mapping. Reactions were run and polymorphism identified as described (Tragoonrung et al. 1992).

Isozymes

Polymorphisms for five isozymes were identified in the 'Steptoe'/'Morex' cross. These are esterase 5 and 9 (*Est5*; *Est9*), aconitate hydratase 2 (*Aco2*), alkaline β-galactosidase 1 (*Bgl1*) and phosphogluconate dehydrogenase 2 (*Pgd2*). The isozyme markers are prefixed with an "i" to differentiate them from RFLP markers. A piece of leaf tissue was cut from young seedlings and ground in 20 μl of extraction buffer (Wendel and Parks 1982). Samples were absorbed to filter paper wicks and run in starch gels. A TRIS-citrate/lithium borate buffer (Selander et al. 1971) was used to separate *Bgl1* allozymes. They were visualized by a staining system described by Muehlbauer et al. (1989) and modified by Hoffman and Goates (1990). The proposed locus designation was changed from *Gal* to *Bgl* since *Gal* has been previously used in barley to designate a different locus. A histidine pH 6.5 system was used to resolve *Aco2* allozymes, and these were stained using the method of Soltis et al. (1983). For *Est5*, *9* and *Pgd2*, gels were run and stained according to Nielsen and Johansen (1986).

Morphological markers

Two morphological marker polymorphisms in the 'Steptoe'/'Morex' cross are rachilla hair length (*Srh* or *S*) and leaf blade pubescence (*Pub*). These designations are prefixed with "m" to differentiate them from RFLP markers. The rachilla hair length is a classical reference locus for chromosome 7 (Sogaard and von Wettstein 1987). Two naturally occurring alleles are known, i.e. long, unicellular hairs and short, multicellular hairs (Smith 1951). The gene *Pub* produces a sparse arrangement of short hairs on leaf blades along the leaf veins. The presence of these hairs is controlled by a single dominant gene (Wolfe and Franckowiak 1991).

A gene (*Rpg1*), conferring resistance to the stem rust pathogen *Puccinia graminis* f. sp. *tritici* (Steffenson et al. 1991), was mapped by evaluating DHLs for resistance to race Pgt-MCC of *P. graminis* f. sp. *tritici* in the seedling stage.

Telomeres

Putative telomere specific clones were obtained and characterized as described in Kilian and Kleinhofs (1992). Polymorphisms detected with these clones were mapped where possible. Also, a 34-nucleotide, telomere-specific primer based on the sequence of *Arabidopsis thaliana* telomere repeats was used at reduced stringency to generate additional polymorphisms.

Centromeres

Centromere positions were identified by testing markers with the barley-wheat telosome addition lines. Markers were hybridized individually to blots containing DNA from the barley-wheat telosome addition lines until the two most closely spaced markers associated with opposite arms were found.

Map construction

The Macintosh II version of MAPMAKER (Lander et al. 1987; supplied by Du Pont, Wilmington, Del.) and G-MENDEL v2, an unreleased update of GMENDEL (Liu and Knapp 1990) were used to construct the maps. Recombination values were converted to cM using the Kosambi function Kosambi 1944).

Results and discussion

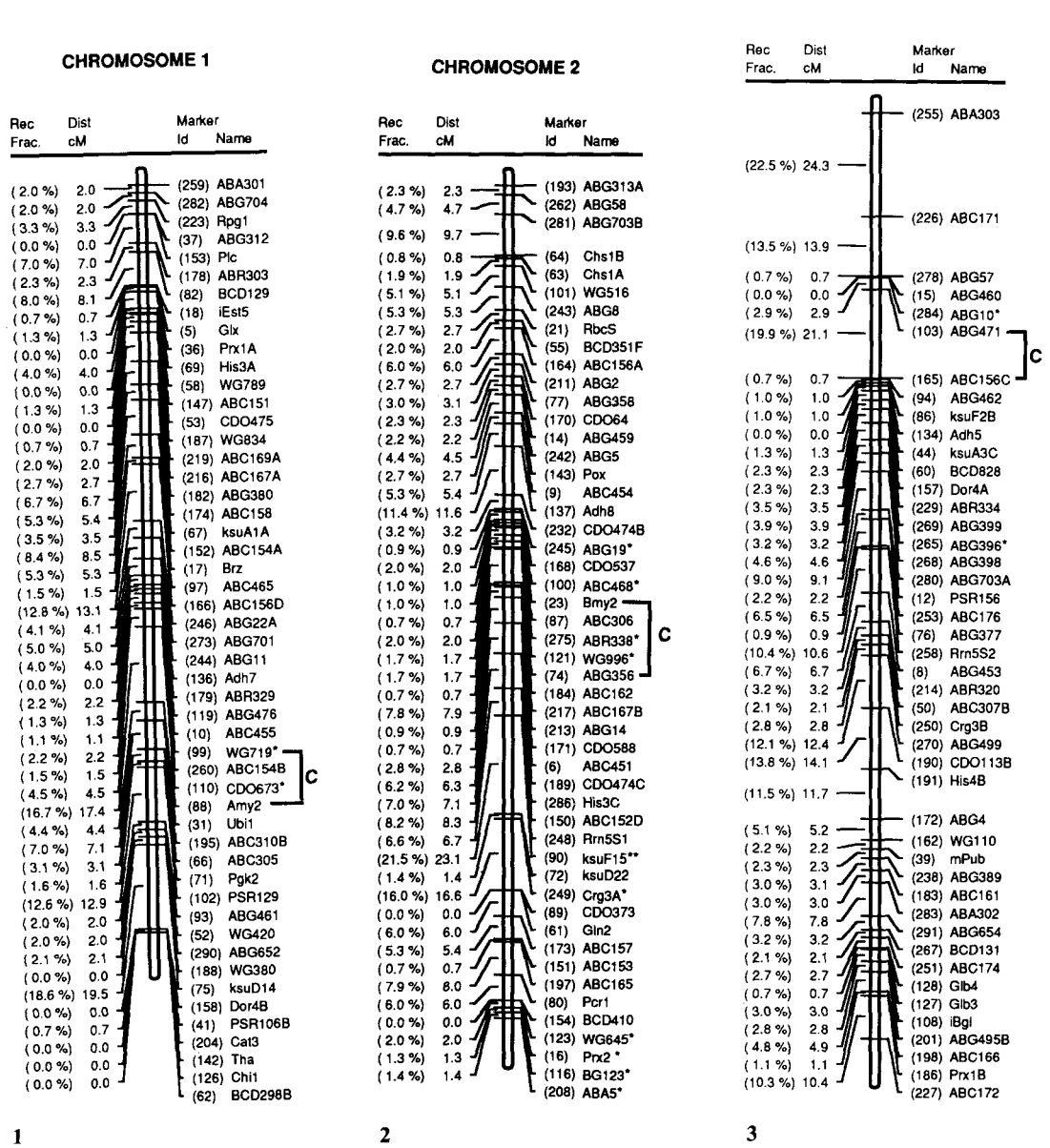
Polymorphism

All probes were screened for polymorphism against the four parents ('Steptoe', 'Morex', 'Harrington' and 'TR306') with 6 restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I). Increasing the number of restriction enzymes to 12 (*Apa*I, *Ase*I, *Nde*I, *Taq*I, *Bcl*I, *Bst*EII added) only slightly increased the number of polymorphisms detected.

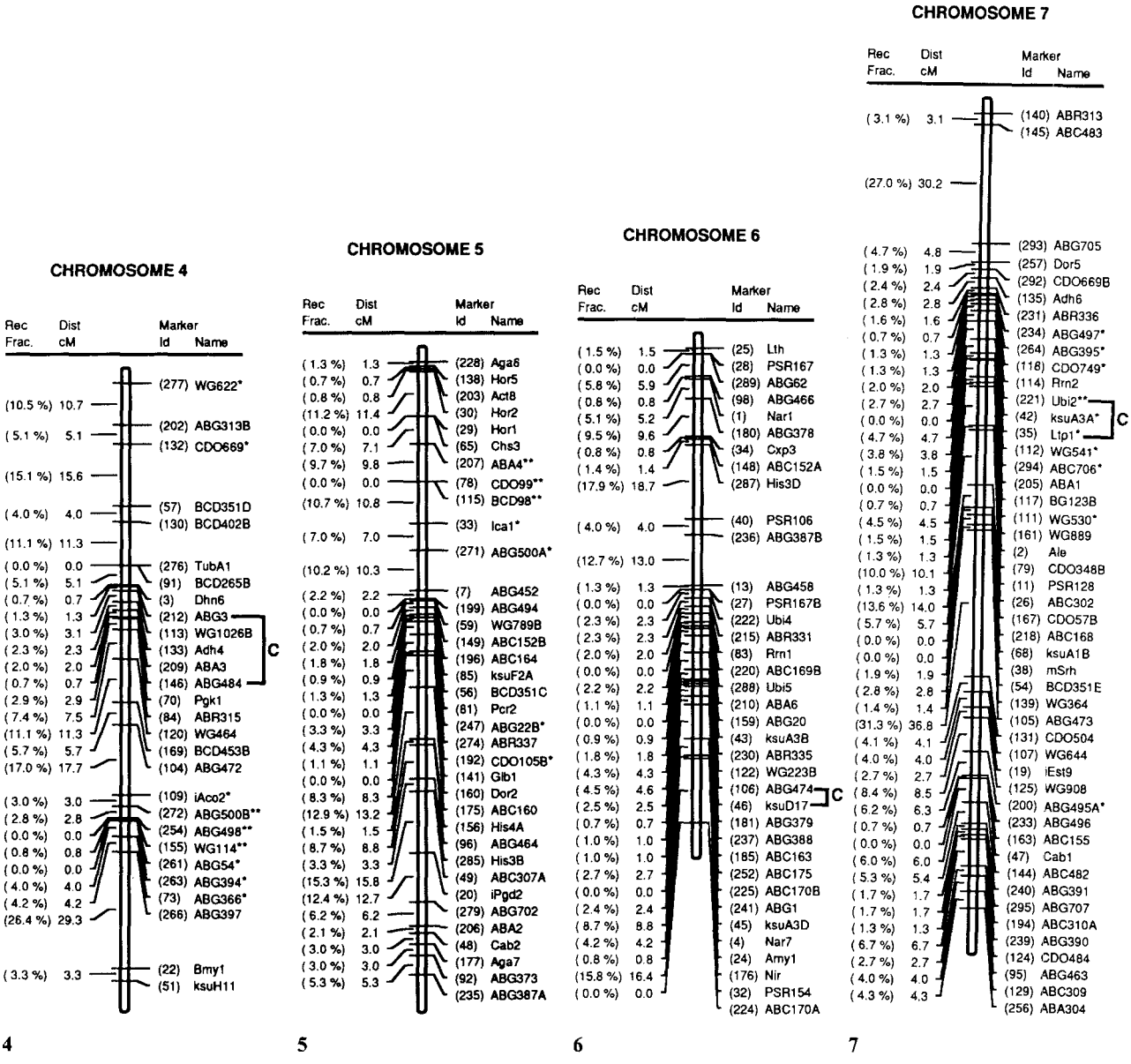
Chromosome maps

The chromosome maps (Figs. 1–7) are shown with recombination fraction and distance in cM indicated between adjacent markers.

Several barley chromosomes are metacentric, which may result in a confusion of the "short" and "long" arm designations, particularly for chromosome 1 (Singh and Tsuchiya 1982). In order to avoid this problem we have adopted the proposal of Linde-Laursen and Jensen (1992) to designate one arm the plus (P)



Figs. 1–3



Figs. 1–7. RFLP maps of the barley genome. Markers showing distorted segregation are indicated by * for $P = 0.05$ and ** for $P = 0.01$. Due to space restrictions by the editor, marker description is not published, but is available by E-mail on the NABGMP bulletin board accessible at "wsuvms 1.csc.wsu.edu", user "barmapbb", password "steptoe". This information is also deposited in the "triticeae data base" and will be placed on the "gopher" network

arm and draw it upwards on the idiogram and the other arm the minus (M) arm and draw it downwards. Thus, the previously designated short and 1β arms become the plus arms and the long and 1α arms becocme the minus arms.

Marker distribution and linkage

The 'Steptoe'/'Morex' map includes 266 RFLP, 14 RAPD, seven SAP, five isozyme, two morphological and one disease resistance markers. The RFLP markers include 152 cDNA probes and 114 genomic DNA probes. Their distribution among the seven barley chromosomes is presented in Table 1.

Among the 295 markers used to construct this map, 3% show segregation distortion at $P = 0.01$ and 14% show segregation distortion at $P = 0.05$. These values are close to what would be expected by random chance. The distorted segregation markers showed clustering on chromosomes 2, 4, 5 and 7. Heun et al. (1991) and

Table 1. Distribution of markers and gentic lengths of the seven barley chromosomes

Chromosome		Markers				
	cM	cDNA	Genom	RAPD	Other[a]	Total
1	180	30	16	2	3	51
2	202	30	18	1	1	50
3	223	18	21	4	2	45
4	154	11	14	1	2	28
5	160	22	10	1	3	36
6	124	19	15	2	1	37
7	207	22	20	3	3	48
Total	1,250	152	114	14	15	295

[a] Other: includes isozymes, morphological markers, disease resistance genes and amplicons

Graner et al. (1991) also observed distorted segregation for some markers, but some of the chromosome regions exhibiting clusters were different. Heun et al. (1991) and Graner et al. (1991) mapped with DHLs obtained by anther culture, while our DHLs were derived by the *bulbosum* method. Thus, it is not possible to differentiate between bias introduced by selection pressure during doubled haploid line development and random chance.

The markers mapped on the 'Steptoe'/'Morex' cross cover 1,250 cM, yielding an average value of 4.2 cM per marker. This establishes barley among the most thoroughly mapped crop plants. The distribution of the markers among the seven barley chromosomes and the genetic length of each chromosome are presented in Table 1. The distribution of markers along the chromosomes and linkage relationships are presented in Figs. 1–7. There are three gaps of approximately 30 cM, one on chromosome 4 and two on chromosome 7. We feel confident that the arrangement of the markers along the chromosomes is correct, based on their arm locations determined with the barley-wheat telosome addition lines. The paucity of markers in the chromosome 4 gap (*ml-o* region) was also noted by Hinze et al. (1991). The gaps on chromosome 7 also occur in comparable position on the 'Proctor'/'Nudinka' map (Heun et al. 1991). Similar large gaps also occur on the chromosome 7 'Igri'/'Franka' and 'Vada'/*Hordeum vulgare* subsp. *spontaneum* maps (Graner et al. 1991), although direct comparisons with the 'Steptoe'/'Morex' map are not possible due to the lack of commonn markers.

Centromere locations

Centromere locations are shown on the maps between brackets connecting the two markers determined to reside on opposite arms of the same chromosome (Figs. 1–7). Centromere location for chromosome 5 could not be determined since telosome addition lines for this chromosome were not available.

Telomere markers

Telomere-specific clones generated using the "vectorette" system (Kilian and Kleinhofs 1992) were used to map the telomeres. Fourteen unique clones have been identified and partially characterized to date. Many of these clones, but not all, are similar to the HvRT family of repetitive sequences isolated from barley (Belostotsky and Ananiev 1990). Using these clones as probes we mapped the telomeric regions of chromosome arms 1P and 2P. These markers have been designated with an arbitrary number ABG312 and ABG313A, respectively, until their telomeric position can be verified. Another putative telomeric marker, ABG313B, mapped proximal to the most distal marker (WG622) on chromosome 4P. The mapping relationship between these two markers deserves further study. Two probable telomeric regions were identified using the telomeric primer at a non-stringent temperature. Annealing at a stringent temperature resulted in a lack of amplification of any DNA bands. Regions identified by the telomere primer were designated ABA303 and ABA304 and map to the telomeric regions of chromosome arms 3P and 7M, respectively. One band, ABA302, however, mapped to an internal region of chromosome 3. A specific primer based on the sequences of one of the telomere-associated clones was designed and used, in association with the telomere primer, to map what appears to be the most telomeric position on chromosome arm 1P. This marker was designated ABA301 (*Tel1P*).

Relation to other published maps

The number of markers placed on each chromosome and the genetic distances are summarized an compared with other published barley RFLP maps in Table 2. In most cases, the genetic lengths of the chromosomes are similar, particularly when the 'Steptoe'/'Morex' map is compared with the 'Vada'/*Hordeum vulgare* subsp. *spontaneum* (V/Hs) map. This observation is significant considering that the V/Hs map is based on an F_2 population derived from a cultivated by wild barley cross and based on much fewer markers (Graner et al. 1991). Unfortunately, there is not a sufficient number of common markers on the 'Steptoe'/'Morex' and V/Hs maps to allow meaningful comparisons of map distances within the chromosomes. Comparisons of map distances between common markers on the 'Steptoe'/'Morex' and 'Proctor'/'Nudinka' maps have been made (Kleinhofs and Kilian 1992). The total number of

Table 2. Comparison among different crosses of the number of markers (M) and cM mapped for each chromosome

Chromosome	Cross[a]							
	S/M		P/N		V/H.s.		I/F	
	M	cM	M	cM	M	cM	M	cM
1	51	180	32	189	19	218	26	186
2	50	202	38	194	29	230	22	225
3	45	223	14	195	30	223	18	207
4	28	154	16	114	18	180	4	32
5	36	160	20	163	19	152	13	109
6	37	124	9	39	20	174	10	135
7	48	207	25	197	28	226	25	245
Total	295	1,250	154	1,091	163	1,403	118	1,130

[a] The crosses are: S/M, 'Steptoe' by 'Morex'; P/N, 'Proctor' by 'Nudinka'; V/H.s., 'Vada' by *Hordeum vulgare* subsp. *spontaneum*; I/F, 'Igri' by 'Franka'

RFLP markers mapped to the barley genome exceeds 800.

Marker choice

RFLPs

cDNA and genomic DNA clones were used as probes with equal success. Slightly more cDNA than genomic probes are on the map primarily due to our interest in known function genes, which are mostly cDNA clones. No apparent bias in the distribution of cDNA or genomic probe markers along the genome was observed.

RAPDs

We have mapped 14 loci using the RAPD technique. These have come at a great expense of time and effort. We find that RAPD markers often showed distorted segregation and were difficult to reproduce, thereby creating problems when determining map locations. These problems seriously detract from their otherwise excellent qualities of speed and high levels of polymorphism. We do not, at this time, recommend the use of RAPDs for mapping of the barley genome. However, we do not exclude the possibility that, with refined techniques, RAPD mapping of the barley genome may be possible in the future.

SAPs

We have limited experience with mapping using specific primers. However, it clearly appears to be the method of choice of tagging specific genes or chromosome regions. The results are reproducible and easily feasible for any laboratory with minimal equipment. This technique also should be transportable among numerous crosses and thus ideal for plant breeding applications. It is probably not the fastest or cheapest method for routine mapping.

Quality control

Due to the large amount of data generated, mapping projects are prone to a significant level or error. We were particularly concerned about this due to the participation of many laboratories and the need to handle the data several times. In order to minimize errors, we rigorously checked all data and feel confident that the map presented is robust.

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