Theor Appl Genet (1993) 86:629–636

Microdissection and microcloning of the barley (*Hordeum vulgare* L.) chromosome 1HS

J. Schondelmaier [2], R. Martin [1], A. Jahoor [2], A. Houben [4], A. Graner [3], H.-U. Koop [1], R. G. Herrmann [1], C. Jung [1]

[1] Institute of Botany, Ludwig-Maximilians-University Munich, Menzinger Straße 67, W-8000 Munich 19, Germany
[2] Institute for Plant Breeding, Technical University Munich, W-8050 Freising-Weihenstephan, Germany
[3] Institute for Resistance Genetics, Federal Biological Research Center for Agriculture and Forestry, W-8059 Grünbach, Germany
[4] Institute of Plant Genetics and Crop Plant Research, Corrensstraße 3, O-4325 Gatersleben, Germany

Received: 26 October 1992 / Accepted: 9 December 1992

Abstract. We have applied a refined microdissection procedure to create a plasmid library of the barley (*Hordeum vulgare* L.) chromosome arm 1HS. The technical improvements involved include synchronization of meristematic root tissue, a metaphase drop-spread technique, paraffin protection of the collection drop to avoid evaporation, and a motorized and programmable microscope stage. Thirteen readily-discernible telocentric chromosomes have been excised from metaphases of synchronized root-tip mitoses. After lysis in a collection drop (2 nl), the DNA was purified, restricted with *Rsa*I, ligated into a vector containing universal sequencing primers, and amplified by the polymerase chain reaction. Finally, the amplified DNA was cloned into a standard plasmid vector. The size of the library was estimated to be approximately 44,000 recombinant plasmids, of which approximately 13% can be utilized for RFLP analysis. Tandem repetitive probes could be rapidly excluded from further analysis after colony hybridization with labelled total barley DNA. Analysis of 552 recombinant plasmids established that: (1) the insert sizes ranged between 70 and 1150 bp with a mean of 250 bp, (2) approximately 60% of the clones contained highly repetitive sequences, and (3) all single- or low-copy probes tested originate from chromosome 1HS. Four probes were genetically mapped, using an interspecific *H. vulgare* × *H. spontaneum* F_2 population. One of these probes was found to be closely linked to the *Mla* locus conferring mildew resistance.

Key words: *Hordeum vulgare* – Microdissection – Microcloning – Chromosome specific library – RFLP

Communicated by F. Salamini

Correspondence to: C. Jung

Introduction

Molecular markers are phenotypically neutral and largely independent of allelic and non-allelic interactions. These attributes have been the principal reason for the construction of RFLP-based genetic maps. Such maps have been established for some important crops including tomato (Tanksley 1988), potato (Bonierbale et al. 1988; Gebhardt et al. 1989), maize (Coe et al. 1988), lettuce (Landry et al. 1987), sugarbeet (Pillen et al. 1992), and barley (Graner et al. 1991; Heun et al. 1991) and applied for uncovering linkage relationships to agronomically important traits in marker-assisted selection as well as for the isolation of genes of economic interest.

The resolution of available linkage maps, usually in the order of a few centimorgans (cM), is generally not sufficient to isolate such genes with a reasonable input of work. Apart from the fact that genetic distances and corresponding physical distances are not always consistent and may vary by one or two orders of magnitude, chromosome-walking techniques constitute a critical hurdle in this DNA size range, even with modern vector systems such as yeast artificial chromosomes (YACs). Therefore, there is an obvious need for high-density RFLP maps to minimize the physical distance between marker loci and the target gene. However, a high marker saturation is not readily attainable with customary techniques involving random selection of recombinant DNAs for RFLPs from 'shot-gun'-cloned libraries. In principle, this can be more easily achieved by chromosome-specific or subchromosomal DNA libraries. At present, two techniques are available which allow the cloning of defined regions of a complex genome: (1) flow-sorting of chromosomes (Davies et al. 1981; Conia et al. 1987; Wang et al. 1992), and (2) microdissection of metaphase chromosomes with glass needles (Lüdecke

et al. 1989) or laser microbeams (Hadano et al. 1991). However, the cloning of subpicogram quantities of DNA has generally been hampered by the availability of sufficient chromosome material, the purity of sorted chromosomes and the low cloning efficiency. Often, more than 100 chromosomes or chromosome segments had to be collected per cloning cycle (Edström et al. 1987). Furthermore, both approaches have almost exclusively been employed in human or animal material (Lüdecke et al. 1990; Senger et al. 1990; Kao and Yu 1991). A substantial improvement was achieved by collecting chromosomes in nl-sized drops and subsequent amplification of restricted DNA fragments using the polymerase chain reaction (PCR, Lüdecke et al. 1989). Recently, we applied this technique to generate a chromosome-specific DNA library from plants via microdissection. Avoiding the acid treatment routinely used for preparation and staining of plant chromosomes, a highly specific marker library was established for a *Beta patellaris* chromosome conferring nematode resistance (Jung et al. 1992).

We are particularly interested in the short arm of the barley chromosome 1H, since various genes of high agronomic importance have been characterized on that linkage group. These include the high amylose locus *amo1* (Schondelmaier et al. 1992), the hordein gene families *Hor1* and *Hor2* (Siedler and Graner 1991), and the locus *Mla* conferring resistance against powdery mildew caused by the fungus *Erysiphe graminis* f. sp. *hordei* (Jahoor and Fischbeck 1987), one of the most important barley diseases in temperate climates. The *Mla* locus, found within the interval (approximately 10 cM) between the *Hor1* and *Hor2* gene clusters, is highly polymorphic and consists of 28 different alleles or genes (Jahoor and Fischbeck 1993). Fifteen discrete RFLP patterns were detected for this locus by the use of a single, closely-linked molecular marker (Jahoor and Fischbeck 1993; Schüller et al. 1992).

Applying a refined microdissection procedure, we present data on the construction and basic characterization of an RFLP marker library from the microdissected barley chromosome arm 1HS.

Materials and methods

Genetic stocks and plant material

The telotrisomic line (2n = 14 + 1t) for chromosome 1HS used for microdissection was kindly provided by Prof. T. Tsuchiya (Fort Collins, USA). The individual barley chromosomes have been designated according to Ainsworth et al. (1986). Chromosomal assignment of DNA inserts was performed with a set of wheat-barley addition lines lacking the addition line for barley chromosome 1H (Islam 1983). The F_2 offspring of a cross between the spring barley cv 'Vada' and the *Hordeum spontaneum* line '1B-87' was used for RFLP mapping (Graner et al. 1991).

Preparation of metaphase spreads

Meristematic root-tip tissue was synchronized according to Doležel et al. (1992) and Pan et al. (1993). Barley seeds or seedlings were incubated on filter paper subsequently soaked with tap water (4 °C, 3 days, followed by 22 °C for 5 h), 1.25 mM hydroxyurea (room temperature, 18 h), distilled water (room temperature, 5 h), and 4 µM APM (room temperature, 3 h). APM [O-methyl-0-(2-nitro-p-tolyl)N-isopropyl-phosphoroamidothioate; amiprophosmethyl] was a gift from Bayer Leverkusen. Root tips of seedlings rinsed in distilled water were collected, incubated in ice water over night, then stored in 70% ethanol for 1 day, and washed in distilled water before incubation in an enzyme solution consisting of 2.5% pectolyase Y23, 2.5% cellulase R10, 75 mM KCl and 7.5 mM EDTA (pH 4) for 45 min. After treatment in 75 mM KCl for 15 min, the protoplast suspension was washed three times with 70% ethanol and centrifuged for 5 min at 75 *g*. The cell sediment was resuspended in 10 ml of fresh fixative (ethanol/glacial acetic acid, 3:1, *v/v*) and centrifuged for 2 min. The supernatant was removed except for 250 µl. This suspension was immediately dropped on ice-cold slides and used for microdissection after drying.

Microdissection and microcloning

An inverted microscope (Zeiss IM35) equipped with a microprocessor-controlled, programmable stage (Spangenberg and Koop 1992), a micromanipulator (Eppendorf 5170), and phase-contrast optics was used to select and manipulate suitable metaphase spreads at a maximum magnification of 640×. A 2 nl collection drop (10 mM Tris-HCl, pH 7.5; 10 mM NaCl, 0.1% SDS, 1% glycerol, 500 µg/ml proteinase K) was deposited on a depression slide overlaid with liquid paraffin (Merck #7161, spectroscopic grade). Thirteen telosomic chromosomes were collected in that drop. All subsequent steps followed essentially the protocols of Lüdecke et al. (1989), Senger et al. (1990) and Jung et al. (1992), except for the inactivation of the restriction enzyme *Rsa*I (15 min, 65 °C). The DNA fragments were ligated overnight at 15 °C and amplified as described by Lüdecke et al. (1990).

Characterization of plasmid clones

Transformed *E. coli* cells were plated onto selection medium containing ampicillin and X-gal. Recombinant plasmids were isolated by alkaline lysis. Inserts were excised by digestion with *Eco*RI or *Pvu*II and subjected to electrophoresis (1.5% agarose slab gel). The 1-kb DNA ladder (BRL) served as a size marker. The gel was then stained with ethidium bromide and the fragments were transferred onto Biodyne B membrane (Pall, Dreieich) using the alkaline technique (manufacturer's protocol). The blots were hybridized with total barley DNA radiolabelled (Feinberg and Vogelstein 1983) in the presence of ^{32}P-dATP and ^{32}P-dCTP. Plasmid inserts were separated in low-melting agarose, and labelled in the presence of ^{32}P-dCTP. A representative number of clones was used for genomic Southern hybridization of restricted barley DNA in order to determine the nature of the cloned inserts.

Genomic Southern analysis

Isolation, restriction, and Southern analysis of genomic DNA were performed according to Graner et al. (1990) and Jahoor et al. (1991). DNA of the barley cultivar 'Marinka' or the parental lines '1B-87' and 'Vada' was digested with the restriction endonucleases *Bam*HI, *Eco*RI, *Eco*RV, *Hind*III, or *Xba*I. 'Marinka' DNA was hybridized for 0.5–4 h with selected plasmid inserts in the presence of 5 × SSPE, 5 × Denhardt's solution, 0.2% SDS and 250 µg/ml of herring sperm DNA. After rinsing

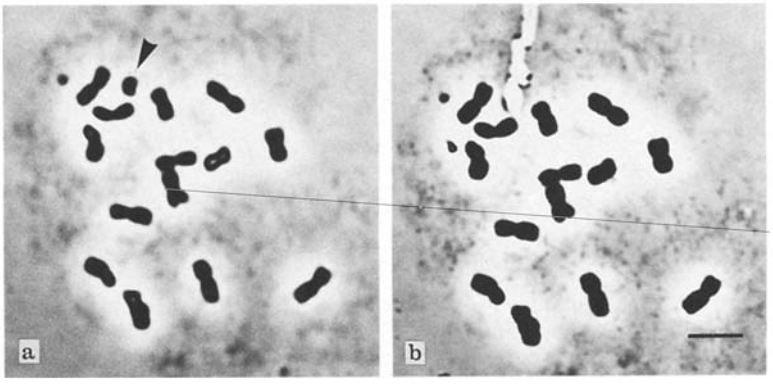

Fig. 1. Mitotic metaphase plate of the barley trisomic line 1HS (2n = 14 + 1t). **A** The telocentric chromosome is indicated by an *arrow*. **B** The univalent is removed from the metaphase plate. The bar represents 10 μm

in 1 × SSC, 0.1% SDS for 20 min, the filters were exposed to X-ray film (Amersham Hyperfilm-MP, −70 °C, overnight). Positive probes were subsequently hybridized with Southern blots of *Hin*dIII-digested DNA of the wheat barley addition lines (complete set except for chromosome 1H), of line '1B-87', and of the cultivar 'Vada'.

Colony hybridizations

Three hundred and sixty six recombinant plasmids were inoculated in duplicate onto 60-well microtiter plates (Nunc, Copenhagen), filled with LB-medium containing 1.5% agar. After overnight growth, filter lifts were taken from the first plate; the second plate was stored at 4 °C. The colony lifts were hybridized with radiolabelled genomic DNA (see above) and exposed for up to 6 days. Plasmid DNA was extracted from colonies which gave weak or even no signals using the minipreparation procedure described above.

Linkage analysis

Autoradiographs were scored visually to identify the chromosomal location of a DNA fragment and to detect fragment polymorphism between the parents. Four polymorphic clones were mapped in the F_2 population. Linkage analysis was performed using the computer programm MAPMAKER (Lander et al. 1987). The linkage groups were formed with linkage criteria of LOD = 3.0 and 0.30 recombination limits. Centimorgan distances were computed using the Kosambi function (Kosambi 1944). Linkage analysis between probe MWG1159 and the *Mla* locus was conducted by comparing the distances on our reference map with those in the segregating population used by Schüller et al. (1992), due to the lack of polymorphism of MWG1159 in that population (DH's of 'Igri' × 'Franka').

Results

Preparation and microdissection of metaphase plates

After monitoring the chromosome number of 18 seedlings from the barley telotrisomic line 1HS in squash preparations of highly-synchronized root tips under phase contrast, approximately 12 root tips of six plants harboring the additional telocentric chromosome (2n = 14 + 1t) were selected, pooled, and enzymatically macerated. Reduction of cytoplasmic background by three wash cycles of the cell suspension in 70% ethanol proved to be essential. Optimal spreading of metaphase plates by drop preparation requires fixation in 3:1 ethanol/glacial acetic acid (*v*/*v*). To minimize acid-depurination of DNA, this treatment was reduced to less than 3 min including centrifugation and dropping.

The number and quality of metaphase spreads could be significantly improved by synchronizing meristematic tissue and the application of the drop preparation technique. Routinely, approximately 20 metaphase cells per slide, in which the additional telocentric chromosome could be unequivocally identified and was spatially well separated from the other chromosomes, were accessible to microdissection (Fig. 1).

The use of a long-distance objective lens (magnification 32 ×, phase contrast) allowed the use of unstained chromosome preparations on microscope slides, thus avoiding the use of thin coverslips which are difficult to handle during microdissection. Microdissection and transfer of dissected chromosome fragments were substantially facilitated by use of a motorized and programmable stage. In an initial microscopical screening step, suitable metaphases were identified on the microscope slide and their positions were stored in the memory of the computer controlling the stage (Golds et al. 1992). Subsequently, these positions and the collection drop were automatically addressed in the sequence required by the microdissection procedure. The use of paraffin oil rather than a moist chamber (Senger et al. 1990) proved to be superior: (1) to prevent evaporation from the collection drop, (2) to ensure that the drop remains accessible throughout the entire procedure; (3) to guarantee that the paraffin layer does not hinder the delivery of the chromatin into the collection drop, and (4) so that transfer and delivery of a chromosome fragment can be easily monitored microscopically.

These modifications allowed the excision of 13 telocentric chromosomes in approximately 3 h, so ensuring that microdissection and digestion of chromatin with proteinase can be readily performed within 1 day.

Characterization of the plasmid library

Of the PCR-amplified DNA used for transformation 1.25% resulted in 552 recombinant plasmids. Thus, the available 1HS library comprised approximately 44,000 recombinant clones. Pre-screening of the library could be substantially accelerated by colony hybridization with radiolabelled total barley DNA (Fig. 2). The results were comparable to those obtained from the Southern hybridization of restricted plasmid inserts (see below). Approximately 43% of the colonies gave medium or strong signals. The remaining clones, which gave only faint or undetectable signals under the chosen conditions (exposure time: up to 6 days), contained a high fraction of single- or low-copy probes as revealed later by RFLP analysis (see below).

Table 1. Characterization of the barley 1HS plasmid library

Recombinant clones	44,000
Insert size (bp)	70–1150
Average size (bp)	250
Repetitives[a]	60%
Vector-like inserts	5%
Inserts >250 bp	18%
No signal	5.3%
Clones useful for mapping	5600 (12.7%)
Polymorphic probes	1580 (3.6%)

[a] Estimated from colony hybridization and genomic Southern hybridization

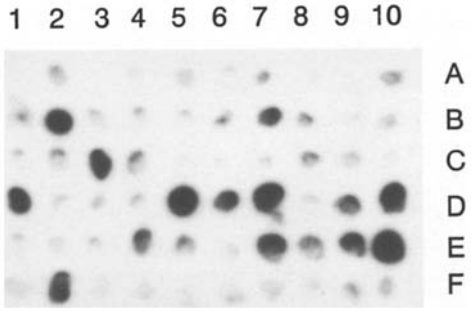

Fig. 2. Colony hybridization of 60 plasmid clones of the 1HS library. Immobilized DNA of the individual colonies was hybridized with labelled total barley DNA (cv 'Marinka'). Filter exposure: 6 days

The results of the plasmid analyses are listed in Table 1 and can be summarized as follows: (1) the insert sizes ranged between 70 and 1150 bp with an average of 250 bp (Fig. 3); (2) 47% of the clones proved to contain medium- or highly-repetitive inserts after hybridization with total barley DNA; (3) approximately 5% of them contained vector-like insertions, as judged from hybridization with radiolabelled plasmid DNA; (4) no signals were found when four randomly chosen (three low-copy, one repetitive) probes were hybridized with excised insert DNA of 167 plasmids indicating that the degree of clone redundancy due to amplification was relatively low.

RFLP analysis

In total, 82 selected clones (>120 bp) representing different levels of repetition were used for Southern analyses in order to determine the nature of the inserts: 'Marinka' DNA was hybridized with 58 single- or low-copy clones as judged from the plasmid and colony hybridization experiments, and of these another 14 clones were found to carry repetitive inserts. Summarizing the results from colony hybridization and genomic Southern analysis, approximately 60% of the plasmid inserts originated from repetitive DNA (Table 1).

Fifty-two clones were hybridized with filter-bound DNA from the wheat-barley addition lines in order to verify the chromosomal origin of the plasmid inserts. Of these, 24 probes hybridized to DNA of more than one

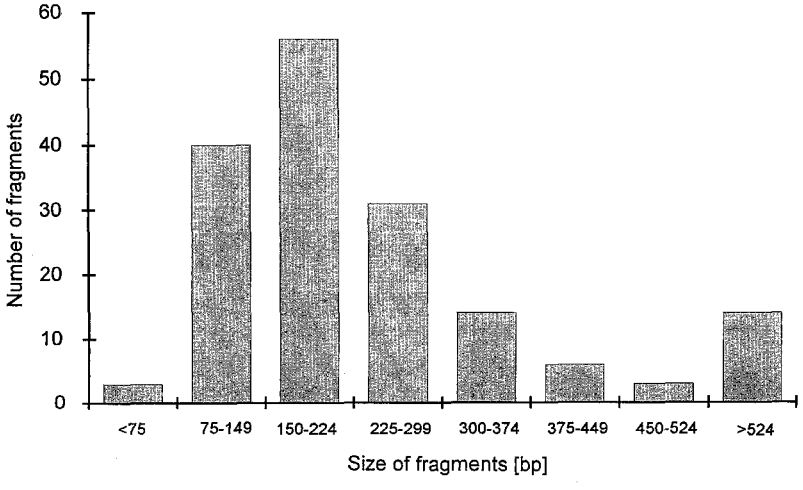

Fig. 3. Size distribution of inserts from 167 randomly chosen clones from the 1HS plasmid library

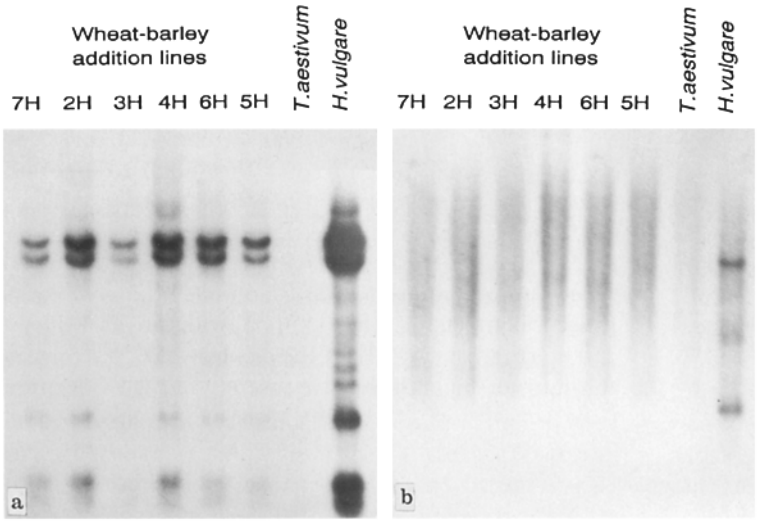

Fig. 4 A, B. Chromosomal localization of clones from the microdissection experiment. *Hin*dIII-digested DNA of six disomic wheat (cv 'Chinese Spring') barley (cv 'Betzes') addition lines was probed with the repetitive probes MWG1059 (**A**) and the single-copy probe MWG1124 (**B**). A wheat line containing a barley chromosome 1H addition was not available. The absence of any RFLP fragments in the addition lines indicates that marker MWG1124 is located on chromosome 1H (**B**)

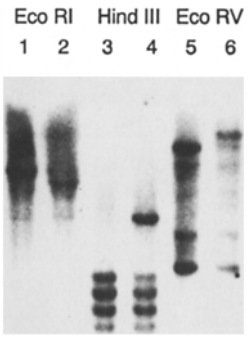

Fig. 5. RFLPs between 'Vada' (*lanes 1, 3, 5*) and '1B-87' (*H. spontaneum*) DNA (*lanes 2, 4, 6*) revealed after hybridization with the probe MWG1159 (130 bp). The restriction endonuclease used were *Eco*RI, *Hin*dIII and *Eco*RV, respectively. Exposure time: 8 days

barley chromosome (Fig. 4A) with patterns characteristic for repetitive DNA; 20 probes did not hybridize with DNA from any of the six addition lines, but clear signals appeared with DNA of the barley cultivar 'Betzes' (Fig. 4B). Since an addition line containing chromosome 1H of barley was not available, this proves that these probes originated from the microdissected 1H chromosome. The remaining probes gave only faint signals.

Finally, 62 out of the 82 clones were tested for RFLPs between the parental lines 'Vada' and '1B-87' (Fig. 5). Twenty-three probes showed repetitive signals, 11 probes showed no signals, 28 probes were single- or low-copy, and of these, eight probes were found to be polymorphic in the mapping population and four of them were chosen for linkage analysis (Fig. 6). All four were localized on linkage group 1H; the probe MWG1159 turned out to be closely linked with the *Mla* locus (3 cM).

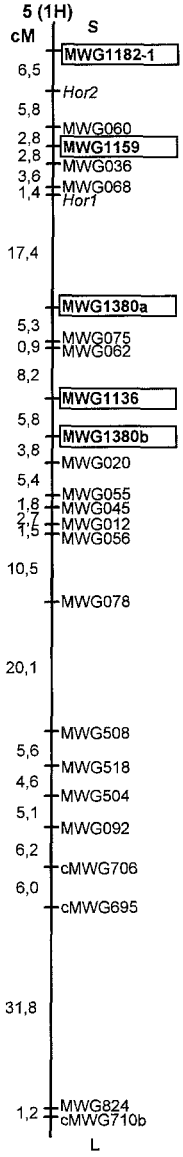

Fig. 6. Linkage group 5 of barley corresponding to chromosome 1H. The top part of the map represents the short arm of the chromosome. The exact position of the centromere is not known. The position of clones selected from the microdissection library are *boxed*. Clone MWG1380 detects two loci indicated by *a* and *b*, respectively

Discussion

Major strategies employed to tag genes which can only be detected by phenotype rest on tightly-linked RFLP markers, fine-scale physical maps around the target gene, and sets of overlapping cosmid or YAC clones (contigs). Although subgenomic DNA libraries offer the possibility to construct high-density linkage maps, so far only two attempts have been published to clone DNA from individual plant chromosomes. Wang et al. (1992) generated a plasmid library from flow-sorted wheat chromosome 4A. Approximately 50% of the inserts when used as probes hybridized as chromosome-specific. An attempt to clone sequences from microdissected B-chromosomes of rye resulted in the recovery of only a single clone (Sandery et al. 1991) probably due to direct cloning into λ vectors.

All single-copy DNA inserts checked in the present study were chromosome-specific, implying that the risk of chromosomal contamination is low. The chosen approach is, therefore, superior to previously described microcloning techniques in plant material for two principal reasons. Firstly, the barley line used in this study carries the additional telosomic chromosome 1HS which is well discernible by its morphology in mitotic metaphase spreads. Secondly, the following technical improvements facilitated microdissection and the construction of subchromosomal libraries: (1) the use of mitotic metaphase spreads compared to meiotic preparations saves time since the plant material is available within 1 week, (2) metaphase spreads of appropriate quality can be obtained with synchronized meristematic root-tip tissue, (3) a drop-spread technique reduces fixation times to less than 3 min, (4) the use of mineral oil has a number of advantages over using a moist chamber (Senger et al. 1990) to prevent evaporation from the collection drop, (5) the instrument set-up allows the user to select suitable metaphases, to store the coordinates of their positions, and to automatically and sequentially address individual metaphases in a given collection drop. This approach is particularly useful when dissection results in several fragments per chromosome arm.

The clear identification of the desired chromosome or chromosome fragment is an indispensible prerequisite for the application of the microdissection technique. Unfortunately, the chromosome complements of many crop species lack pronounced morphological differences. The G-banding technique, routine in human chromosome microdissection (Lüdecke et al. 1989, 1990; Senger et al. 1990), is of little use in plants (Greilhuber 1977; Anderson et al. 1982). The HCl treatment required for C-banding in plants excludes the use of this approach in the identification of individual chromosomes for microdissection due to DNA damage. Aneuploid lines with readily identifiable chromosomes in metaphase plates can bypass these drawbacks, as demonstrated recently for univalent *B. patellaris* chromosomes from a monosomic sugar beet/wild beet addition line (Jung et al. 1992).

The cloning efficiency of our microcloning experiment (44,000 clones/5 pg DNA) is comparable to, or even higher than, that of equivalent libraries reported from man (Lüdecke et al. 1989) and beet (Jung et al. 1992). Barley has a relatively large genome, 5.5 pg per haploid genome corresponding to 5.3×10^9 bp. One barley chromosome arm contains approximately 0.4 pg of DNA which is equivalent to 3.8×10^8 bp. Approximately 12.7% of the clones were found to be of use for further characterization. Some 28.6% of the single- and low-copy probes displayed RFLPs between the parents of the interspecific cross. This is less than the polymorphism of 76% reported by Graner et al. (1991) from shot-gun-cloned *Pst*I barley libraries. Only a small fraction of the probes (5.3%) showed weak signals which could not be evaluated, possibly due to small insert sizes or the cloning of depurinated DNA fragments.

The selection of appropriate clones from the library is easy, since repetitive sequences can be readily identified by colony hybridization and excluded from further analysis. The barley genome consists of approximately 70% repetitive sequences (Rimpau et al. 1980) which agrees fairly well with the proportion of repetitive clones in the microdissection library and in shot-gun *Eco*RI-based barley libraries (unpublished results). Since transcribed sequences in higher plants are usually not methylated (Antequera and Bird 1988), the fraction of repetitive clones can probably be reduced if methylation-sensitive restriction endonucleases are used. Although the optimal insert size for RFLP analysis in barley has been suggested to be >400 bp (Jahoor et al. 1991), smaller inserts from the subchromosomal library gave appropriate signals. However, insert sizes may be increased using enzymes with hexamer recognition sequences in combination with appropriate linker adapters (Kao and Yu 1991).

There is substantial interest and potential in applying RFLP technology to barley improvement. The development of genetic maps of barley and their use in marker-assisted selection with a view to isolate genes or to detect traits valuable for barley breeding is of obvious value. Principal interests focus on genes conferring powdery mildew resistance (*Mla*, Schüller et al. 1992; *mlo*, Hinze et al. 1991), and high amylose content in the endosperm (*amo*1, Schondelmaier et al. 1992). The data presented indicate that the available 1HS library should be suitable for generating a high-density map around the *Mla* region. A set of established near-isogenic lines (Schüller et al. 1992) and tightly-linked RFLP markers, in combination with a recently constructed YAC library of the barley genome (Kleine et al. in preparation), will serve as starting points for gene characterization and isolation. Furthermore, experiments are in progress to establish a

full set of chromosome-arm-specific libraries for the barley genome.

Acknowledgements. The authors thank Mrs. Anina Schaefer and Mrs. Sabine Steiner for excellent technical assistance. This work was supported by the Bundesministerium für Forschung und Technologie (BMFT grant No 0318990F) and by the Fonds der Chemischen Industrie.

References

Ainsworth CC, Miller TE, Gale MD (1986) The genetic control of grain esterases in hexaploid wheat. II. Homoeologous loci in related species. Theor Appl Genet 72:219–225

Anderson KL, Stack SM, Mitchel JB (1982) An investigation of the basis of a current hypothesis for the lack of G-banding in plant chromosomes. Exp Cell Res 138:433–436

Antequera F, Bird AP (1988) Unmethylated CpG islands associated with genes in higher plant DNA. EMBO J 7:2295–2299

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Coe EH, Neuffer MG, Hoisington DA (1988) The genetics of corn. In: Sprague GF, Dudley JW (eds) Corn and corn improvement, vol 3. American Society of Agronomy Madison Wisconsin, pp 81–258

Conia J, Bergounioux C, Perennes C, Muller P, Brown S, Gadal P (1987) Flow-cytometric analysis and sorting of plant chromosomes from *Petunia hybrida* protoplasts. Cytometry 8:500–508

Davies KE, Young BD, Elles RG, Hill ME, Williamson R (1981) Cloning of a representative genomic library of the human X chromosome after sorting by flow cytometry. Nature 293:374–376

Doležel J, Čihaliková J, Lucretti S (1992) A high-yield procedure for isolation of metaphase chromosomes from root tips of *Vicia faba* L. Planta 188:93–98

Edström J-E, Kaiser R, Röhme D (1987) Microcloning of mammalian metaphase chromosomes. Methods Enzymol 151:503–516

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

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP-analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–77

Golds TJ, Babczinsky J, Rauscher G, Koop H-U (1992) Computer-controlled tracking of single cell development in *Nicotiana tabacum* L. and *Hordeum vulgare* L. protoplasts embedded in agarose/alginate films. J Plant Physiol 140:582–587

Graner A, Siedler H, Jahoor A, Herrmann RG, Wenzel G (1990) Assessment of the degree and the type of restriction fragment length polymorphism in barley (*Hordeum vulgare*). Theor Appl Genet 80:826–832

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

Greilhuber J (1977) Why plant chromosomes do not show G-bands. Theor Appl Genet 50:121–124

Hadano S, Watanabe M, Yokoi H, Kogi M, Kondo I, Tsuchiya H, Kanazawa I, Wakasa K, Ikeda J-E (1991) Laser microdissection and single unique primer PCR allow generation of regional chromosome DNA clones from a single human chromosome. Genomics 11:364–373

Heun M, Kennedy AE, Anderson JA, Sorrels ME, Tanksley SD (1991) Construction of a restriction-fragment-length-polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Hinze K, Thompson RD, Ritter E, Salamini F, Schulze-Lefert P (1991) RFLP-mediated targeting of the *ml-o* resistance locus in barley (*Hordeum vulgare*). Proc Natl Acad Sci USA 88:3691–3695

Islam AKMR (1983) Ditelosomic additions of barley chromosomes to wheat. Proc 6th Int Wheat Genet Symp Kyoto, Japan, pp 233–238

Jahoor A, Fischbeck G (1987) Source of resistance to powdery mildew in barley lines derived from *Hordeum spontaneum* collected in Israel. Plant Breed 99:274–281

Jahoor A, Fischbeck G (1993) Identification of new genes for mildew resistance of barley at the *Mla* locus in *Hordeum spontaneum*-derived lines. Plant Breed (in press)

Jahoor A, Backes G, Graner A, Herrmann RG, Fischbeck G (1991) Development of RFLP markers for barley. Plant Breed 107:73–76

Jung C, Fischer F, Claussen U, Horsthemke B, Herrmann RG (1992) A DNA library from an individual *Beta patellaris* chromosome conferring nematode resistance obtained by microdissection of meiotic metaphase chromosomes. Plant Mol Biol 20:503–511

Kao F-T, Yu J-W (1991) Chromosome microdissection and cloning in human genome and genetic disease analysis. Proc Natl Acad Sci USA 88:1844–1848

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

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

Landry BS, Kesseli RV, Farrara B, Michelmore RW (1987) A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331–337

Lüdecke H-J, Senger G, Claussen U, Horsthemke B (1989) Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification. Nature 338:348–350

Lüdecke H-J, Senger G, Claussen U, Horsthemke B (1990) Construction and characterization of band-specific DNA libraries. Hum Genet 84:512–516

Pan WH, Houben A, Schlegel R (1993) Highly effective cell synchronization in plant roots by hydroxyurea, amiprophosmethyl and colchicine. Genome (in press)

Pillen K, Steinrücken G, Wricke G, Herrmann RG, Jung C (1992) A linkage map of sugar beet (*Beta vulgaris* L.). Theor Appl Genet 84:129–135

Rimpau I, Smith DB, Flavell RB (1980) Sequence organization in barley and oat chromosomes revealed by interspecies DNA/DNA hybridization. Heredity 44:131–149

Sandery MJ, Forster JW, Macadam Sr, Blunden R, Jones RN, Brown SDM (1991) Isolation of a sequence common to A- and B-chromosomes of rye (*Secale cereale*) by microcloning. Plant Mol Biol Rep 9:21–30

Schondelmaier J, Jacobi A, Fischbeck G, Jahoor A (1992) Genetical studies on mode of inheritance and localization of the *amo1* (high amylose) gene in barley. Plant Breeding 109:274–280

Schüller C, Backes G, Fischbeck G, Jahoor A (1992) RFLP markers to identify the alleles on the *Mla* locus conferring

powdery mildew resistance in barley. Theor Appl Genet 84:330–338

Senger G, Lüdecke H-J, Horsthemke B, Claussen U (1990) Microdissection of banded human chromosomes. Hum Genet 84:507–511

Siedler H, Graner A (1991) Construction of physical maps at the *Hor1* locus of two barley cultivars by pulsed-field gel electrophoresis. Mol Gen Genet 226:177–181

Spangenberg G, Koop H-U (1992) Low-density cultures: microdroplets and single-cell nurse cultures. In: Lindsey K (ed) Plant tissue culture manual, supplement 1, section A. Kluwer Academic Publishers Dordrecht, Boston London, pp 1–28

Tanksley SD (1988) Molecular mapping of plant chromosomes. In: Gustafson P, Appels R (eds) Chromosome structure and function. Plenum Publ Corp, New York, pp 157–173

Wang ML, Leitch AR, Schwarzacher T, Heslop-Harrison JS (1992) Construction of a chromosome-enriched *Hpa*II library from flow-sorted wheat chromosomes. Nucleic Acids Res 20:1897–1901