Theor Appl Genet (2006) 113:651–659
DOI 10.1007/s00122-006-0329-8

ORIGINAL PAPER

Dissection of the nuclear genome of barley by chromosome flow sorting

Pavla Suchánková · Marie Kubaláková · Pavlína Kovářová · Jan Bartoš · Jarmila Číhalíková · Márta Molnár-Láng · Takashi R. Endo · Jaroslav Doležel

Received: 6 March 2006 / Accepted: 27 May 2006 / Published online: 30 June 2006

Abstract Isolation of mitotic chromosomes using flow cytometry is an attractive way to dissect nuclear genomes into their individual chromosomal components or portions of them. This approach is especially useful in plants with complex genomes, where it offers a targeted and hence economical approach to genome analysis and gene cloning. In several plant species, DNA of flow-sorted chromosomes has been used for isolation of molecular markers from specific genome regions, for physical mapping using polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH), for integration of genetic and physical maps and for construction of chromosome-specific DNA libraries, including those cloned in bacterial artificial chromosome vectors. Until now, chromosome analysis and sorting using flow cytometry (flow cytogenetics) has found little application in barley ($2n = 14$, 1C ~ 5, 100 Mbp) because of the impossibility of discriminating and sorting individual chromosomes, except for the smallest chromosome 1H and some translocation chromosomes with DNA content significantly different from the remaining chromosomes. In this work, we demonstrate that wheat–barley ditelosomic addition lines can be used to sort any arm of barley chromosomes 2H–7H. Thus, the barley genome can be dissected into fractions representing only about 6–12% of the total genome. This advance makes the flow cytogenetics an attractive tool, which may greatly facilitate genome analysis and gene cloning in barley.

Communicated by J. W. Snape

P. Suchánková · M. Kubaláková · P. Kovářová · J. Bartoš · J. Číhalíková · J. Doležel (✉)
Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovská 6, 77200 Olomouc, Czech Republic
e-mail: dolezel@ueb.cas.cz

M. Kubaláková · J. Číhalíková · J. Doležel
Department of Cell Biology and Genetics, Palacký University, Olomouc, Czech Republic

M. Molnár-Láng
Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary

T. R. Endo
Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Introduction

Barley (*Hordeum vulgare*, L.) is a globally important cereal crop, ranking the fourth most important after wheat, rice and maize. A variety of its uses includes malt, food and feed. In 2004, barley was grown on 57 million ha and produced 154 million tons of grain, with a world export value of $3.3 billion (http://www.apps.fao.org/default.jsp). In order to counteract the negative effects of global climate changes and the loss of cultivated land for barley production, new cultivars with improved yield, quality and resistance to biotic and abiotic stress are needed. To this aim, advances in genomics, in combination with exploitation of natural genetic diversity, are expected to provide important tools.

Unlike the genomes of *Arabidopsis* and rice, which have already been sequenced (Arabidopsis Genome

Initiative 2000; Goff et al. 2002), the genome of barley is large (1C ~ 5,100 Mbp, Doležel et al. 1998) and complex because of the prevalence of repetitive DNA sequences (Rimpau et al. 1980; Shirasu et al. 2000). Despite these features, a great progress was achieved recently in barley genome analysis, including the development of genetic maps (Hori et al. 2003; Karakousis et al. 2003), the identification of quantitative trait loci (QTLs) for many agronomic and quality traits (Li et al. 2005; von Korff et al. 2005) and the integration of genetic and physical maps (Künzel et al. 2000). Over 420,000 expressed sequence tags (ESTs) have been deposited in a public database (http://www.ncbi.nlm.nih.gov/dbEST/), a 22,700-gene Affymetrix Barley1 GeneChip is publicly available (Close et al. 2004), and some important genes have been cloned (Brueggeman et al. 2002; Buschges et al. 1997; Collins et al. 2003).

These advances demonstrate that the barley genome can be accessed using the current genomic tools. However, further efforts are needed to isolate and characterize additional genes, to understand their genome organization, and to reveal the complex pathways that govern their expression. The availability of the complete genome sequence would make these studies feasible and also provide tools to study the genetic mechanisms that shaped the genome of cultivated barley and its agronomically relevant genes.

The rapid progress in DNA sequencing technology indicates complete sequencing of even complex plant genomes as a potentially feasible target. With the sequencing of the large genome of maize in progress, plans to sequence the genomes of barley and bread wheat are being discussed (Gill et al. 2004; http://www.pgrc.ipk-gatersleben.de/etgi/). However, because of the sheer size and prevalence of repetitive DNA sequences, sequencing and ordering the sequenced clones to faithfully represent the whole genomes would be a daunting challenge. Although it has been argued that sequencing only the expressed portion of the genome after cot selection (Peterson et al. 2002) and methylation filtering (Rabinowicz et al. 1999) might be sufficient, these approaches do not provide contextual information on the surrounding gene-poor regions (Mayer and Mewes 2001). It is also not clear whether the small clones thus obtained could be ordered to produce a linearly ordered set of sequences. According to Meyers et al. (2004), any sequencing strategy would eventually require a robust physical map.

A time-proven approach to produce physical chromosome maps is to order large-insert DNA clones, typically bacterial artificial chromosome (BAC) clones, on the basis of clone fingerprint patterns (Luo et al. 2003). This might be done in barley, as genomic BAC libraries are available (Yu et al. 2000). Considering the barley genome size, about half million BAC clones need to be fingerprinted to achieve the required genome coverage. Although it is possible to fingerprint such a large number of clones with the automated fingerprinting technique of Luo et al. (2003), development of contigs and physical maps from the resulting fingerprints will not be a trivial task. An attractive option would be to develop physical maps for individual chromosome arms that represent only 5–9% of the entire genome (Marthe and Künzel 1994).

With the aim to generate chromosome- and chromosome arm-specific BAC resources from hexaploid wheat, we have developed an original procedure for purification of particular chromosomes and chromosome arms by laser flow cytometry (Vrána et al. 2000; Kubaláková et al. 2002; Kubaláková et al. 2005), and a protocol for preparation of intact DNA from sorted chromosomes suitable for cloning (Šimková et al. 2003). These advances, together with the improved protocol for BAC cloning (Chalhoub et al. 2004), allowed us to generate BAC libraries specific for wheat chromosomes 1D, 4D and 6D (Janda et al. 2004), chromosome 3B (Šafář et al. 2004) and the short arm of chromosome 1B (Janda et al., submitted), as well as for the short arm of rye chromosome 1R (Šafář et al. 2006).

As indicated by our previous experiences, sorting and subsequent development of chromosome- and chromosome arm-specific BAC resources is feasible for barley, provided the sorting target(s) can be distinguished on the basis of size from the rest of the chromosome complement. Previously, we developed protocols for the preparation of liquid suspension of intact mitotic chromosomes of barley, and for their analysis and sorting using flow cytometry (Lysák et al. 1999). Because of the similarity in size, out of the seven barley chromosomes, only chromosome 1H could be purified by flow sorting. However, our ability to prepare chromosome suspensions from wheat (Vrána et al. 2000) opens a new possibility for dissecting the barley genome using wheat–barley chromosome addition lines. We demonstrated that wheat–rye chromosome addition lines facilitate sorting of all chromosomes of rye, which are larger than any of the wheat chromosomes (Kubaláková et al. 2003). In the same way, a wheat–rye ditelosomic addition line was used to sort the short arm of chromosome 1R for BAC library construction (Šafář et al. 2006).

This success stimulated us to explore the wheat–barley chromosome and telosome addition lines. Use of the latter materials allowed purification of the separate arms of each of the chromosomes 2H–7H by flow cytometry. This advance opens new avenues for

targeted and hence more rapid and cost-effective analysis of the barley genome. We envisage the use of flow-sorted barley chromosomes in a number of applications, including the creation of BAC resources, physical mapping and map-based cloning.

Materials and methods

Plant material

Seeds of barley (*H. vulgare* L., $2n = 2x = 14$) cv. 'Betzes' were obtained from the Agricultural Research Institute, Kroměříž, Czech Republic. Seeds of wheat (*Triticum aestivum* L., $2n = 6x = 42$) cv. 'Chinese Spring' were kindly provided by Prof. A.J. Lukaszewski (University of California, Riverside, USA). Seeds of two wheat–barley disomic addition lines carrying barley chromosomes 2H and 6H (Linc and Molnár-Láng 2003) were obtained from the Agricultural Research Institute, Martonvásár, Hungary. Seeds of 12 wheat–barley ditelosomic addition lines carrying arms of barley chromosomes 2H–7H were obtained from the National Bioresource Project/Wheat in Japan. All telosomic addition lines used in this study were originally developed by Islam et al. (1981) and Islam (1983).

Cell cycle synchronization and accumulation of metaphases

The procedures of Lysák et al. (1999) and Vrána et al. (2000) were used for barley and wheat, respectively. Seeds were germinated in the dark at 25 ± 0.5°C on moistened filter paper in glass petri dishes for 2–3 days to achieve optimal root length (2–3 cm). Seedlings with the optimal root length were transferred to an open mesh basket positioned on a tray filled with 900 ml of Hoagland's nutrient solution (Gamborg and Wetter 1975) containing 2 mM hydroxyurea (HU) and incubated for 18 h. Then the roots were washed in distilled water and cultured in HU-free Hoagland's solution. To accumulate cells at metaphase, the roots were treated for 2 h with 2.5 μM of amiprophos-methyl (APM) in Hoagland's solution after 4.5 h (wheat) or 6.5 h (barley) of recovery from the HU treatment. All incubations were performed in the dark at 25 ± 0.5°C, and all solutions were aerated. Finally, the synchronized roots were incubated overnight in ice water (1–2°C).

Preparation of chromosome suspensions

Liquid suspensions of intact chromosomes were prepared according to Vrána et al. (2000). Briefly, 30 roots were cut 1 cm from the root tip, rinsed in deionized water and fixed in 2% (v/v) formaldehyde in Tris buffer at 5°C for 20 min. After three washes in Tris buffer for 5 min at 5°C, the meristem tips were excised and transferred to a tube containing 1 ml of LB01 buffer (Doležel et al. 1989) at pH 9. Metaphase chromosomes were released after homogenization with a Polytron PT1300D homogenizer (Kinematica AG, Littau, Switzerland) at 20,000 rpm for 13 s. Large cellular debris was removed after passing through a 50-μm pore size nylon mesh.

Chromosome analysis and sorting

Chromosome suspensions were stained with 2 μg/ml of DAPI (4',6-diamino-2-phenylindole) and analyzed using a FACSVantage SE flow cytometer (Becton Dickinson, San José, USA). Approximately 5,000 chromosomes were analyzed from each sample and data were collected using Cell Quest software (Becton Dickinson). Chromosomes were sorted at rates of approximately 5–15 chromosomes per second. For fluorescence in situ hybridization (FISH), 1,000 chromosomes were sorted onto microscope slides into 10 μl drop of primed in situ DNA labeling (PRINS) buffer containing 5% (w/v) sucrose (Kubaláková et al. 1997), air-dried and stored at room temperature until use. For polymerase chain reaction (PCR), 300–500 chromosomes were sorted into 0.5-ml PCR tubes containing 10 μl of sterile deionized water. The tubes with sorted chromosomes were frozen and kept at −20°C until use.

Fluorescence in situ hybridization

A digoxigenin-labeled probe for GAA microsatellites was prepared by PCR with $(GAA)_7$ and $(CTT)_7$ primers and wheat genomic DNA as a template; a biotin-labeled probe for telomeric repeats was prepared using PCR with $(TTTAGGG)_3$ and $(CCCTAAA)_5$ primers. Hybridization mix consisting of 40% formamide, 25% dextran sulfate, 0.1 x SSC, 250 ng/μl of sheared calf thymus DNA and 1 ng/μl of labeled probe was dropped on slides and covered with cover slips. The slides were placed on a thermal cycler for 1.5 min at 80°C and exposed at 37°C overnight. After a stringent wash (Kubaláková et al. 2003), the sites of digoxigenin-labeled probe hybridization were detected using anti-digoxigenin-fluorescein isothiocyanate (FITC) raised in sheep (Roche Molecular Biochemicals, Mannheim, Germany). The biotin-labeled probe was detected using Cy3-labeled streptavidin (Sigma, Saint Louis, USA). Chromosome preparations were counterstained with 0.2 μg/ml of DAPI and mounted in Vectashield antifade solution (Vector Laboratories, Burlingame, USA).

Fluorescence microscopy

The preparations were evaluated using Olympus BX60 microscope equipped with optical filter sets appropriate for DAPI, fluorescein and Cy3 fluorescence. The images of DAPI, fluorescein and Cy3 fluorescence were acquired separately with a b/w CCD camera, which was interfaced to a PC running the ISIS software (Metasystems, Altlussheim, Germany). The images were superimposed after contrast and background optimization.

Polymerase chain reaction

The chromosomes were denatured at 94°C for 4 min and PCR mix was added to reach the reaction volume of 25 µl. The final concentrations of the reagents were: 10 mM of Tris-HCl (pH 8.3), 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 2 U/100 µl of DyNAzyme II DNA polymerase (Finnzymes, Espoo, Finland). The following primers for chromosome-specific restriction fragment length polymorphism (RFLP) markers (Künzel et al. 2000) were used at 0.5 µM: MWG0889 (2HS), MWG0866 (2HL), MWG2158 (3HS), MWG0549 (3HL), MWG2033 (4HS), MWG2163 (4HL), MWG 0618 (5HS), cMWG0770 (5HL), MWG2264 (6HS), MWG0798 (6HL), MWG2232 (7HS) and MWG0539 (7HL). PCR was performed under the following conditions: denaturation for 2 min at 94°C, followed by 35 cycles consisting of 0.5 min denaturation at 94°C, 1-min annealing at 55°C, 2-min extension at 72°C and final extension at 72°C for 10 min. PCR products were analyzed by electrophoresis on 1.5% agarose gels.

Results

Our current work was stimulated by the observation that wheat–rye chromosome addition lines facilitate flow sorting of particular chromosomes of rye (Kubaláková et al. 2003). We reasoned that a similar approach could be used to sort barley chromosomes. We first analyzed DAPI-stained chromosome suspensions prepared from barley cv. 'Betzes'. Resulting distribution of relative fluorescence intensity (flow karyotype) comprised one large peak and one minor peak (Fig. 1). FISH on flow-sorted chromosomes with the GAA microsatellite probe confirmed that the large peak was composite and represented chromosomes 2H–7H, with chromosome 2H forming a shoulder. The small and well-separated peak was assigned to chromosome 1H. This chromosome could be sorted with over 85% purity.

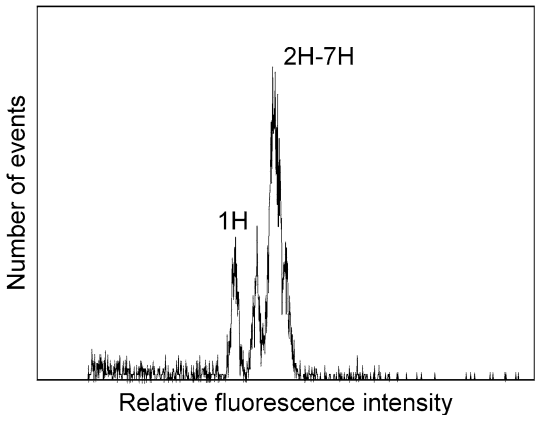

Fig. 1 Histogram of relative fluorescence intensities (flow karyotype) obtained after the analysis of DAPI-stained chromosome suspension prepared from barley cv. 'Betzes'. The karyotype consists of a large composite peak representing chromosomes 2H–7H with the chromosome 2H forming a shoulder on the left side of the peak. Note that the peak representing chromosome 1H is well resolved from the remaining chromosomes

As the next step, we analyzed chromosome suspensions prepared from wheat cv. 'Chinese Spring' and obtained a flow karyotype comprising three large peaks and one minor peak (Fig. 2a). We had previously established that the large peaks (labeled I–III) were composite and represented groups of various chromosomes, while the small peak was assigned to chromosome 3B (Kubaláková et al. 2002). We calculated the range of peak positions of barley chromosomes and chromosome arms corresponding to their different sizes (Table 1) within the flow karyotype of wheat and concluded that barley chromosomes should overlap with those of wheat (Fig. 2a). However, peaks of barley chromosome arms were expected to be well separated from those of wheat chromosomes.

These predictions were verified experimentally by analyzing wheat–barley disomic addition lines carrying barley chromosomes 2H and 6H, respectively. As expected, no major alteration as to the presence and position of peaks within the flow karyotype was observed, that is, the barley chromosomes did not yield separate peaks (not shown). Microscopic analysis of chromosomes sorted from composite peaks I–III revealed barley chromosomes 2H and 6H within peak II. This observation was independently confirmed by analyzing a mixture of mitotic chromosomes from hexaploid wheat cv. 'Chinese Spring' and barley cv. 'Betzes'. The resulting 'flow karyotype' revealed a dominant peak at the position of peak II of wheat (Fig. 2b).

Next we analyzed the wheat–barley ditelosomic addition line 2HL. We obtained a flow karyotype on

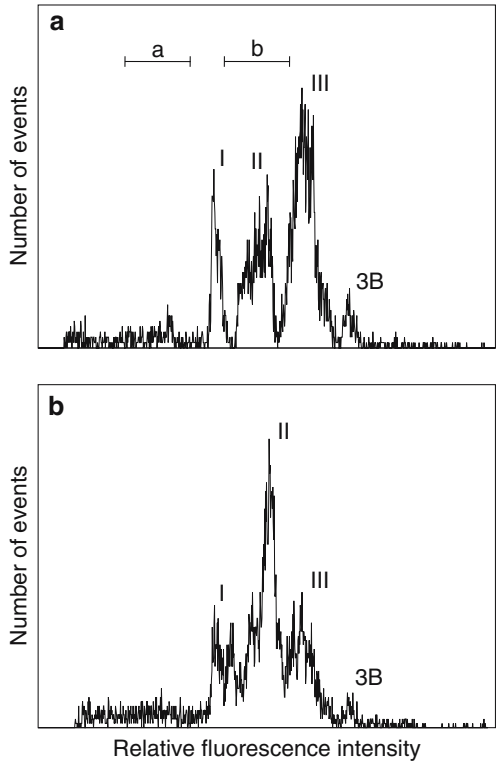

Fig. 2 Flow karyotypes obtained after flow cytometric analysis of DAPI-stained suspensions of mitotic chromosomes. **a** The flow karyotype of hexaploid wheat cv. 'Chinese Spring' consists of three composite peaks (I, II and III) representing specific groups of chromosomes, and a peak representing chromosome 3B. *Horizontal bars* labeled 'a' and 'b' indicate a range of estimated positions of peaks representing barley chromosomes and chromosome arms, respectively. **b** 'Flow karyotype' obtained after analysis of a mixture of mitotic chromosomes from hexaploid wheat cv. 'Chinese Spring' and barley cv. 'Betzes'. The largest peak (II) represents wheat chromosomes and barley chromosomes 2H–7H

Table 1 Molecular sizes of barley chromosome arms

Chromosome	Chromosome size (Mbp)[a]						
	1H	2H	3H	4H	5H	6H	7H
Entire chromosome	622	790	755	729	760	689	755
Short arm	255	362	336	336	301	332	382
Long arm	367	428	419	393	459	357	373

[a] Calculated considering the relative chromosome arm lengths given by Marthe and Künzel (1994) and barley 1C genome size of 5,100 Mbp (Doležel et al. 1998)

which the peak representing 2HL was unambiguously resolved (Fig. 3a). The distance from the composite peak I of wheat chromosomes was sufficient to avoid any overlap. We confirmed the identity of 2HL by PCR using specific primers (Fig. 3b). Subsequently we analyzed the remaining 11 wheat–barley ditelosomic addition lines. For each of them, the peak of a barley telocentric chromosome was clearly resolved.

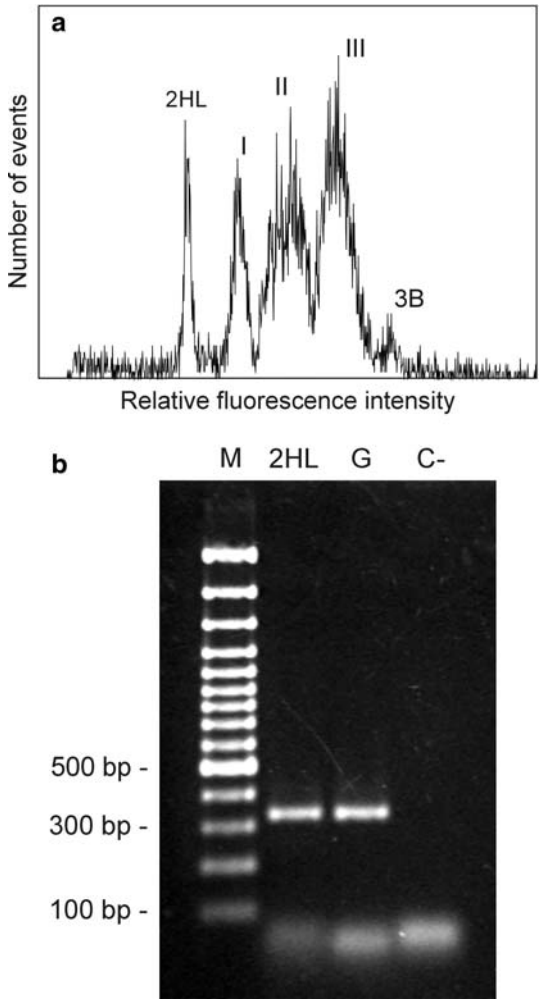

Fig. 3 Flow cytometric sorting of the long arm of barley chromosome 2H. **a** Flow karyotype obtained after the analysis of DAPI-stained chromosome suspensions prepared from wheat–barley ditelosomic addition line 2HL. The karyotype consists of four peaks I, II, III and 3B of wheat chromosomes, and an additional well-resolved peak of 2HL. **b** Agarose-gel electrophoresis of PCR products obtained with primers specific for 2HL: *M* 100-bp size marker ladder; *2HL* 300 copies of flow-sorted 2HL; *G* genomic DNA; *C-* negative control

Representative examples of flow karyotypes are shown in Figs. 4a and b. As in case of 2HL, the identity of all sorted telosomes was confirmed by PCR with primers for arm-specific markers (data not shown). We conclude that the use of wheat–barley ditelosomic addition lines enables the sorting of any arm of barley chromosomes 2H–7H.

To evaluate the extent of contamination of sorted barley chromosome arms by wheat chromosomes and their fragments, we sorted 1,000 particles from peaks representing barley chromosome arms onto microscope slides and performed two-color FISH with probes for GAA microsatellites and telomeric repeats. While the use of GAA microsatellites allowed unambiguous

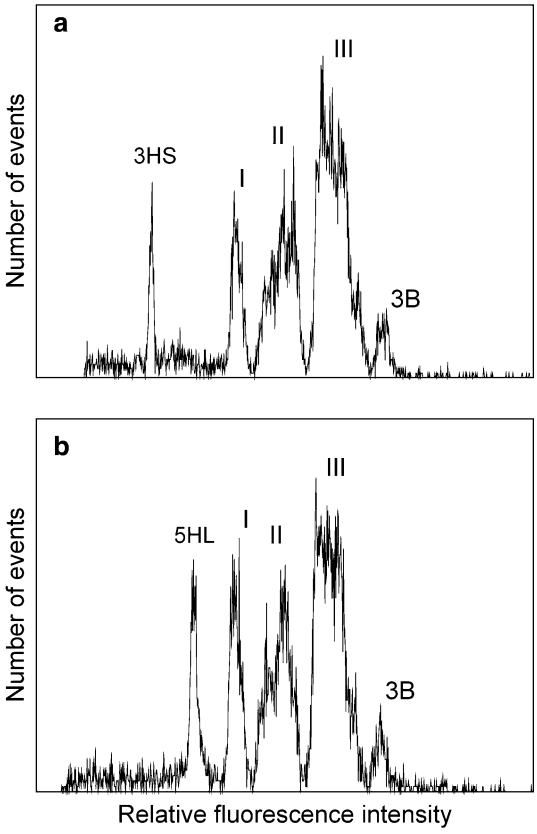

Fig. 4 Flow karyotypes obtained after the analysis of DAPI-stained chromosome suspensions prepared from: **a** wheat–barley ditelosomic addition line 3HS (one of the smallest barley chromosome arms) and **b** wheat–barley ditelosomic addition line 5HL (the biggest barley chromosome arm). Peaks representing barley telosomes are well discriminated

identification of any barley chromosome arm, FISH with telomeric repeats was used to verify that sorted chromosomes were barley telocentric chromosomes with two telomeres, and not fragments of wheat chromosomes without telomere sequences at the centromere end. This analysis confirmed sorting of barley telocentrics (Fig. 5). The purity of sorted barley chromosome arm fractions ranged from 85 to 95%.

Discussion

This work represents the progress in flow cytogenetics of barley, considering the fact that only wild-type chromosome 1H could be isolated by flow sorting until now (Lysák et al. 1999). A novel strategy enables the purification of the remaining barley chromosomes 2H–7H in the form of chromosome arms. Because of the clear-cut size difference between the complete wheat chromosomes and the added barley arms, the latter could be easily identified and sorted. Similarly, ditelosomic lines and other particular cytogenetic stocks enabled purification of defined wheat chromosome arms and rye chromosomes using flow sorting (Kubaláková et al. 2002, 2003, 2005). The wide array of wheat and alien chromosomal structural variants available in the wheat background represents an extremely valuable tool to dissect the large genomes of important Triticeae species into individual and well-defined parts.

As chromosome 1H represents only 12.2% of the barley genome, and can be sorted directly from standard barley lines (Lysák et al. 1999), we did not attempt to sort its short and long arms. In fact, because of the sterility of telosomic addition for 1HL (Islam and Shepherd 1990, 2000), only the short arm of 1H (1HS) is accessible. Arms of the remaining barley chromosomes can be sorted and represent only about 6–9% of the entire genome. The use of DNA obtained from sorted chromosome 1H and from the arms of chromosomes 2H–7H can greatly simplify genome analysis and sequencing in barley. It is worth mentioning that the use of gametocidal chromosomes can produce sub-arm

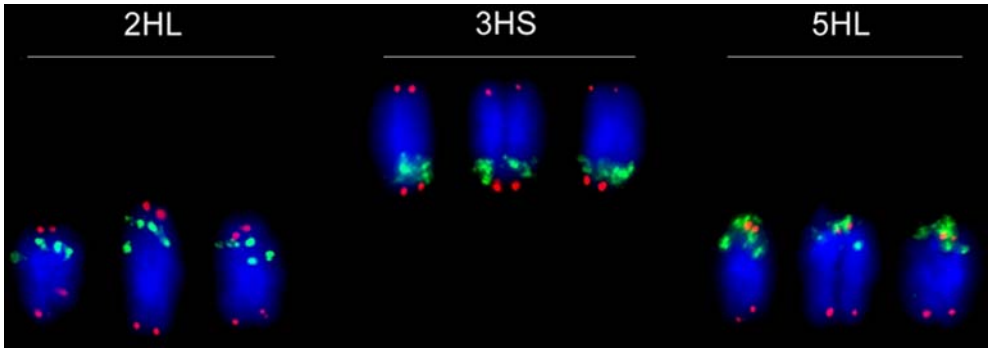

Fig. 5 Identification of flow-sorted barley chromosome arms by FISH. Chromosomes were sorted onto microscope slides, air-dried and subjected to FISH with probes for the GAA microsatellite (*green*) and telomeric repeats (*red*). The chromosomes were counterstained with DAPI (*blue*). Patterns of the GAA signals confirmed the identity of the sorted telocentric chromosomes. Presence of telomeric signals proved that intact barley telocentrics were sorted

chromosome fragments (Shi and Endo 1997; Yoshino et al. 1998). Thus, in future it might be possible to use flow sorting to isolate portions of the barley genome smaller than a chromosome arm.

We have shown previously that DNA of sorted plant chromosomes according to our protocol is of high molecular weight (Šimková et al. 2003). In this work, we show that barley chromosome arms can be sorted at high purity, which makes chromosome sorting in barley an attractive tool for a broad range of studies.

With the sort rates of 5–15 chromosomes/s it is possible to purify several millions of barley chromosome arms, which are needed to prepare microgram quantities of DNA for direct cloning and construction of BAC libraries. The feasibility of creating BAC libraries from flow-sorted chromosomes was demonstrated recently in wheat and rye. In wheat, three chromosome-specific BAC libraries were generated (Janda et al. 2004; Šafář et al. 2004; Janda et al., submitted), and in rye a BAC library specific for the short arm of chromosome 1R (1RS) was produced (Šafář et al. 2006). With an average insert size of 100 kb, BAC libraries created from barley chromosome arms should comprise not more than 50,000 clones to provide a 10 × coverage. Current high-throughput methods can fingerprint a library of this size in a few months (Luo et al. 2003). Furthermore, the reduced sample complexity should greatly simplify the assembly of BAC contigs. Recent results obtained with the development of a physical contig map of the largest wheat chromosome 3B (Paux et al. 2006) support these assumptions.

Cytogenetic mapping is another attractive application of sorted chromosomes. In addition to analyzing the long-range molecular structure of barley chromosomes, cytogenetic mapping will be needed for development of physical contig maps (Harper and Cande 2000). We demonstrated that flow-sorted plant chromosomes could be stretched longitudinally and used for FISH mapping with an increased physical resolution (Valárik et al. 2004). Furthermore, the absence of cell wall and cytoplasmic remnants makes DNA of sorted chromosomes easily accessible to probes and facilitates localization of sequences as short as 2 kb (Janda et al., submitted). Finally, a possibility to sort 1,000 copies of a particular chromosome arms on one slide offers a higher-throughput alternative to FISH mapping on mitotic metaphase spreads.

Although not yet explored, we expect DNA of flow-sorted chromosomes and chromosome arms to be suitable for high-throughput mapping of DNA sequences on DNA arrays, HAPPY mapping (Thangavelu et al. 2003) and optical mapping (Aston et al. 1999). At first sight, the great potential of flow cytogenetics may seem to be compromised by the need to invest in expensive equipment. However, this is not needed, as one or a few laboratories should be capable to prepare and distribute sub-genomic DNA libraries, DNA isolated from sorted chromosomes and microscope slides with fixed sorted chromosomes dried on for cytogenetic mapping.

Acknowledgments We are grateful to our colleagues Jitka Weiserová, Radka Tušková, Michaela Libosvárová and Romana Nováková for excellent technical assistance, and Dr Hana Šimková for improvement of this manuscript. This work was supported by research grants from the Czech Science Foundation (grant award 521/05/H013), Ministry of Education, Youth and Sports of the Czech Republic (grant awards ME884 and LC06004), and was performed within a framework of a collaborative agreement No. 14 between the Academy of Sciences of the Czech Republic and the Hungarian Academy of Sciences. We thank two anonymous reviewers for valuable suggestions.

References

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Aston C, Mishra B, Schwartz DC (1999) Optical mapping and its potential for large-scale sequencing projects. Trends Biotechnol 17:297–302

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002) The barley stem rust-resistance gene Rpg1 is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328–9333

Buschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, van Daelen R, van der Lee T, Diergaarde P, Groenendijk J, Topsch S, Vos P, Salamini F, Schulze-Lefert P (1997) The barley *Mlo* gene: a novel control element of plant pathogen resistance. Cell 88:695–705

Chalhoub B, Belcram H, Caboche M (2004) Efficient cloning of plant genomes into bacterial artificial chromosome (BAC) libraries with larger and more uniform insert size. Plant Biotechnol J 2:181–188

Close TJ, Wanamaker SI, Caldo RA, Turner SM, Ashlock DA, Dickerson JA, Wing RA, Muehlbauer GJ, Kleinhofs A, Wise RP (2004) A new resource for cereal genomics: 22K Barley GeneChip comes of age. Plant Physiol 134:960–968

Collins NC, Thordal-Christensen H, Lipka V, Bau S, Kombrink E, Qiu JL, Huckelhoven R, Stein M, Freialdenhoven A, Sommerville SC, Schulze-Lefert P (2003) SNARE-protein-mediated disease resistance at the plant cell wall. Nature 425:973–977

Doležel J, Binarová J, Lucretti S (1989) Analysis of nuclear DNA content in plant cells by flow cytometry. Biol Plant 31:113–120

Doležel J, Greilhuber J, Lucretti S, Meister A, Lysák MA, Nardi L, Obermayer R (1998) Plant genome size estimation by flow cytometry: inter-laboratory comparison. Ann Bot 82(Suppl. A):17–26

Gamborg OL, Wetter LR (1975) Plant tissue culture methods. National Research Council of Canada, Saskatoon

Gill BS, Appels R, Botha-Oberholster AM, Buell CR, Bennetzen JL, Chalhoub B, Chumley F, Dvorak J, Iwanaga M, Keller B,

Li W, McCombie WR, Ogihara Y, Quetier F, Sasaki T (2004) A workshop report on wheat genome sequencing: international genome research on wheat consortium. Genetics 168:1087–1096

Goff SA, Ricke D, Lan TH, Presting G, Wang RL, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchinson D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong JP, Miguel T, Paszkowski U, Zhang SP, Colbert M, Sun WL, Chen LL, Cooper B, Park S, Wood TC, Mao L, Quail P, Wing R, Dean R, Yu YS, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A, Briggs S (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 296:92–100

Harper LC, Cande WZ (2000) Mapping a new frontier: development of integrated cytogenetic maps in plants. Review Funct Integr Genomics 1:89–98

Hori K, Kobayashi T, Shimizu A, Sato K, Takeda K, Kawasaki S (2003) Efficient construction of high-density linkage map and its application to QTL analysis in barley. Theor Appl Genet 107:806–813

Islam AKMR (1983) Ditelosomic additions of barley chromosomes to wheat. In: Sakamoto S (ed) Proceedings of 6th International Wheat Genetics Symposium. Kyoto University Press, Kyoto, pp 233–238

Islam AKMR, Shepherd KW (1990) Incorporation of barley chromosomes into wheat. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, 13 (Wheat). Springer, Berlin Heidelberg New York, pp 128–151

Islam AKMR, Shepherd KW (2000) Isolation of a fertile wheat–barley addition line carrying the entire barley chromosome 1H. Euphytica 111:145–149

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

Janda J, Bartoš J, Šafář J, Kubaláková M, Valárik M, Číhalíková J, Šimková H, Caboche M, Sourdille P, Bernand M, Chalhoub B, Doležel J (2004) Construction of a subgenomic BAC library specific for chromosomes 1D, 4D and 6D of hexaploid wheat. Theor Appl Genet 109:1337–1345

Karakousis A, Gustafson JP, Chalmers KJ, Barr AR, Langridge P (2003) A consensus map of barley integrating SSR, RFLP, and AFLP markers. Austr J Agric Res 54:1173–1185

von Korff M, Wang H, Leon J, Pillen K (2005) AB-QTL analysis in spring barley. I. Detection of resistance genes against powdery mildew, leaf rust and scald introgressed from wild barley. Theor Appl Genet 111:583–590

Kubaláková M, Macas J, Doležel J (1997) Mapping of repeated DNA sequences in plant chromosomes by PRINS and C-PRINS. Theor Appl Genet 94:758–763

Kubaláková M, Vrána J, Číhalíková J, Šimková H, Doležel J (2002) Flow karyotyping and chromosome sorting in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 104:1362–1372

Kubaláková M, Valárik M, Bartoš J, Vrána J, Číhalíková J, Molnár-Láng M, Doležel J (2003) Analysis and sorting of rye (*Secale cereale* L.) chromosomes using flow cytometry. Genome 46:893–905

Kubaláková M, Kovářová P, Suchánková P, Číhalíková J, Bartoš J, Lucretti S, Watanabe N, Kianian SF, Doležel J (2005) Chromosome sorting in tetraploid wheat and its potential for genome analysis. Genetics 170:823–829

Künzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics 154:397–412

Li J, Huang XQ, Heinrichs F, Ganal MW, Röder MS (2005) Analysis of QTLs for yield, yield components, and malting quality in a BC3-DH populations of spring barley. Theor Appl Genet 110:356–363

Linc G, Molnár-Láng M (2003) Two methods for the development of wheat/barley disomic additions in winter wheat varieties, and identification using molecular cytogenetic techniques (C-banding, GISH). Növénytermelés 52:3–13

Luo MC, Thomas C, You FM, Hsiao J, Shu OY, Buell CR, Malandro M, McGuire PE, Anderson OD, Dvorak J (2003) High-throughput fingerprinting of bacterial artificial chromosomes using the SNaPshot labeling kit and sizing of restriction fragments by capillary electrophoresis. Genomics 82:378–389

Lysák MA, Číhalíková J, Kubaláková M, Šimková H, Künzel G, Doležel J (1999) Flow karyotyping and sorting of mitotic chromosomes of barley (*Hordeum vulgare* L.). Chromosome Res 7:431–444

Marthe F, Künzel G (1994) Localization of translocation breakpoints in somatic metaphase chromosomes of barley. Theor Appl Genet 89:240–248

Mayer K, Mewes HW (2001) How can we deliver the large plant genomes? Strategies and perspectives. Curr Opin Plant Biol 5:173–177

Meyers BC, Scalabrin S, Morgante M (2004) Mapping and sequencing complex genomes: let's get physical! Nat Rev Genet 5:578–581

Paux E, Sourdille P, Salsa J, Leroy P, Bartoš J, Mestiri I, Chalhoub B, Quetier F, Bernard M, Doležel J, Feuillet C (2006) Chromosome-based strategies to decipher the hexaploid wheat genome: chromosome 3B, a case study. In: Final Abstracts Guide of the International Conference Plant and Animal Genome XIV. Sherago International, Inc., San Diego, p 18

Peterson PG, Schulze SR, Sciara EB, Lee SA, Bowers JE, Nagel A, Jiang N, Tibbitts DC, Wessler SR, Paterson AH (2002) Integration of Cot analysis, DNA cloning and high-throughput sequencing facilitates genome characterization and gene discovery. Genome Res 12:795–807

Rabinowicz PD, Schulze SR, Sciara EB, Lee SA, Bowers JE, Nagel A, Jiang N, Tibbitts DC, Wessler SR, Paterson AH (1999) Differential methylation of genes and retrotransposons facilitates shotgun sequencing of the maize genome. Nat Genet 23:305–308

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

Šafář J, Šimková H, Suchánková P, Kovářová P, Bartoš J, Janda J, Kubaláková M, Číhalíková J, Lelley T, Doležel J (2006) A novel resource for genomics of rye and wheat: BAC library specific for the short arm of chromosome 1R (1RS). In: Final Abstracts Guide of the International Conference Plant and Animal Genome XIV. Sherago International, Inc., San Diego, p 115

Šafář J, Bartoš J, Janda J, Bellec A, Kubaláková M, Valárik M, Pateyron S, Weiserová J, Tušková R, Číhalíkova J, Vrána J, Šimková H, Faivre-Rampant P, Sourdille P, Caboche M, Bernand M, Doležel J, Chalhoub B (2004) Dissecting large and complex genomes: flow sorting and BAC cloning of individual chromosomes from bread wheat. Plant J 39:960–968

Shi F, Endo TR (1997) Production of wheat–barley disomic addition lines possessing an *Aegilops cylindrica* gametocidal chromosome. Genes Genet Syst 72:243–248

Shirasu K, Schulman AH, Lahaye T, Schulze-Lefert P (2000) A contiguous 66-kb barley sequence provides evidence for reversible genome expansion. Genome Res 10:908–915

Šimková H., Číhalíková J, Vrána J, Lysák MA, Doležel J (2003) Preparation of HMW DNA from plant nuclei and chromosomes isolated from root tips. Biol Plant 46:369–373

Thangavelu M, James AB, Bankier A, Bryan GJ, Dear PH, Waugh R (2003) HAPPY mapping in a plant genome: reconstruction and analysis of a high-resolution physical map of a 1.9 Mbp region of *Arabidopsis thaliana* chromosome 4. Plant Biotechnol J 1:23–31

Valárik M, Bartoš J, Kovářová P, Kubaláková M, de Jong H, Doležel J (2004) High-resolution FISH on super-stretched flow-sorted plant chromosomes. Plant J 37:940–950

Vrána J, Kubaláková M, Šimková H, Číhalíková J, Lysák MA, Doležel J (2000) Flow-sorting of mitotic chromosomes in common wheat *(Triticum aestivum* L.). Genetics 156:2033–2041

Yoshino M, Nasuda S, Endo TR (1998) Detection of terminal deletions in barley chromosomes by the PCR-based method. Genes Genet Syst 73:163–168

Yu Y, Tomkins JP, Waugh R, Frosch DA, Kudrna D, Kleinhofs A, Brueggeman RS, Muehlbauer GJ, Wise RP, Wing RA (2000) A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. Theor Appl Genet 101:1093–1099