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# Development and genetic mapping of 127 new microsatellite markers in barley

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**Abstract** To enhance the marker density of existing genetic maps of barley (*Hordeum vulgare* L.), a new set of microsatellite markers containing dinucleotide motifs was developed from genomic clones. Out of 254 primer pairs tested, a total of 167 primer pairs were classifed as functional in a panel of six barley cultivars and three *H. spontaneum* accessions, and of those, 127 primer pairs resulting in 133 loci were either mapped or located onto chromosomes. The polymorphism information content (PIC) ranged from 0.05 to 0.94 with an average of 0.60. The number of alleles per locus varied from 1 to 9. On average, 3.9 alleles per primer pair were observed. The RFLP frameworks of two previously published linkage maps were used to locate a total of 115 new microsatellite loci on at least one mapping population. The chromosomal assignment of 48 mapped loci was corroborated on a set of wheat-barley chromosome addition lines; 18 additional loci which were not polymorphic in the mapping populations were assigned to chromosomes by this method. The microsatellites were located on all seven linkage groups with four significant clusters in the centromeric regions of 2H, 3H, 6H and 7H. These newly developed microsatellites improve the density of existing barley microsatellite maps and can be used in genetic studies and breeding research.

**Keywords** *Hordeum vulgare* · Linkage map · SSRs · Segregation distortion · PIC

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## Introduction

Barley (*Hordeum vulgare* L.) is not only an important crop worldwide but also an excellent system for genome mapping and map-based analyses (Costa et al. 2001). It is a diploid (2n = 14) self-pollinated species and has a large genome of approximately $5.3 \times 10^9$ bp/1C (Bennett and Smith 1976). Marker-dense genetic maps of cereals contribute substantially to the positional cloning of important genes and provide a tool for evolutionary studies, as well as the characterization of germplasm and genetic research. A well-developed classical genomic map has been constructed for barley using isozyme and morphological markers (Sogaard and von-Wettstein-Knowles 1987). Later, the first genetic map using restriction fragment length polymorphism (RFLP) as molecular markers in barley emerged (Shin et al. 1990). Consequently, more detailed and high-density RFLP maps were published (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993). Molecular linkage maps in many model plants and crops were improved rapidly by the addition of new types of molecular markers. Compared with hybridization-based markers, markers based on the polymerase chain reaction (PCR) are advantageous, since they require significantly smaller amounts of genomic DNA as a template and do not need the use of radioactivity. Further advantages are low costs and, in particular, the rapid and easy assay allows high-throughput genotyping in marker-assisted selection and the detection of both qualitative and quantitative traits. The informative value of microsatellite markers for genetic studies and as a powerful tool for barley breeding was confirmed in several studies (Saghai-Maroof et al. 1994; Becker and Heun 1995; Liu et al. 1996; Struss and Plieske 1998). A second-generation linkage map of barley using only PCR-based microsatellite markers was constructed (Ramsay et al. 2000). Besides microsatellites derived from genomic clones, ESTs were also exploited for the development of PCR-based SSR-markers (Pillen et al. 2000; Holton et al. 2002; Thiel et al. 2003). Meanwhile, the physical mapping of the seven barley chromosomes

was published and indicated a severe suppression of recombination around the centromeres leading to extensive clustering of markers in genetic linkage maps (Künzel et al. 2000; Künzel and Waugh 2002). Despite these extensive mapping efforts, large gaps are still present on the barley linkage map with PCR-based microsatellites, also called SSRs; the coverage is still incomplete because of some clustering around the centromeres. Here, we present the development and genetic mapping of a new set of barley microsatellites from genomic small-insert libraries and the integration into previously published maps. In total, 127 functional microsatellite markers were developed and mapped onto the seven barley chromosomes using two mapping populations and/or wheat-barley addition-lines.

## Materials and methods

### Microsatellite marker development and analysis

The variety Franka was used as the DNA source for constructing a small-insert plasmid library. Genomic DNA was pre-digested with the methylation-sensitive restriction enzyme *Pst*I. *Pst*I-digested DNA was separated on preparative agarose gels, and the size range of 2 to 6 kb was excised and isolated using the GeneClean kit (Dianova). The size-selected DNA was further digested with the second restriction enzyme *Mbo*I. DNA fragments of 300 to 1,000 bp in length were size-fractionated and cloned into the plasmid vector pUC18 *Bam*HI/BAP (Amersham Biosciences). The high efficiency *Escherichia coli* XL2-Blue cells (Stratagene) were used for the transformation procedure according to the manufacturer's instructions. The transformation mixture was plated onto 22 × 22 cm plates and incubated overnight at 37 °C. Using the BioPick system (BioRobotics Ltd., U.K.), single colonies were transferred into 384-well microtiter plates as described previously (Pestsova et al. 2000). The libraries obtained were stored at –80 °C.

High-density nylon filters (Hybond $N^+$, Amersham Biosciences) were prepared from the 48 microtiter plates using the robotic gridding system BioGrid (BioRobotics Ltd., U.K.) resulting in 18,432 clones per filter. The filters were hybridized with synthetic dinucleotide repeats poly(GA) and poly(GT) (Amersham Biosciences). Positive clones were selected and used for the plasmid DNA isolation according to the plasmid mini-protocols (Qiagen). Plasmid clones were sequenced with M13 primers. Primer pairs flanking the microsatellite sites were designed using the computer program PRIMER v.0.5. One primer of each pair was labeled with Cy5 for fragment analysis.

These microsatellite markers were tested for functionality and polymorphism against a panel of nine barley lines: the *Hordeum spontaneum* lines, HS213, HS277 and HS584, and the varieties Brenda, Trasco, Steptoe, Morex, Igri and Franka. PCR reactions were performed as described in Röder et al. (1998). Fragment analysis was carried out on automated laser fluorescence (ALF-II express) sequencers (Amersham Biosciences) using short-gel cassettes. Fragment sizes were calculated using the computer program Fragment Analyser (Amersham Biosciences) by comparison with internal size standards.

### Map construction

Two mapping populations were used in the present study. The first population was the principal mapping population of the North American Barley Genome Mapping Project, constructed from the cross of Steptoe × Morex (S/M), and consisted of 80 doubled-haploids (Kleinhofs et al. 1993). The second population contained 65 DH plants derived from the cross of the winter barley cultivars Igri × Franka (I/F) by Foroughi-Wehr and Friedt (1984). A set of wheat (cv 'Chinese Spring')/*H. vulgare* (cv 'Betzes') barley chromosome addition lines was used to confirm the assignment of each new microsatellite to the seven chromosomes in barley (Islam et al. 1981). The addition lines for chromosomes 3H, 4H, 5H, 6H and 7H were disomic, the addition lines for 2HS and 2HL were ditelosomic, respectively. Microsatellites which were not polymorphic in the two mapping populations were assigned to specific chromosomes and to specific arms of chromosomes using these addition lines. Aliquots of genomic DNA of both mapping populations and the addition lines were kindly provided by A. Graner (IPK Gatersleben).

Mapped barley microsatellites isolated from the genomic library were designed as GBMS for 'Gatersleben Barley MicroSatellite' and numbered consecutively. A small letter was added after the number if a microsatellite identified more than one locus. Besides the newly developed GBMS microsatellites, 36 microsatellites described in Ramsay et al. (2000) were used in the S/M or I/F maps as reference markers.

The segregation data for the polymorphic microsatellites in S/M and I/F populations were tested for segregation distortion against the expected 1:1 ratio by calculating chi-square values using Joinmap (version 3.0, Stan P. and van Ooijen JW., CPRO-DLO, Wageningen, The Netherlands). The software package MAPMAKER/Exp version 3.0b (Lander et al. 1987) was then used to construct genetic maps in the two mapping populations. Markers mapping to the same chromosome were considered as linked if they showed a LOD score of at least 3.0. The final order of markers in each chromosome was confirmed with the 'Ripple' command. Recombination fractions were converted to centiMorgans with the Kosambi mapping function (Kosambi 1944). The data for the RFLP makers have been kindly provided by A. Graner (IPK Gatersleben).

In order to measure the informativeness of a DNA marker in the analyzed material, the polymorphic information content (PIC) for each loci was calculated according to Weber (1990) and Anderson et al. (1993).

## Results and discussion

### Development of microsatellite markers

The plasmid library for marker development was enriched for single- and low-copy DNA according to Röder et al. (1998). Based on hybridization with the dinucleotide repeats $(GA)_n$ and $(GT)_n$, a total of 455 positive clones (0.3%) was isolated which were sequenced (Table 1). Based on an average insert size of 500 bp for the plasmid clones, our results indicate the presence of a $(GA)_n$ or $(GT)_n$ microsatellite approximately every 222 kb. This result is comparable to the estimates found by Liu et al. (1996) with one $(GA)_n$ motif every 330 kb and one $(GT)_n$ motif every 620 kb. Taking into account all types of SSRs, Cardle et al. (2000) estimated a frequency of one SSR per 7.4 kb in the barley genome. Eliminating duplicated clones, false positives and clones for which sequence information was only available from one side of the microsatellite, 254 sequences were suitable for primer

**Table 1** Frequency of microsatellite sequences in the genome of barley cultivar Franka

| | |
|---|---|
| Size of the plasmid library | 148,000 clones |
| Probes | $(GA)_n$, $(GT)_n$ |
| Positive clones | 455 |
| Primer pairs designed | 254 |
| Functional primer pairs | 167 (66%) |
| Primer pairs mapped or located on chromosomes | 127 (50%) |

**Table 2** List of barley microsatellites including repeat motif, PIC, allele numbers and chromosomal location

| Marker | Motif types | PIC | Alleles | S/M[a]I/F[a] | W/B[b] | Marker | Motif types | PIC | Alleles | S/M[a]I/F[a] | W/B[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GBMS2 | $(GA)_{14}$ | 0.79 | 6 | 2H | | GBMS119 | $(CA)_{22}$ | 0.68 | 4 | 5H | 5H |
| GBMS3 | $(AG)_{14}$ | 0.67 | 4 | 7H | 7H | GBMS120 | $(AT)_{35}$ | 0.83 | 5 | 7H | |
| GBMS9 | $(GA)_{23}$ | | 1 | | 3H | GBMS121 | $(GA)_{13}$ | 0.47 | 5 | 6H | |
| GBMS11 | $(AG)_{14}$ | 0.75 | 2 | 2H | | GBMS125 | $(GA)_{6}$ | 0.49 | 2 | 6H | |
| GBMS12 | $(CT)_{11}(CA)_{20}$ | 0.85 | 4 | 1H | | GBMS128a | $(GT)_{12}$ | 0.79 | 3 | 4H | |
| GBMS13 | $(CT)_{10}$ | 0.59 | 3 | 5H | | GBMS128b | | 0.74 | 4 | 2H | |
| GBMS14 | $(CT)_{15}$ | 0.83 | 5 | 1H | | GBMS129 | $(CA)_{11}$ | 0.69 | 4 | 7H | 7H |
| GBMS15 | $(GT)_{19}$ | 0.49 | 3 | 4H | | GBMS133 | $(CA)_{17}(AT)_{12}$ | 0.74 | 6 | 4H | 4H |
| GBMS17 | $(TG)_{11}$ | 0.32 | 4 | 1H | | GBMS135 | $(GT)_{23}$ | 0.75 | 6 | 6H | |
| GBMS20 | $(TC)_{6}$ | 0.54 | 2 | | 3H | GBMS137 | $(GA)_{40}$ | 0.86 | 8 | 2H | 2H |
| GBMS22 | $(GT)_{20}$ | 0.72 | 5 | 3H | | GBMS138 | $(GT)_{11}$ | 0.52 | 4 | 3H | 3H |
| GBMS28 | $(GT)_{11}$ | 0.26 | 5 | 4H | 4H | GBMS139 | $(CA)_{10}$ | 0.85 | 6 | 7H | 7H |
| GBMS29 | $(AC)_{12}(AT)_{14}$ | 0.84 | 7 | 4H | | GBMS140 | $(CA)_{19}(AT)_{15}$ | 0.89 | 6 | 3H | 3H |
| GBMS31 | $(AG)_{14}$ | 0.44 | 3 | 2H | | GBMS141 | $(CA)_{16}$ | 0.79 | 4 | 7H | |
| GBMS32 | $(GT)_5CT(GT)_{11}$ | 0.64 | 5 | 5H | 5H | GBMS143 | $(CA)_{8}$ | 0.79 | 4 | 1H | |
| GBMS33 | $(AT)_5AG(AC)_{19}(AT)_7$ | 0.28 | 5 | 6H | | GBMS147 | $(GT)_{18}$ | 0.20 | 2 | 3H | 3H |
| GBMS35 | $(GA)_{12}$ | 0.75 | 5 | 7H | | GBMS149 | $(CAT)_{24}$ | 0.64 | 5 | 3H | 3H |
| GBMS37 | $(TG)_{27}$ | 0.41 | 7 | 1H | | GBMS150 | $(CT)_{12}$ | 0.35 | 3 | 4H | |
| GBMS38 | $(TC)_{22}$ | 0.83 | 5 | 3H | | GBMS154 | $(CAT)_{7}$ | 0.74 | 5 | | 5H |
| GBMS40 | $(AG)_{18}$ | 0.23 | 3 | 2H | | GBMS156 | $(GT)_{11}$ | 0.69 | 4 | 5H | |
| GBMS41 | $(GT)_{25}$ | 0.93 | 3 | 7H | | GBMS157 | $(GT)_{10}$ | 0.54 | 2 | 3H | 3H |
| GBMS45 | $(AC)_{11}$ | 0.72 | 5 | 3H | 3H | GBMS160 | $(GA)_{14}$ | 0.72 | 5 | 2H | |
| GBMS46 | $(AG)_{32}$ | 0.60 | 5 | 3H | 3H | GBMS163 | $(GT)_{15}$ | 0.37 | 3 | | 3H |
| GBMS48 | $(AG)_8CA(AG)_{10}$ | 0.72 | 4 | 3H | 3H | GBMS164 | $(GT)_{16}$ | 0.48 | 4 | 7H | |
| GBMS49 | $(AG)_{14}$ | 0.72 | 5 | 4H | | GBMS166 | $(CA)_6TCGCT(CA)_9$ | 0.74 | 5 | 3H | 3H |
| GBMS50 | $(AC)_{13}(AG)_{14}$ | 0.72 | 5 | 3H | 3H | GBMS174 | $(GT)_{19}$ | 0.38 | 2 | 5H | 5H |
| GBMS53 | $(GT)_{11}$ | 0.36 | 5 | 1H | | GBMS178 | $(GT)_{11}$ | 0.05 | 5 | 6H | |
| GBMS54 | $(CA)_{9}$ | 0.69 | 4 | 1H | | GBMS180 | $(GA)_{30}$ | 0.85 | 9 | 6H | 6H |
| GBMS56 | $(AC)_{10}$ | 0.20 | 2 | | 6H | GBMS183 | $(GT)_5(CT)_3(GT)_8$ | 0.57 | 5 | 7H | 7H |
| GBMS57 | $(CT)_{9}$ | 0.49 | 2 | 3H | 3H | GBMS184 | $(GT)_5(GA)_{14}$ | 0.62 | 4 | 1H | |
| GBMS58 | $(CA)_{7}$ | 0.44 | 2 | | 6H | GBMS185 | $(GA)_{14}$ | 0.51 | 4 | 3H | |
| GBMS60 | $(GT)_{11}$ | 0.64 | 3 | 5H | 5H | GBMS187 | $(GT)_{20}$ | 0.94 | 2 | 1H | |
| GBMS61 | $(TG)_{10}$ | 0.68 | 3 | | 7H | GBMS188 | $(GA)_{19}$ | 0.68 | 5 | 2H | |
| GBMS62 | $(AG)_{10}$ | 0.68 | 5 | 1H | | GBMS189 | $(GA)_{32}$ | 0.64 | 5 | 3H | 3H |
| GBMS63 | $(AC)_{11}$ | 0.60 | 6 | 7H | 7H | GBMS190 | $(GA)_{10}$ | 0.79 | 2 | 4H | 4H |
| GBMS65 | $(AG)_{10}$ | 0.35 | 2 | 1H | | GBMS192 | $(GT)_{14}$ | 0.47 | 5 | 7H | 7H |
| GBMS66 | $(CA)_{10}$ | 0.65 | 5 | 2H | | GBMS196 | $(GT)_{10}$ | 0.49 | 3 | 5H | 5H |
| GBMS67 | $(AT)_8(GA)_{12}$ | 0.11 | 3 | | 5H | GBMS198 | $(CA)_4GA\ (CA)_3(GA)_6$ | 0.57 | 3 | 3H | 3H |
| GBMS68 | $(CT)_{11}$ | 0.53 | 3 | 5H | | GBMS201 | $(GT)_{15}$ | 0.35 | 2 | 6H | |
| GBMS69 | $(TC)_{12}$ | 0.38 | 2 | | 2HS | GBMS202 | $(GT)_{12}$ | 0.89 | 3 | 2H | |
| GBMS70 | $(AT)_8(GA)_{12}$ | 0.51 | 2 | 5H | | GBMS203a | $(GT)_{14}$ | 0.06 | 5 | 3H | 3H |
| GBMS72 | $(TG)_{11}$ | 0.73 | 5 | 6H | 6H | GBMS203b | | 0.07 | 2 | 4H | 4H |
| GBMS74 | $(CA)_{22}$ | 0.77 | 7 | 3H | 3H | GBMS204 | $(GT)_{10}$ | 0.64 | 3 | 3H | 3H |
| GBMS75 | $(AT)_8(GT)_{16}$ | 0.94 | 5 | 5H | | GBMS206 | $(GA)_{14}$ | | 1 | | 3H |
| GBMS77 | $(GT)_{18}$ | 0.84 | 7 | 5H | 5H | GBMS211 | $(GT)_{10}$ | 0.35 | 2 | | 4H |
| GBMS79 | $(GT)_{11}$ | 0.52 | 4 | | 4H | GBMS212 | $(GA)_{14}$ | 0.49 | 3 | 3H | 3H |
| GBMS81 | $(AG)_{12}$ | 0.65 | 4 | 4H | 4H | GBMS214 | $(GT)_{10}$ | 0.37 | 3 | | 4H |
| GBMS83 | $(AC)_{20}(AT)_5$ | 0.59 | 3 | 6H | | GBMS216 | $(GA)_{33}$ | 0.77 | 5 | 2H | |
| GBMS87 | $(CT)_{15}$ | 0.74 | 6 | 4H | 4H | GBMS219a | $(GT)_{14}$ | 0.84 | 2 | 1H | |
| GBMS88 | $(GT)_8(GC)_6G_4(GT)_6$ | 0.44 | 2 | 7H | 7H | GBMS219b | | 0.28 | 5 | 5H | 5H |
| GBMS89 | $(GA)_{9}$ | 0.69 | 4 | 3H | 3H | GBMS219c | | 0.51 | 2 | 7H | 7H |
| GBMS90 | $(GA)_{17}GTGT(G)_{11}$ | 0.95 | 4 | 2H | | GBMS219d | | 0.23 | 2 | 5H | |
| GBMS93 | $(CT)_{12}$ | 0.05 | 3 | 1H | | GBMS222 | $(AC)_3TC(AC)_8$ | 0.51 | 2 | 6H | 6H |
| GBMS94 | $(GT)_{11}$ | 0.79 | 2 | 7H | | GBMS223a | $(CA)_{10}$ | 0.30 | 3 | | 7H |
| GBMS95 | $(CT)_4CCA(CT)_{10}$ | 0.64 | 3 | 2H | | GBMS223b | | 0.91 | 4 | 3H | 3H |
| GBMS96 | $(GA)_{11}$ | 0.35 | 2 | | 4H | GBMS226 | $(GT)_{9}$ | 0.63 | 3 | 7H | 7H |
| GBMS102 | $(CA)_{10}$ | 0.52 | 4 | 3H | | GBMS229 | $(GT)_{10}$ | 0.67 | 4 | 2H | |
| GBMS103 | $(GT)_{19}$ | 0.52 | 4 | 2H | | GBMS230 | $(GT)_{9}$ | 0.72 | 8 | 2H | |
| GBMS105 | $(CT)_{17}(AT)_{29}$ | 0.84 | 5 | | 3H | GBMS233 | $(TG)_8T_3(GT)_3$ | 0.79 | 3 | 2H | |
| GBMS106 | $(GA)_{38}$ | 0.84 | 7 | 5H | | GBMS235 | $(TC)_{12}T(TC)_{23}$ | 0.80 | 5 | 2H | |
| GBMS107 | $(GT)_8T(GT)_4$ | 0.62 | 4 | 6H | | GBMS238 | $(CA)_{12}$ | | 1 | | 6H |
| GBMS110 | $(GA)_{21}$ | 0.67 | 4 | 3H | 3H | GBMS240 | $(GA)_{10}$ | 0.73 | 4 | 7H | |
| GBMS111 | $(CA)_{15}$ | 0.74 | 5 | 7H | | GBMS244 | $(GT)_{10}$ | 0.64 | 3 | 2H | |
| GBMS112 | $(CA)_{20}(AT)_{23}$ | 0.07 | 4 | 7H | | GBMS245 | $(CA)_{11}\ldots(CA)_{14}$ | 0.74 | 3 | | 2H |
| GBMS114 | $(CT)_{9}$ | 0.62 | 5 | 4H | 4H | GBMS247 | $(GT)_{9}$ | 0.72 | 5 | 2H | |
| GBMS115 | $(AT)_{38}$ | 0.84 | 2 | 5H | | GBMS254 | $(GT)_{12}$ | 0.94 | 2 | 7H | |
| GBMS117 | $(CT)_{9}$ | 0.58 | 2 | 3H | 3H | | | | | | |

[a] Chromosomal location by linkage mapping on the population Steptoe/Morex or Igri/Franka
[b] Chromosomal location on wheat-barley chromosome addition lines

design and contained the expected microsatellite motif. Of these sequences, 98 contained $(GA)_n$ repeats and 128 contained $(GT)_n$ repeats, while the remaining microsatellite sequences were of a compound nature containing several repeat types including $(AT)_n$. The longest dinucleotide repeat was $(GA)_{40}$. The average repeat number was 16 for $(GA)_n$ loci and 13 for $(GT)_n$ loci. The number of repeats ranged from 6 to 40 for $(GA)_n$ and from 9 to 27

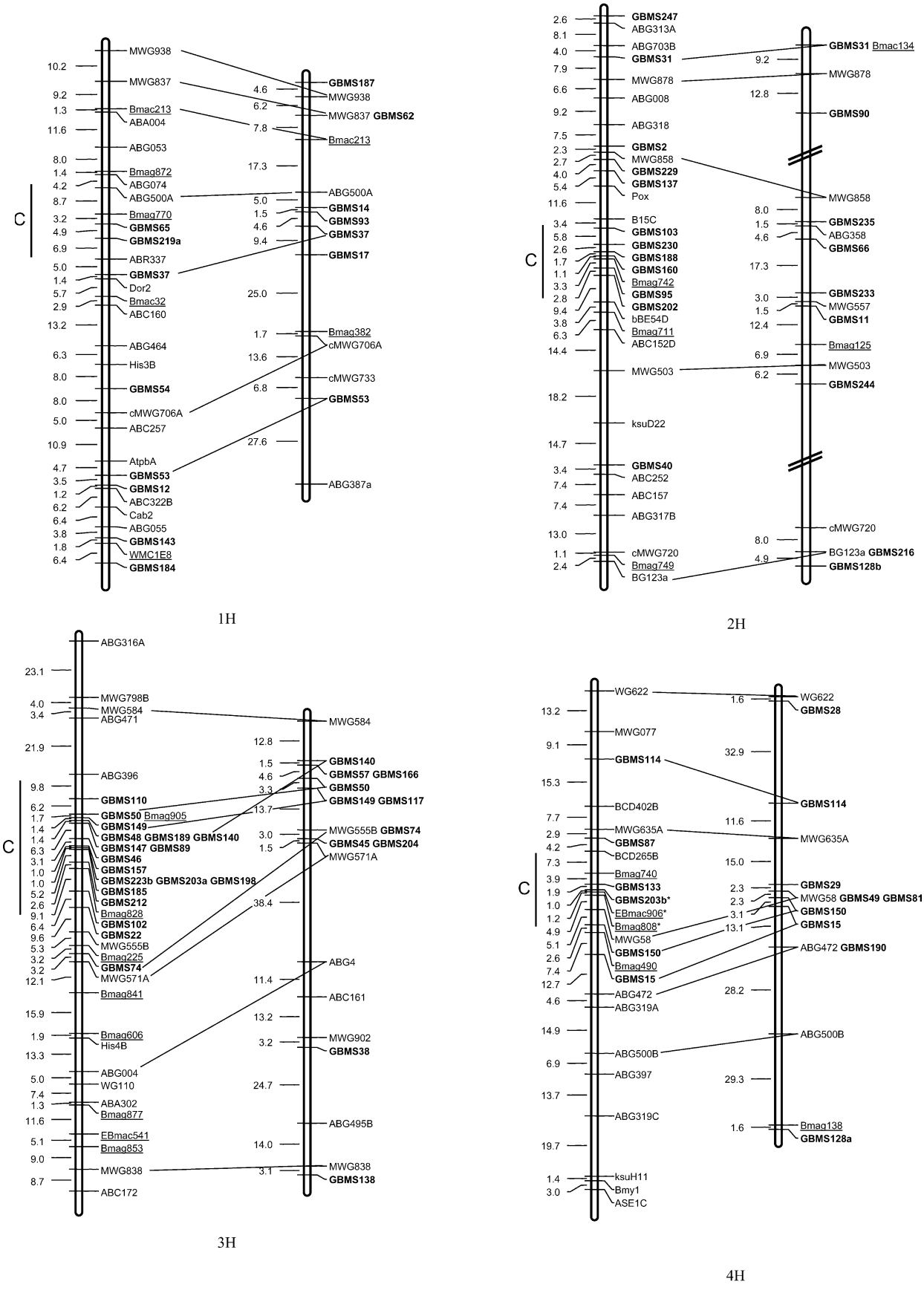
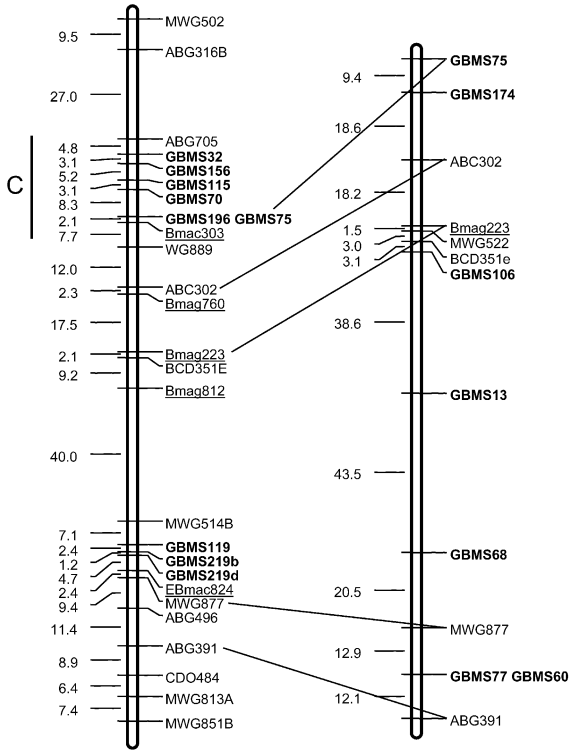
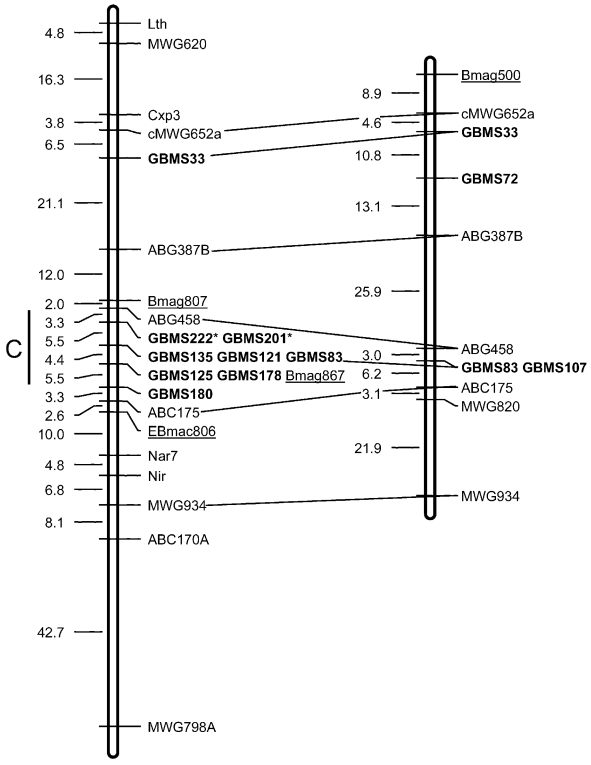


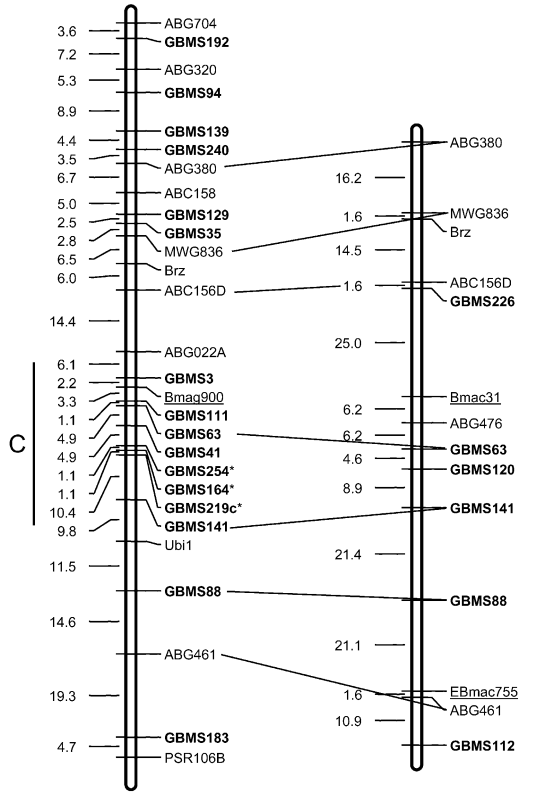

**Fig. 1** Linkage map of barley based on the data from the Steptoe/Morex cross (Kleinhofs et al. 1993) lefthand and the Igri/Franka cross (Graner et al. 1991) righthand. The new microsatellite loci mapped in this study are shown in *boldface type*, while eight loci mapped with a LOD score <3.0 are indicated with an *asterisk*. The SSRs previously published in Ramsay et al. (2000) are *underlined*. The centromeres are indicated based on a barley map published in http://barleygenomics.wsu.edu and Künzel et al. (2000)




5H




6H




7H

**Fig. 1** (continued)

for $(GT)_n$ (Table 2). A total of 167 microsatellite markers (66%) were classified as functional and amplified fragments of the expected size based on sequence data. Non-functional primers amplified either a smear, monomorphic fragments of the wrong size, many fragments or nothing. A proportion of 66% functional primers is in agreement with results obtained for wheat using libraries enriched for single- and low-copy sequences by pre-digestion with a methylation-sensitive restriction enzyme (Röder 1998). For primer pairs derived from libraries enriched for microsatellites, a level of functionality of 83% was reported based on tests with agarose gels (Ramsay et al. 2000) which is not directly comparable to the analysis on DNA sequence analyzers. In Ramsay et al. (2000) 488 (83%) out of 585 tested primer pairs were classified as functional, while only 242 primer pairs (41%) were genetically mapped. From the primer pairs described in this research out of 254 tested primer pairs 167 (66%) were classified as functional, but 127 primer pairs (50%) resulting in 133 loci could be mapped or assigned to chromosomes (Table 1).

## Screening for polymorphism and diversity analysis

The amplification quality of these newly developed primers was evaluated on a panel of six barley varieties and three *H. spontaneum* lines, according to the classification by Pepin et al. (1995) and Smulders et al. (1997). Of the microsatellites tested, 95 amplified well-scorable

fragments consisting of one expected strong band, while 72 amplified a weaker fragment of the expected size or relatively strong stutter bands. Denaturing-PAGE electrophoresis in combination with a laser fluorescence sequencer yielded a high resolution of the products differing in steps of two base pairs. In order to demonstrate the informativeness of the microsatellites, the PIC values were calculated. They ranged from 0.05 to 0.94 with 0.60 on average (Table 2). The number of alleles ranged from one to nine with an average of 3.9 alleles per locus. Only three microsatellites were monomorphic on the test panel. No significant correlation between the PIC value and number of repeats was detected. Out of the 254 primer pairs, 86 (34%) and 76 (30%) detected polymorphism between two parental lines of I/F and S/M, respectively. Forty seven (19%) primer pairs were polymorphic in both mapping populations. Few primer pairs amplified some additional monomorphic or polymorphic fragments, in addition to the expected microsatellite fragment. The microsatellites GBMS128, GBMS203, GBMS219 and GBMS223 produced two or more polymorphic loci which could be mapped. According to the literature data, the microsatellites developed from genomic DNA libraries generally have longer repeat sequences and were more polymorphic than those from ESTs. Pillen et al. (2000) reported an average PIC value of 0.38 for EST-derived barley microsatellites, a similar value of 0.45 was reported by Thiel et al. (2003).

## Mapping of microsatellite loci

Of the 86 markers surveyed in the progeny of S/M, 14 (16.3%) deviated significantly ($P < 0.05$) from the 1:1 allele frequency. Twelve loci were skewed toward 'Morex' whereas two were skewed toward 'Steptoe'. In contrast to the low level of segregation distortion in the S/M population, 34 (44.8%) loci were found significantly ($P < 0.05$) deviated from the expected ratio of 1:1 in the I/F DH population. Out of the 34 SSR loci, 27 were skewed toward 'Igri' while seven were toward 'Franka'. This high level of segregation distortion was also observed with RFLPs in the same mapping population and has possibly been caused during the development of the doubled-haploids (Graner et al. 1991). Deviations from the expected Mendelian segregation ratios were previously reported for mapping in many different plants based on both co-dominant and dominant marker types (Lyttle 1991; Xu et al. 1997; Chani et al. 2002; Knox and Ellis 2002). In a study of SSR mapping in maize (Sharopova et al. 2002), no evidence was found that the order of markers was influenced by distorted segregation. These findings were consistent with a previous report for *Arabidopsis* (Liu et al. 1996).

Overall, 127 primer pairs resulting in 133 microsatellite loci were mapped on the seven barley chromosomes. In total, 78 loci were integrated in the S/M map and 53 loci in the I/F map, while 16 microsatellites were integrated in both maps. In all cases, the mapping positions corresponded in the two maps. The assignment of 48 mapped loci was reconfirmed by a set of wheat-barley chromosome addition lines. In addition, 18 microsatellites that were monomorphic in the two mapping populations were assigned to chromosomes by this method. In order to facilitate the comparison with the microsatellite map produced by Ramsay et al. (2000) 29 previously mapped microsatellites were integrated into the S/M map and nine microsatellites into the I/F map. The total map deduced contained 204 loci for S/M and 105 loci for I/F including the framework markers. In this study, out of the 109 polymorphic primer pairs, only four amplified more than one locus. These results are in contrast with about 20% in bread wheat (Röder et al. 1998; Varshney et al. 2000; Gupta et al. 2002) and 11% of the markers detecting more than one locus in barley (Ramsay et al. 2000).

Clustering of microsatellites was observed around the centromeres, especially on chromosomes 2H, 3H, 6H and 7H. Strong centromeric clustering was also described in the microsatellite map of Ramsay et al. (2000) and is most likely the result of suppressed recombination around the centromeres as has been described in the physical map of Künzel et al. (2000). No significant clusters of SSR markers were detected in centromeric or telomeric regions of the high-density genetic map of rice (Temnykh et al. 2000).

The improved coverage of the barley genetic map with microsatellite markers will facilitate the mapping of genes and QTLs which are of economic importance in barley, and support studies of genetic diversity, pedigree analysis and the display of graphical genotypes (Russell et al. 1997, 2000; Macaulay et al. 2001; Koebner et al. 2003; Sjakste et al. 2003).

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