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Integration of microsatellite markers into the translocation-based physical RFLP map of barley chromosome 3H

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Abstract PCR with the DNA of translocation chromosomes and marker-specific primers has been used to merge genetically mapped microsatellite (MS) markers into the physically integrated restriction fragment length polymorphism (RFLP) map of barley chromosome 3H. It was shown that the pronounced clustering of MS markers around the centromeric region within the genetic map of this chromosome results from suppressed recombination. This yielded a refinement of the physically integrated RFLP map of chromosome 3H by subdivision of translocation breakpoints (TBs) that were previously not separated by markers. The physical distribution of MS markers within most of the subchromosomal regions corresponded well with that of the RFLP markers, indicating that both types of markers are similarly valuable for a wide range of applications in barley genetics.

Keywords *Hordeum vulgare* · Microsatellite markers · Physical mapping · Microisolated chromosomes · Reciprocal translocations

Introduction

Microsatellite (MS) markers based on short-repeating DNA nucleotide motifs have proven useful for the genetic analysis of plant genomes and breeding purposes because of their high levels of polymorphism and suitability for PCR amplification (Weising et al. 1989; Morgante and Olivieri 1993; Gupta et al. 1996; Cardle et al. 2000). Recently, MS-based linkage maps, partly integrated into existing restriction fragment length polymorphism (RFLP) maps, have been reported for a number of agronomically important crops such as rice (Panaud et al. 1996; Chen et al. 1997; Temnykh et al. 2000), wheat (Röder et al. 1995, 1998a; Stephenson et al. 1998), barley (Saghai Maroof et al. 1994; Liu et al. 1996; Ramsay et al. 2000), maize (Senior et al. 1996; Taramino and Tingey 1996), soybean (Akkaya et al. 1995), potato (Provan et al. 1996; Milbourne et al. 1997), tomato (Smulders et al. 1997; Areshchenkova and Ganal 1999), sugar beet (Schmidt and Heslop-Harrison 1996; Rae et al. 2000) and others. For positional gene cloning and other genetic objectives, the usefulness of microsatellites as molecular markers depends on the genomic distribution of detectable loci. Ideally, the marker distribution should coincide with regions of high gene and marker density, which are often characterized by high recombination frequency and correspond to only small chromosomal areas in plants with large genomes such as wheat (Gill et al. 1996; Faris et al. 2000) and barley (Künzel et al. 2000). For MS markers, both pericentric as well as gene-like distributions have been described. In sugar beet (Schmidt and Heslop-Harrison 1996) and tomato (Arens et al. 1995; Broun and Tanksley 1996; Areshchenkova and Ganal 1999) MS markers have been reported to be predominantly associated with centromeric regions. In barley the $(GAA)_7$ repeat physically maps to gene-poor chromosomal regions that represent heterochromatic Giemsa C-bands (Pedersen and Linde-Laursen 1994). However, on rice chromosomes a large set of MS markers have been shown to have a distribution similar to RFLP markers (Chen et al. 1997; Temnykh et al. 2000). In hexaploid wheat, an apparently random genomic distribution of MS markers was concluded from the deletion-based physical mapping of homoeologous group-2 chromosomes (Röder et al. 1998b).

For barley, Perovic et al. (2000) placed 45 MS loci on the Igri × Franka molecular linkage map (I/F map) which are genetically clustered around the centromeres. Only seven of the 45 loci (16%) mapped 20 cM outside the

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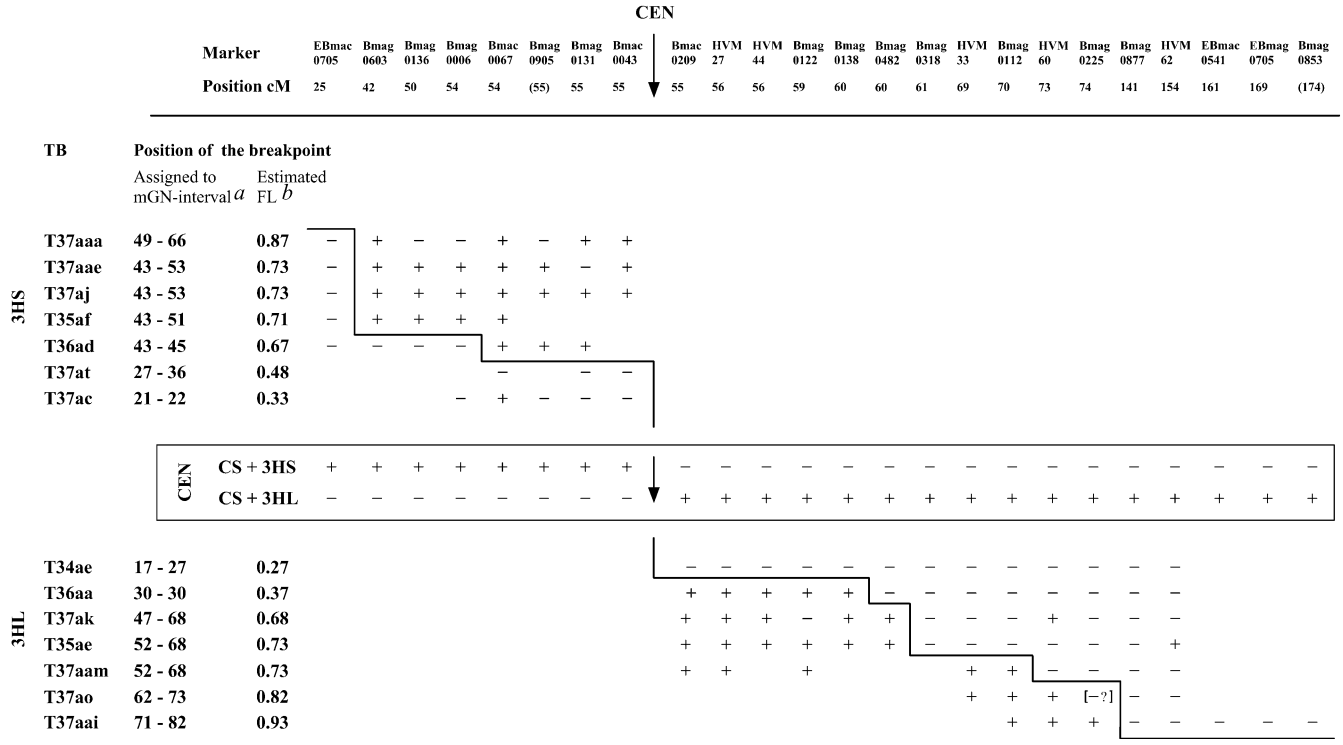


Fig. 1 Assignment of MS markers to subregions of chromosome 3H. Presence (+) and absence (–) of the 24 markers within the DNA of translocated (*T*) barley chromosomes and the DNA of wheat-barley telosome addition lines for the short arm (CS+3HS) and the long arm (CS+3HL). *Blanks* represent unchecked combinations. [a] The position of each translocation breakpoint (*TB*) is defined as an interval in milliGeNome units (1 mGN = 1/1,000 mitotic-metaphase genome length) away from the centromere according to Jensen and Linde-Laursen (1992). [b] The FL positions represent fraction lengths of chromosome arms (length of the non-translocated arm segment relative to the whole arm) calculated on the basis of the midpoints of refined mGN intervals to which the TBs were assigned. For further explanation see section results

centromeres, as compared to 354 (74%) of the 477 previously mapped RFLP loci. A tendency to cluster around centromeres was also observed by Liu et al. (1996) for 40 MS loci incorporated into barley RFLP maps. Recently, Ramsay et al. (2000) published a comprehensive linkage map of barley on the basis of 242 MS markers. A prominent feature of this map is again the strong clustering of MS loci within the centromeric regions of all seven linkage groups.

Here, we have used the translocation-(T)-based PCR technique, previously applied to physical mapping of the I/F map (Künzel et al. 2000), to integrate the MS markers of Ramsay et al. (2000) into the physical map of barley chromosome 3H. The goals of this study were: (1) to identify the physical positions of the MS markers which genetically clustered around the centromere, (2) to integrate the MS markers into the I/F map, and (3) to improve the physical map for chromosome 3H by refining chromosomal locations of translocation breakpoints (TBs) which previously could not be resolved by RFLP-derived sequence-tagged sites (STS).

Materials and methods

Out of the 20 T lines used for physical mapping of the RFLP-derived STS markers in chromosome 3H of the I/F map (see http://www.genetics.org/cgi/content/full/154/1/397/DC1/3 Table 3 and Künzel et al. 2000), 14 lines were selected based on TBs which were expected to be most informative for physical positioning of the 24 MS markers specified in Fig. 1. Additionally, we used the DNA of wheat (cv Chinese Spring), barley (cv Betzes) and wheat-barley disomic addition lines (Islam 1983) for chromosome 3H, 3HS (short arm) and 3HL (long arm) for PCR with the primers of the respective MS markers.

PCR analyses with the DNA of microdissected T chromosomes as a template were performed using primer sets of the MS markers. By ascertaining the presence or absence of marker-specific PCR products according to Sorokin et al. (1994), it was tested which part of chromosome 3H harbours the respective loci. For details of the protocol, designation of chromosomes and T lines, preparation and microdissection of chromosomes, positioning of markers in chromosomal subregions, refinement of cytological TB positions by mapping between markers of linkage groups and construction of the physically integrated map, see Künzel et al. (2000).

The PCR (35–45 cycles) was performed in volumes of 25 µl. Either 50 ng of genomic DNA, or ten microisolated chromosomes within a drop of approximately 1 µl of buffer (10 mM Tris–HCl, pH 9.0, 10 mM NaCl and 0.1% SDS), were transferred into PCR tubes. The final reaction mixture contained 100 mM of Tris–HCl, pH 8.8, 500 mM of KCl, 0.8% Nonidet P40, 0.3 µM of each primer, 0.2 µM of each dNTP, 2.0 mM of $MgCl_2$ and 1.5 units of *Taq* polymerase (MBI Fermentas). For individual markers, the PCR conditions were used as specified by Ramsay et al. (2000). Amplified DNA fragments were electrophoresed in 1.5–1.8% agarose gels and stained with ethidium bromide. Fragment sizes were estimated using a pUC marker (MBI Fermentas).

Results

With all 24 MS markers it was possible to distinguish the genomic DNA of barley (cv Betzes) and wheat (cv Chi-

nese Spring). Except for two markers that detected a barley/wheat fragment polymorphism (Bmag0112: 196 bp/140 bp; Bmag0318: 173 bp/190 bp), no PCR products were obtained from wheat DNA. Using the DNA of wheat-barley telosome addition lines for PCR, the markers were assigned to the short and the long arm of chromosome 3H. This identified the centromere within the cluster of cosegregating markers at the cM position 55, flanked by a group of markers (Bmac0043, Bmag0131 and Bmag0905) in the short arm, and Bmac0209 in the long arm (Fig. 1).

In 3HS, the seven TBs divided the eight MS markers into three groups which were placed into the FL intervals 0.48–0.67, 0.67–0.71 and 0.87–1.0 (Fig. 2). One TB split the two cosegregating markers Bmag0067 and Bmag0006 at cM position 54. None of the markers occurred between the breakpoints T37ac and T37at, nor between T35af, T37aj, T37aae and T37aaa. The five TBs, T37ac, T37at, T36ad, T35af and T37aj, that were not separated by markers of the I/F map became subdivided into three groups by inserted MS markers. This enabled us to identify the physical positions of T35af and T37aj more precisely. In 3HL, the seven TBs separated the 16 MS markers into six groups which were assigned to the FL intervals 0.27–0.37, 0.37–0.68, 0.73–0.74, 0.73–0.82, 0.82–0.93 and 0.93–1.0. There was no marker within the FL interval 0.68–0.73 between the TBs of T37ak and T35ae (Figs. 1 and 2).

The localization of MS markers within T chromosomes involving 3H yielded a few inconsistencies. The chromosomes T37aaa and T37aae, with breakpoints in 3HS, and the chromosome T37ak, with a breakpoint in 3HL, lacked three (T37aaa) or one (T37aae and T37ak) of the interstitial markers, although the distally flanking markers Bmag0603 (T37aaa), Bmag0905 (T37aae) and Bmag0138 (T37ak) were present. The chromosomes T37ac, T37ak and T35ae contained the markers Bmac0067, HVM60 and HVM62, respectively, but lacked all flanking markers. Genotype-specific nucleotide divergences preventing primer binding or generating new primer binding sites (by transposition?) could explain these contradictions since the markers, as well as the individual T lines, were derived from different genotypes. The absence of the marker Bmag0225 from the long arm of chromosome T37ao is assumed to be a non-specific result based on the cM data of the I/F map.

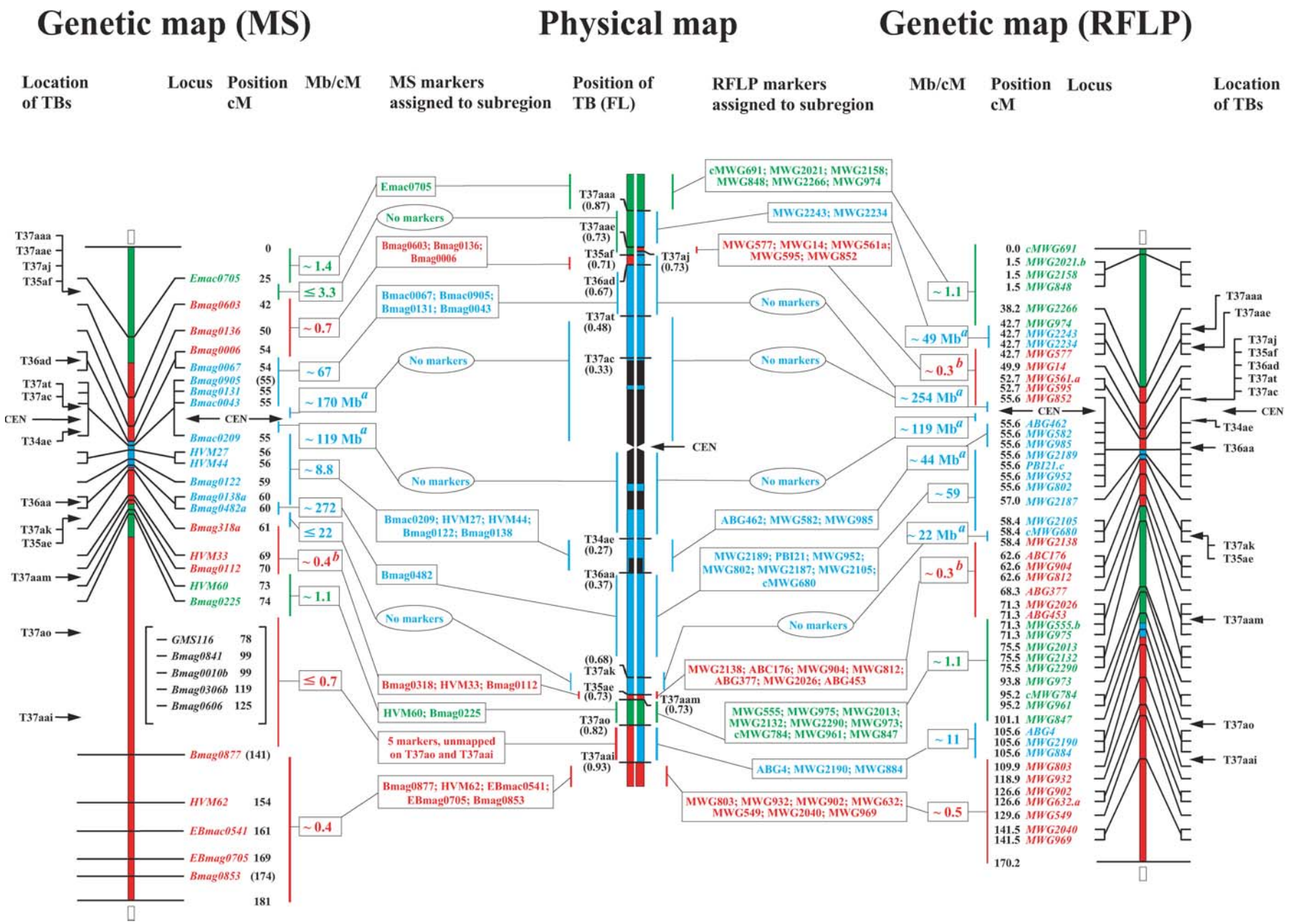
Discussion

Ramsay et al. (2000) suggested three potential explanations for the pronounced clustering of MS markers observed in the Lina × *Hordeum spontaneum* Canada Park (L/*Hs*) population: (1) the non-random distribution of SSRs in the barley genome, perhaps the result of an observed association between MS and mobile genetic elements (Ramsay et al. 1999); (2) an artifact of the enrichment procedure used to isolate the MS markers which may preferentially select for longer repeat lengths; these may, in turn, be predominantly associated with centromeric regions (Areshchenkova and Ganal 1999); and (3) the distribution of recombination events which may be exaggerated in the interspecific L/*Hs* cross used to map the MS genetically (Messeguer et al. 1991). Based on preliminary experiments in intra-specific crosses, Ramsay et al. (2000) suggested that (3) was likely to be the major contributing factor. Determining which is the real explanation for the observed distribution has significant practical importance for the effective deployment of MS markers in genetical studies ranging from estimates of genetic diversity and population biology through to linkage mapping, linkage disequilibrium studies and map-based cloning. Clearly, if barley MSs are predominantly located in pericentric locations, their practical value would be severely challenged.

The results presented here show that the centromeric clustering of MS markers observed for barley chromosome 3H on the L/*Hs* linkage map results from suppressed recombination within the pericentric region of this chromosome. The centromere is located between cosegregating markers at cM position 55. The centromere-flanking markers are separated at least by approximately 36% of the entire chromosome length. This region showed no recombination in the L/*Hs* MS map (Fig. 2). This result corresponds well with previous findings for the RFLP-based I/F map localizing the centromere of 3H at cM position 55.6, flanked by co-segregating markers which are separated by approximately 45% of the chromosome length (Künzel et al. 2000).

In general, the physical distribution of MS markers corresponds well with that of the RFLP markers, albeit that in 3HS two of the seven chromosomal subregions differ as to their content of MS and RFLP markers. The

Fig. 2 Integration of the microsatellite-based linkage map of Ramsay et al. (2000) into the cytologically integrated physical RFLP map of barley chromosome 3H according to Künzel et al. (2000). The left and the right parts of the bipartite physical map relate to the MS-based and RFLP-based linkage maps, respectively. The positions of TBs are given as estimated fraction lengths (*FL*). Megabase/centimorgan (*Mb/cM*) ratios were calculated on the basis of the DNA content of a 5,350 Mb/haploid barley genome (Bennett and Smith 1976) and relative chromosome measurements of Marthe and Künzel (1994). Taking the entire genetic length of the I/F map of 1,214.2 cM as a reference base, an average recombination frequency of 4.4 Mb/cM is assumed for the barley genome. Subregions of similar recombination rates are shown in the *same color*. *Blue* indicates suppressed (>4.4 Mb/cM), *green* increased (1.0–4.4), and *red* strongly increased (≤1.0 Mb/cM) recombination. *Black regions* of the physical map mark Giemsa N-bands. For individual subregions, the Mb/cM calculations are based on the midpoints of the cM intervals within which the TBs were mapped. For TBs mapped between the same loci, the Mb-distance between the two physically most distant TBs was divided by the cM-distance of the two flanking markers and marked by the preceding symbol ≤. [a]Indicates that the genetic distance is not definable since the TBs are located between cosegregating markers; [b]denotes a physical subregion arbitrarily assigned to an 0.01 FL interval because its two TBs occur within the same FL estimate but are located in different regions of the genetic map


Genetic map (MS)
Physical map
Genetic map (RFLP)
Location of TBs
Locus
Position cM
Mb/cM
MS markers assigned to subregion
Position of TB (FL)
RFLP markers assigned to subregion
Mb/cM
Position cM
Locus
Location of TBs
T37aaa
T37aae
T37aj
T35af
T36ad
T37at
T37ac
CEN
T34ae
T36aa
T37ak
T35ae
T37aam
T37ao
T37aai
Emac0705 25
Bmag0603 42
Bmag0136 50
Bmag0006 54
Bmag0067 54
Bmag0905 (55)
Bmag0131 55
Bmac0043 55
Bmac0209 55
HVM27 56
HVM44 56
Bmag0122 59
Bmag0138a 60
Bmag0482a 60
Bmag318a 61
HVM33 69
Bmag0112 70
HVM60 73
Bmag0225 74
GMS116 78
Bmag0841 99
Bmag0010b 99
Bmag0306b 119
Bmag0606 125
Bmag0877 (141)
HVM62 154
EBmac0541 161
EBmag0705 169
Bmag0853 (174)
181
0
~ 1.4
≤ 3.3
~ 0.7
~ 67
~ 170 Mb a
~ 119 Mb a
~ 8.8
~ 272
≤ 22
~ 0.4 b
~ 1.1
≤ 0.7
~ 0.4
Emac0705
No markers
Bmag0603; Bmag0136; Bmag0006
Bmac0067; Bmac0905; Bmag0131; Bmag0043
No markers
No markers
Bmac0209; HVM27; HVM44; Bmag0122; Bmag0138
Bmag0482
No markers
Bmag0318; HVM33; Bmag0112
HVM60; Bmag0225
5 markers, unmapped on T37ao and T37aai
Bmag0877; HVM62; EBmac0541; EBmag0705; Bmag0853
T37aaa (0.87)
T37aae (0.73)
T35af (0.71)
T37aj (0.73)
T36ad (0.67)
T37at (0.48)
T37ac (0.33)
CEN
T34ae (0.27)
T36aa (0.37)
(0.68)
T37ak
T35ae (0.73)
T37aam (0.73)
T37ao (0.82)
T37aai (0.93)
cMWG691; MWG2021; MWG2158; MWG848; MWG2266; MWG974
MWG2243; MWG2234
MWG577; MWG14; MWG561a; MWG595; MWG852
No markers
No markers
No markers
ABG462; MWG582; MWG985
MWG2189; PBI21; MWG952; MWG802; MWG2187; MWG2105; cMWG680
No markers
MWG2138; ABC176; MWG904; MWG812; ABG377; MWG2026; ABG453
MWG555; MWG975; MWG2013; MWG2132; MWG2290; MWG973; cMWG784; MWG961; MWG847
ABG4; MWG2190; MWG884
MWG803; MWG932; MWG902; MWG632; MWG549; MWG2040; MWG969
~ 1.1
~ 49 Mb a
~ 0.3 b
~ 254 Mb a
~ 119 Mb a
~ 44 Mb a
~ 59
~ 22 Mb a
~ 0.3 b
~ 1.1
~ 11
~ 0.5
0.0 cMWG691
1.5 MWG2021.b
1.5 MWG2158
1.5 MWG848
38.2 MWG2266
42.7 MWG974
42.7 MWG2243
42.7 MWG2234
42.7 MWG577
49.9 MWG14
52.7 MWG561.a
52.7 MWG595
55.6 MWG852
CEN
55.6 ABG462
55.6 MWG582
55.6 MWG985
55.6 MWG2189
55.6 PBI21.c
55.6 MWG952
55.6 MWG802
57.0 MWG2187
58.4 MWG2105
58.4 cMWG680
58.4 MWG2138
62.6 ABC176
62.6 MWG904
62.6 MWG812
68.3 ABG377
71.3 MWG2026
71.3 ABG453
71.3 MWG555.b
71.3 MWG975
75.5 MWG2013
75.5 MWG2132
75.5 MWG2290
93.8 MWG973
95.2 cMWG784
95.2 MWG961
101.1 MWG847
105.6 ABG4
105.6 MWG2190
105.6 MWG884
109.9 MWG803
118.9 MWG932
126.6 MWG902
126.6 MWG632.a
129.6 MWG549
141.5 MWG2040
141.5 MWG969
170.2
T37aaa
T37aae
T37aj
T35af
T36ad
T37at
T37ac
CEN
T34ae
T36aa
T37ak
T35ae
T37aam
T37ao
T37aai

region between FL 0.48 and 0.67 contains a group of MS markers but is free of RFLP markers and, conversely, the FL interval 0.73–0.87 contains several RFLPs but no MS markers. This could be interpreted as a bias toward a more-proximal location of the MS markers. Whether this represents a general tendency for MS loci, a larger number of MS markers needs to be physically localized at a high level of resolution in other barley chromosomes. Recently, Cardle et al. (2000) performed *in silico* surveys of MS distribution in large insert DNA clones from a number of species, and concluded that, in gene-rich regions, the MS frequency can be as high as one every 6 kb. These conclusions were confirmed experimentally in barley by sample-sequencing BAC clones, which had been selected by hybridisation to 'gene' probes. The high relative occurrence of MS sequences in the gene-rich regions was reflected in the frequency with which MSs were discovered in the DNA sequences of random subclones of the selected BACs. In contrast, the frequency of MSs in clones from a nebulised genomic DNA library of barley is in the order of one every 200–300 kb, suggesting a disjunct genomic distribution of MSs between repetitive and low-copy sequences (our unpublished results). Taken together, those results would predict the colocalisation of MS and RFLP markers. Accordingly, in 3HL, MS and RFLP, markers showed a comparable density within the seven defined subregions.

Using the map positions of TBs as reference points, the MS markers were integrated into the I/F linkage map as shown in Fig. 2.

In barley, TBs often can be assigned only roughly to larger chromosomal segments based on Giemsa-banding of the corresponding karyotypes (Linde-Laursen 1988; Kakeda and Yamagata 1991; Marthe and Künzel 1994). This situation was improved by mapping the TBs between markers of a defined genetic distance. In this case the genetic and physical TB positions determine each other mutually (see Fig. 8 of Künzel et al. 2000). Software developed to process the corresponding data for that purpose proved crucial for the high-level of physical resolution achieved for the I/F map. The localization of MS markers between the previously unseparated TBs T37ac, T37at, T36ad, T35af and T37aj provided the basis for further refinement of chromosomal FL positions for T35af and T37aj from 0.66 to 0.71 and 0.64 to 0.73, respectively, as ascertained by computer-aided data analysis.

The L/*Hs* map and the I/F map are based on 86 and 71 F_1-derived doubled-haploid (DH) lines resulting from an interspecific cross (*Hordeum vulgare* × *H. spontaneum*) and an intraspecific cross (*H. vulgare* × *H. vulgare*), respectively. Although the L/*Hs* map contains less markers (242) than the I/F map (477), and in interspecific crosses reduced-recombination may occur in genomic regions of limited homology, the total map lengths of 1,173 cM (L/*Hs*) and 1,214 cM (I/F) are nearly equal. To provide a basis for comparison of the distribution of recombination rates within individual subregions of chromosome 3H, the 1,214.2 cM-length of the I/F map was chosen to define a genome-wide average of recombination frequency (4.4 Mb/cM). The distribution of recombination rates over the physical subregions of chromosome 3H corresponds reasonably well between the two genetic maps. In 3HS, four TBs (T36ad, T35af, T37aj and T37aae) identified a small region (FL interval 0.67–0.73) of high marker density and recombination frequency within which the MS and RFLP markers are physically separated into three subregions (Fig. 2). Since the FL estimations were based on midpoints of the mGN-intervals to which the TBs were assigned, and these intervals are overlapping for T36ad, T35af, T37aj and T37aae (see Fig. 1), the three segments between T36ad and T37aae may be considered as one region of high recombination activity characterized by approximately 1.0 Mb/cM for the L/*Hs* map and approximately 1.6 Mb/cM for the I/F map. In 3HL, two FL intervals showed remarkable differences as to their recombination rates calculated from both maps. The segment between FL 0.37 and 0.68 is characterized by approximately 272 Mb/cM in the L/*Hs* map versus approximately 59 Mb/cM in the I/F map. This region seems to be more strongly affected by suppressed recombination in the L/*Hs* map than in the I/F map. The high recombination frequency within the L/*Hs* map defined for the FL interval 0.82–0.93 (approximately 0.7 Mb/cM) is in striking contrast to that of the I/F map (approximately 11 Mb/cM). The reason is that none of the five additional MS markers which mapped within the 74 to 141-cM interval of the L/*Hs* map of Ramsay et al. (2000) could be physically mapped on the T-lines. Therefore, the true genetic positions of T37ao and T37aai could not be determined on the L/*Hs* map, as was possible for the I/F map.

Altogether, the results of this study suggest that, from the viewpoint of physical distribution, the MS and RFLP markers are of similar utility for genetic objectives such as map-based gene cloning, and, for applications such as marker-assisted crop improvement, the value of the MS markers remains high due to their inherent practicality and high information content.

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