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ORIGINAL PAPER

A high density barley microsatellite consensus map with 775 SSR loci

R. K. Varshney · T. C. Marcel · L. Ramsay · J. Russell · M. S. Röder · N. Stein · R. Waugh · P. Langridge · R. E. Niks · A. Graner

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Abstract A microsatellite or simple sequence repeat (SSR) consensus map of barley was constructed by joining six independent genetic maps based on the mapping populations 'Igri × Franka', 'Steptoe × Morex', 'OWB_{Rec} × OWB_{Dom}', 'Lina × Canada Park', 'L94 × Vada' and 'SusPtrit × Vada'. Segregation data for microsatellite markers from different research groups including SCRI (Bmac, Bmag, EBmac, EBmag, HV*GeneName*, scsssr), IPK (GBM, GBMS), WUR (GBM), Virginia Polytechnic Institute (HVM), and MPI for Plant Breeding (HV*GeneName*), generated in above mapping populations, were used in the computer program RECORD to order the markers of the individual linkage data sets. Subsequently, a framework map was constructed for each chromosome by integrating the 496 "bridge markers" common to two or more individual maps with the help of the computer programme JoinMap® 3.0. The final map was calculated by following a "neighbours" map approach. The integrated map contained 775 unique microsatellite loci, from 688 primer pairs, ranging from 93 (6H) to 132 (2H) and with an average of 111 markers per linkage group. The genomic DNA-derived SSR marker loci had a higher polymorphism information content value (average 0.61) as compared to the EST/gene-derived SSR loci (average 0.48). The consensus map spans 1,068 cM providing an average density of one SSR marker every 1.38 cM. Such a high-density consensus SSR map provides barley molecular breeding programmes with a better choice regarding the quality of markers and a higher probability of polymorphic markers in an important chromosomal interval. This map also offers the possibilities of thorough alignment for the (future) physical map and implementation in haplotype diversity studies of barley.

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R. K. Varshney and T. C. Marcel contributed equally to this work.

R. K. Varshney · M. S. Röder · N. Stein · A. Graner
Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstrasse 3, 06466 Gatersleben, Germany

R. K. Varshney (✉)
Applied Genomics Laboratory,
International Crops Research Centre for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324 AP, India
e-mail: r.k.varshney@cgiar.org

T. C. Marcel · R. E. Niks
Laboratory of Plant Breeding,
Graduate School for Experimental Plant Sciences,
Wageningen University, Droevendaalsesteeg 1,
6708 PB Wageningen, The Netherlands

L. Ramsay · J. Russell · R. Waugh
Genetics, Scottish Crop Research Institute (SCRI),
Invergowrie, Dundee DD2 5DA, UK

P. Langridge
Australian Centre for Plant Functional Genomics (ACPFG), University of Adelaide,
PMB1 Glen Osmond, South Australia

Introduction

Molecular genetic maps of crop species find a variety of uses not only in breeding but also in genomics research. For instance, molecular genetic maps have been

extensively used for comparative genomic studies, throwing light on genome organization in grasses in general and in cereal crops in particular. Molecular genetic maps are also used for the identification and mapping of genes and quantitative trait loci (QTLs) for morphological, physiological and economic traits of crop species.

In barley, the first molecular genetic maps comprised RFLP markers (Graner et al. 1991; Kleinhofs et al. 1993) and over time, PCR based molecular markers became the dominant marker type (see Varshney et al. 2004). Among different types of molecular markers available for barley, microsatellite or simple sequence repeats (SSRs) have proven to be the markers of choice for marker-assisted selection (MAS) in breeding and genetic diversity studies. This is largely because they require small amounts of sample DNA, are easy to detect by PCR, are amenable to high-throughput analysis, co-dominantly inherited, multi-allelic, highly informative and abundant in genomes (Powell et al. 1996; Gupta and Varshney 2000). The value of microsatellite markers for both genetic diversity studies and for barley breeding was demonstrated as early as 1994 (Saghai Maroof et al. 1994; Becker and Heun 1995; Liu et al. 1996; Struss and Plieske 1998). Later, comprehensive microsatellite genetic maps integrating 242 SSR loci and 127 SSR loci were prepared by Ramsay et al. (2000) and by Li et al. (2003), respectively. In the majority of the studies mentioned above, the SSR markers were developed after screening small insert or microsatellite enriched genomic libraries for SSR motifs. In recent years, however, because of the availability of large expressed sequence tag (EST) datasets for a number of plant species and the development of several bioinformatics tools, it has been possible to identify and develop SSR markers from ESTs (Pillen et al. 2000; Thiel et al. 2003; Ramsay et al. 2004; Varshney et al. 2006a). The SSR markers derived from ESTs are commonly known as ‘‘EST-SSRs’’. The development of such markers, in contrast to the earlier genomic SSRs, is easier, faster and cheaper (Varshney et al. 2005a).

Ideally, a molecular genetic map should be densely populated with PCR-based markers. This is especially important as barley genomics research increasingly involves map-based gene cloning projects that require accurate, fine genetic maps to correctly position a gene of interest between closely linked flanking markers (Stein and Graner 2004). To further facilitate such studies, efforts are currently underway to prepare subgenomic physical maps with the eventual objective of capturing and sequencing the barley gene-space (http://phymap.ucdavis.edu:8080/barley/index.jsp).

Although several hundreds of microsatellite markers have been developed, they have been mapped in several mapping populations that vary in their level of polymorphism (Varshney et al. 2004). To increase the density of microsatellite markers available on the overall barley genetic map and to provide relative locations, the present study aimed to construct a consensus genetic map integrating all available SSR-marker data. This goal was achieved by employing common markers (RFLP, AFLP and SSR) on each chromosome to anchor the chromosome maps from different populations. The final consensus map included 775 microsatellite marker loci offering a significant improvement over any single population genetic map. The distribution of different types of SSR loci and the PIC values for the markers are discussed.

Materials and methods

Mapping populations

A total of six mapping populations were integrated into a single consensus map. These included two recombinant inbred line (RIL) populations and four doubled haploid (DH) populations (Table 1). The RIL populations have been developed at the Laboratory of Plant Breeding, Wageningen University, The Netherlands, and consist of L94 × Vada (L × V) developed by Qi et al. (1998) and of SusPtrit × Vada (Su × V) developed by Jafary et al. (2006). The two DH populations Steptoe × Morex (St × M) and the Oregon Wolfe Barleys (OWBs), developed in North America, are reference mapping populations and subjects of extensive genotyping and phenotyping. The St × M population is the product of the North American Barley Genome Mapping Project (NABGMP) (Kleinhofs et al. 1993) and the OWB population was developed by Costa et al. (2001). The Igri × Franka DH population (I × F) was developed by Graner et al. (1991). The Lina × *Hordeum spontaneum* Canada Park (Li × Hs) is a DH population from Svalof Weibull and was used by SCRI (Ramsay et al. 2000) to genetically map 242 SSR marker loci.

SSR markers and segregation data

Several sources of SSR markers, listed in Table 2, and mapped in different mapping populations were used to prepare the barley microsatellite consensus map. These markers included both marker types, derived from genomic DNA as well as from genes or ESTs. More than ten designations have been assigned to these

Table 1 Summary of individual mapping data used to construct the microsatellite consensus map of barley

Population number	Name of the mapping population	Type of population	Number of lines	Total number of markers	Predominant marker type	Number of SSR markers	Number of SSR markers in common with n other mapping populations				
							$n = 0$	$n = 1$	$n = 2$	$n = 3$	$n = 4$
1	L94 × Vada (L × V)	F_9 RIL[a]	103	968	AFLP[c]	138	57	38	19	17	7
2	SusPtrit × Vada (Su × V)	F_8 RIL	152	450	AFLP	24	12	0	2	8	2
3	Steptoe × Morex (St × M)	DH[b]	150	694	RFLP[d]	218	110	70	17	15	6
4	OWBrec × OWBdom (OWB)	DH	94	995	AFLP	230	156	34	17	16	7
5	Igri × Franka (I × F)	DH	71	695	RFLP	139	54	60	10	9	6
6	Lina × *H. spontaneum* (Li × Hs)	DH	84	418	SSR[e]	307	195	68	22	15	7
	Total						584	135	29	20	7

[a] Recombinant inbred line, [b]Doubled haploid, [c]Amplified fragment length polymorphism, [d]Restriction fragment length polymorphism, [e]Simple sequence repeat

Table 2 Details on microsatellite loci integrated into the consensus map

Microsatellite code	Source of markers	Number of loci	Developing laboratory	References
AF, BAC	BAC end sequences	4	SCRI (R. Waugh)	Ramsay et al. (2000), Cardle et al. (2000)
Bmac, EBmac	Genomic DNA libraries (AC repeats)	157	SCRI (R. Waugh)	Ramsay et al. (2000)
Bmag, EBmag	Genomic DNA libraries (AG repeats)	135	SCRI (R. Waugh)	Ramsay et al. (2000)
Bmg	Genomic DNA library	2	SCRI (R. Waugh)	Ramsay et al. (2000)
EBmatc	Genomic DNA library (ATC repeats)	6	SCRI (R. Waugh)	Ramsay et al. (2000)
GMS	Genomic DNA libraries (GA and GT repeats)	12	IPK (D. Struss)	Struss and Plieske (1998), Li et al. (2003)
GBMS	Genomic DNA libraries (GA and GT repeats)	119	IPK (M. Röder, M. Ganal)	Li et al. (2003)
HVM	Majority from genomic DNA and some from genes	34	VPISU (M.A. Saghai Maroof)	Saghai Maroof et al. (1994), Liu et al. (1996), Li et al. (2003)
HV*GeneName*	Barley genes	7	MPIZ (M. Heun)	Becker and Heun (1995)
HV*EMBLName*	Barley genes	17	SCRI (R. Waugh) Univ. Bonn (K. Pillen)	Ramsay et al. (2000) Pillen et al. (2000)
GBM	Barley ESTs	246	IPK (A. Graner) WUR (R.E. Niks)	Thiel et al. (2003), Varshney et al. (2006a) Marcel et al. (2007)
scssr	Barley ESTs	34	SCRI (R. Waugh)	Ramsay et al. (2004), Rostoks et al. (2005)
WM	Wheat microsatellites from genomic DNA libraries	2	SCRI (R. Waugh) VPISU (M.A. Saghai Maroof)	Ramsay et al. (2000) Liu et al. (1996)

markers by the laboratory that developed the markers (Table 2, Tables ESM S1, S2).

The segregation data of 968 marker loci mapped in L × V and of 450 marker loci mapped in Su × V were obtained from Marcel et al. (2007). Those data sets predominantly consisted of AFLP markers, but also included 138 and 24 microsatellite loci, respectively (Table 1). Two barley segregation data sets were downloaded from the publicly available GrainGenes 2.0 database (http://wheat.pw.usda.gov/GG2/index.shtml), for the St × M and I × F populations, respectively. Those two data sets predominantly consisted of RFLP markers, to which the segregation data for 218 and 139 microsatellite loci (Table 1) were added, respectively. Another set of segregation data was downloaded from the Oregon State University (OSU) Barley Project web site (http://www.barleyworld.org/), for the OWBs. Most of the markers mapped in the OWB population are AFLP markers, but the segregation data for 230 microsatellite loci could also be obtained (Table 1). Within the latter set of 230 microsatellite loci, 34 are new scssr (SCRI-SSR) loci recently integrated into a SNP map of barley (Rostoks et al. 2005) and provided by Joanne Russell. Finally, the segregation data of 418

marker loci, 307 being microsatellite loci, mapped in Li × Hs were provided by Luke Ramsay (Table 1).

The genotyping data for all the SSR loci mapped in different mapping populations have been appended as Table ESM S3.

Marker ordering in the individual maps

The recently developed computer program RECORD (Van Os et al. 2005a) was used to order the markers from the six individual linkage data sets, which comprised from 400 to 1,000 markers per set (Table 1). RECORD employs a marker-ordering algorithm based on minimization of the total number of recombination events in any given marker order. The linkage groups were sorted by graphical genotyping in Microsoft® Office Excel 2003. The ordering of markers with RECORD was repeated three times for each individual linkage map. Between each two marker orderings, singletons and other potential errors in the marker segregation data were identified by visual inspection of graphical genotypes. The identified singletons (a single locus in one progeny line that appears to have recombined with both its directly neighbouring loci) were replaced by missing values as suggested by Isidore et al. (2003) and Van Os et al. (2005b).

Production of the framework map

The RECORD software package does not offer the possibility to integrate different marker data sets. The integration module of the software package JoinMap® 3.0 (Van Ooijen and Voorrips 2001) could also not be used directly because it cannot handle sets of several thousands of segregating markers. Then, the integrative function of JoinMap® 3.0 was used to construct a framework map for each chromosome containing only the bridge markers identified between two or more populations. A bridge marker was considered as such when it had an (almost) identical name and a similar map position in the different mapping populations concerned. Markers with the same name that mapped to different positions in different populations were not considered to be common. The obtained framework maps contained 45, 86, 82, 54, 69, 68 and 79 integrated bridge markers for the barley linkage groups 1H to 7H, respectively. Those 496 bridge markers consist of 191 SSRs, 160 AFLPs, 139 RFLPs and 6 genes mapped by function spanning 1,024 cM with an average density of one marker every 2.1 cM. All markers were assigned to a chromosome during the marker ordering procedure. For each chromosome, the identified bridge markers were assembled and the corresponding framework map calculated separately in JoinMap® 3.0. The values used to calculate the maps ranged from 0.2 to 1.0 for the LOD (logarithm of odds) threshold and from 0.400 to 0.490 for the recombination threshold, depending on the linkage group. The map distances were calculated using the Kosambi mapping function.

Construction of the SSR consensus map

The final map comprising all 3,610 markers was calculated based on the ‘‘neighbours’’ map approach described by Cone et al. (2002). A new improved version of JoinMap based on a faster algorithm (Jansen et al. 2001) was kindly provided by Dr. van Ooyen (www.kyazma.nl). The six individual barley maps were recalculated by adding the order of the framework markers, as given by JoinMap® 3.0, as a ‘‘fixed order file’’ into this improved version of JoinMap. Then, the framework map served as a fixed backbone onto which the unique loci of each newly calculated individual map were added. For a target locus, the two nearest flanking bridge markers shared by the framework map and by the map to integrate were identified and the coordinate of this locus was calculated relative to the ratio of the intervals defined by the flanking bridge markers on the two maps. In such a way, an integrated map of 3,610 markers was obtained from which the coordinates of 775 unique microsatellite loci were extracted. In the final microsatellite integrated map of barley the position of BIN markers, as defined by Marcel et al. (2007), are given as reference. Mostly, the same BIN-defining markers and numbers as defined by Kleinhofs and Graner (2001) were maintained. Each 10 cM BIN was subdivided into two 5 cM subBINs.

Polymorphism information content (PIC)

The PIC is a tool to measure the informativeness of a given DNA marker. The PIC value is generally calculated using the following formula (Anderson et al. 1993).

$$\mathrm{PIC} = 1 - \sum_{i=1}^{k} P_i^2,$$

where k is the total number of alleles detected for a microsatellite and P_i the frequency of the ith allele in germplasm investigated.

The PIC value for the SSR markers developed at IPK and WUR was calculated using the above formula. However, the PIC value for a majority of the other markers integrated into the microsatellite consensus

map was taken from the original publications in which the corresponding markers were first reported (Table 2). Other publications reported PIC values of SSR markers calculated on different sets of barley lines and cultivars (Matus and Hayes 2002; Ivandic et al. 2003; Karakousis et al. 2003; Sjakste et al. 2003; Malysheva-Otto et al. 2006). Those PIC values were compiled in Microsoft® Office Excel and identical microsatellites (identical name) between marker sets were identified and aligned. For each set of values, microsatellites in common with the ones reported in this paper were used to calculate a correlation coefficient.

Results

Consensus microsatellite map

The present barley microsatellite consensus map contains a total of 775 microsatellite loci mapped with 688 microsatellite primer combinations in one or more of the six barley populations used (Fig. 1, Tables ESM S1, S2). In total 191 SSR markers were in common, i.e. they were mapped in at least two mapping populations (Table 1). A total of 584 SSR marker loci were mapped only once in a particular mapping population, while seven SSR marker loci were mapped in five mapping populations. The RECORD order of those markers that segregated in more than one population was highly consistent between the six individual mapping data sets. On the consensus map, linkage group 2H had the highest number of markers (132) with an average marker density 1/1.19 cM followed by linkage group 7H (127) with an average marker density 1/1.24 cM (Table 3). Linkage group 6H had the smallest number of markers (93) and the lowest marker density (1/1.75 cM) was observed on linkage group 5H. Although all linkage groups had a more or less uniform distribution of SSR loci, some gaps of 14–22 cM without microsatellite marker were observed on the distal ends of linkage groups 5H and 6H (Fig. 1). Clustering of microsatellite markers at centromeric regions was observed with 33.5% of the markers found in 5.6% of the BINs. In total, the consensus microsatellite map of barley had 1,068 cM genome coverage with an average density of one microsatellite per 1.38 cM. The BIN marker order of the present consensus map was inspected for inconsistencies with the order of the same markers on the BIN map of Kleinhofs and Graner (2001) and of the Steptoe × Morex and Igri × Franka linkage maps. The marker orders between the maps were in good agreement with only two inversions of markers on chromosome 3HS and at the distal end of chromosome 5HL. Chromosomes 3HS and 5HL were recalculated by adding the BIN markers of Kleinhofs and Graner (2001) as fixed order in JoinMap® 3.0. The present SSR consensus map was also aligned with the SSR maps developed by Ramsay et al. (2000) (GrainGenes: "Barley, LxHs") and by Li et al. (2003) (GrainGenes: "Barley, Steptoe × Morex, SSR"). The SSR marker orders were highly consistent between all maps. Nevertheless, differences in the order of markers were observed within the centromeric BINs of the linkage groups from the present consensus map and from the map of Ramsay et al. (2000). The primer sequences for the SSR loci integrated into the consensus map, wherever possible, are given in Table ESM S2 and the genotyping data for all the SSR loci are given in Table ESM S3.

Nomenclature of SSR loci

Several SSR developing laboratories have designated their SSR markers by their own codes (or code systems) (Table 2). The SSR markers that mapped in more than one mapping population are in the present study termed as common bridge markers, as these have been used to prepare the consensus map. In fact, the integration of several genetic maps depends on the number and on the distribution of common bridge markers between the individual maps. However, while checking the segregation data for markers in different mapping populations, several inconsistencies were found in the designation of the same SSR marker mapped in more than one mapping population. In order to maintain the uniformity and avoid confusion, we made some slight changes in the designations of mapped SSR loci and recommend the community to use the same in the future (Table ESM S1). For example, the Bmac, Bmag, EBmac, EBmag, EBmatc and GBM microsatellite loci were all identified with a suffix of four digits (e.g. Bmac29 becomes Bmac0029). Similarly, the GMS and GBMS microsatellite loci were identified with a suffix of three digits (e.g. GBMS2 becomes GBMS002), and the HVM microsatellite loci were identified with a suffix of two digits (e.g. HVM4 becomes HVM04). Multiple segregating bands identified with one microsatellite primer pair have been usually indicated with lower case letters; for example, two bands (loci) for the Bmac0040 SSR marker (primer pair) became Bmac0040a and Bmac0040b. However, the same letter was often assigned to different loci identified with the same microsatellite primer pair in different populations. Those markers were renamed in a way that distinctive letters were assigned to different loci (Table ESM S1).

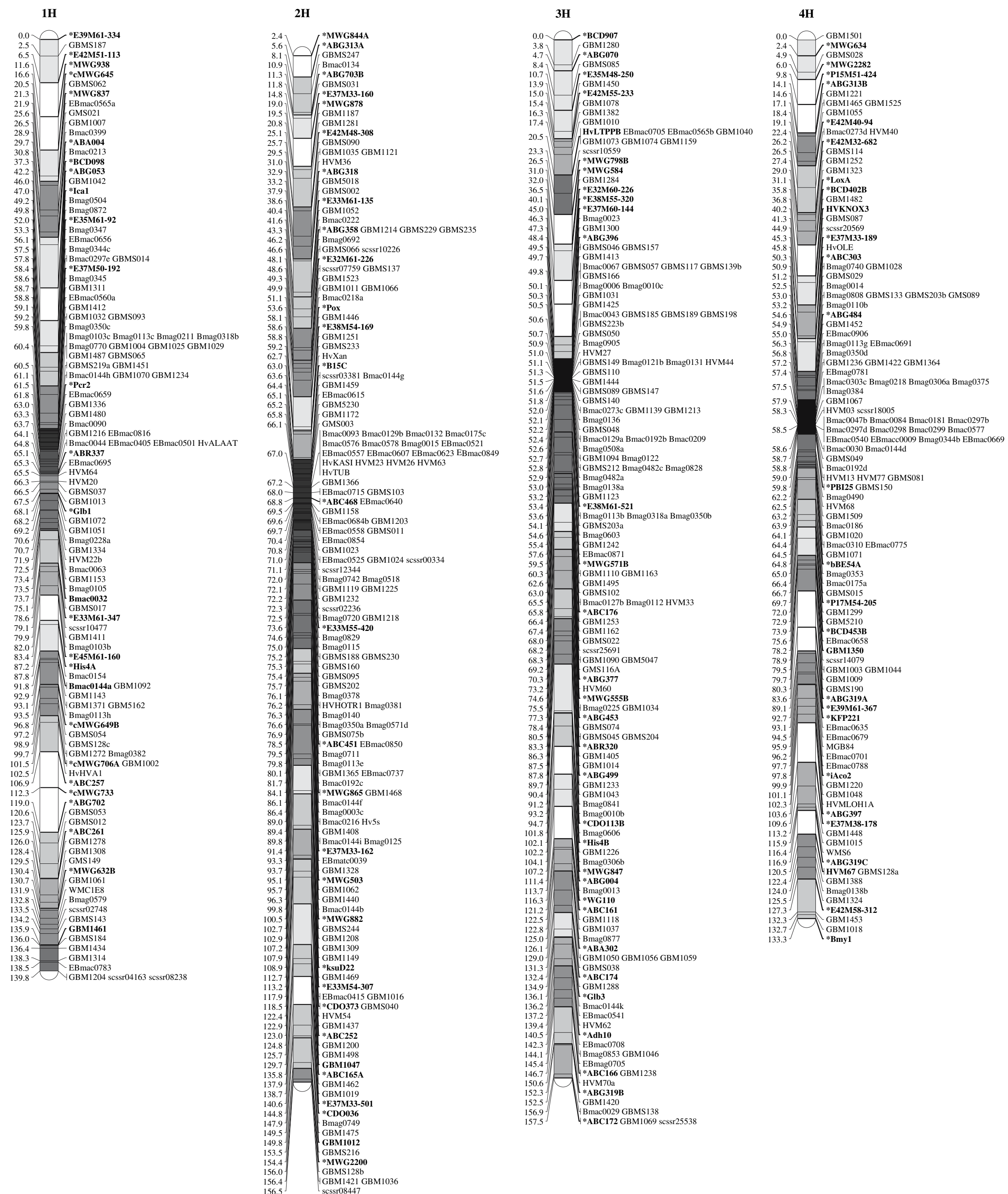

Fig. 1 A microsatellite consensus map of barley (*Hordeum vulgare*) derived from six independent genetic maps. A skeleton map with common markers was constructed using JoinMap® 3.0 and used to fit the markers from the six individual maps. The BIN markers, as defined by Marcel et al. (2007) are in *bold*. The *loci preceded by an asterisk* are BIN markers, which are not microsatellites. The remaining loci are microsatellite markers. Co-segregating markers are listed next to each other in a vertical line on the right side of the chromosome. *Numbers on the left side* show the distance in centiMorgans from the top of each chromosome. *Colour intensity inside the bars* indicates the density of microsatellite markers per BIN. Detailed information about these markers including the name of microsatellite loci, the chromosome position, the repeat motif, the PIC value (if available) and the contact of the developing laboratory are available in Table ESM S1 while the primer sequences for the mapped SSR loci are available in Table ESM S2. The genotyping data for all the mapped SSR loci are available in Table ESM S3. Additionally, all the supplementary data are available at GrainGenes under the URL http://wheat.pw.usda.gov/pubs/2007/varshney/

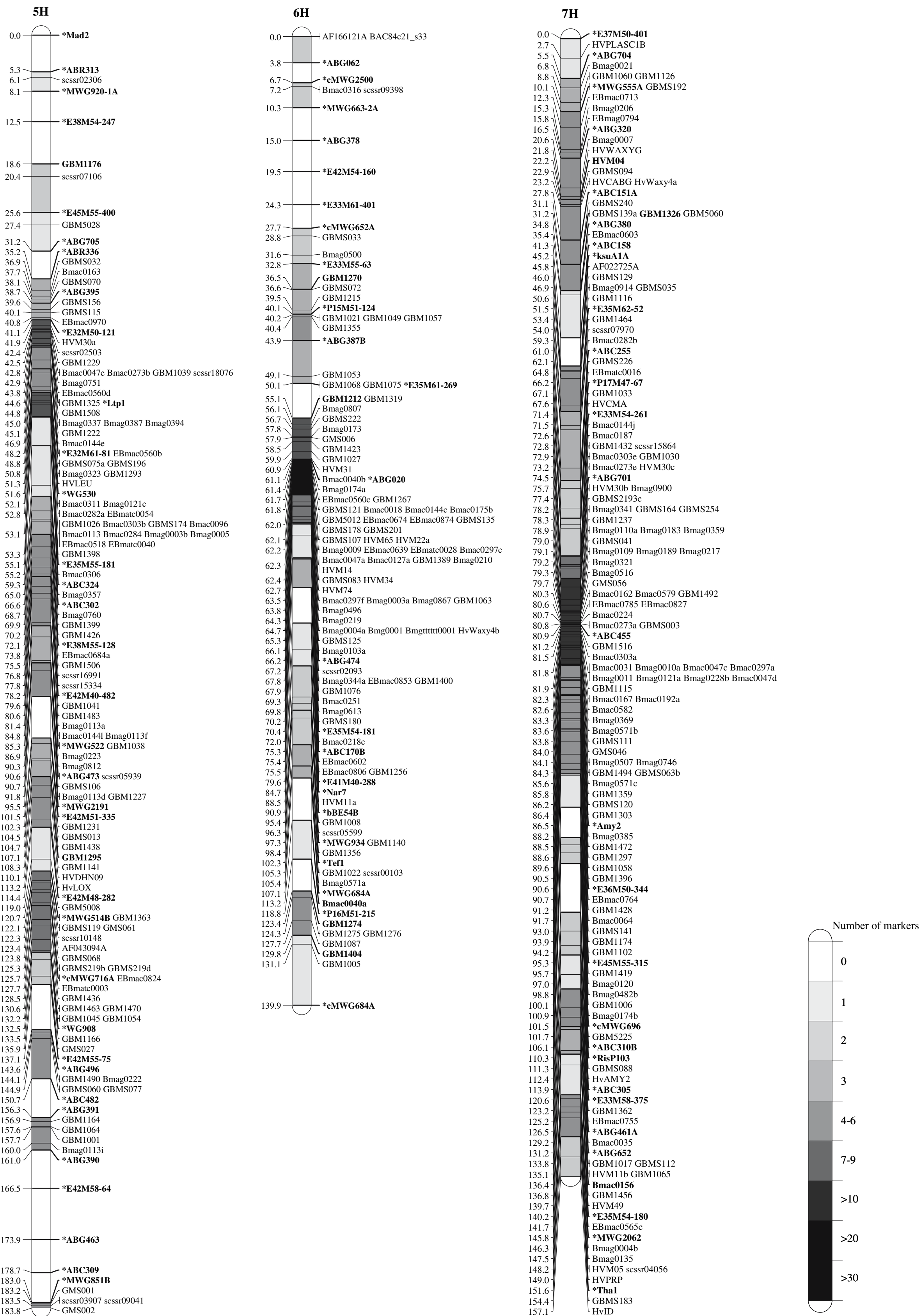

Fig. 1 continued

Table 3 Summary of the number of SSR markers integrated into the barley microsatellite consensus map

Linkage group (chromosome)	Mapping populations						Consensus map	Genome coverage (cM)	Marker density
	1. L × V	2. Su × V	3. St × M	4. OWB	5. I × F	6. L × Hs			
1H (5)	13	4	23	29	18	37	96	139.8	1.46
2H (2)	27	7	34	41	15	50	132	156.5	1.19
3H (3)	17	4	45	38	23	42	122	157.5	1.29
4H (4)	14	2	27	34	20	43	100	133.3	1.33
5H (5)	23	1	27	36	14	34	105	183.7	1.75
6H (6)	25	2	26	26	24	37	93	139.9	1.50
7H (1)	19	4	36	26	25	64	127	157.1	1.24
Total	138	24	218	230	139	307	775	1067.8	1.38

Microsatellite repeat motifs

Out of 775 SSR loci integrated into the consensus map, information on occurrence of the SSR repeat motif was available for 768 SSR loci. More than 56% of SSR loci (435) for which repeat information was available, consisted of dinucleotide repeat motifs (NN) (Table - ESM S1). Compound microsatellites occur when two different SSRs, separated by a few base pairs, are amplified with the same primer pair. In the present study, compound microsatellites consisted in a majority of NNs and were the second most common type of SSR loci (163 loci, 21%) integrated to the consensus map. The trinucleotide (NNN) and tetranucleotide (NNNN) repeat motifs were present only in 16.5% (128) and 3.6% (28) of the SSR loci, respectively. The remaining repeat classes, i.e. mononucleotide (N), pentanucleotide (NNNNN) and hexanucleotide (NNNNNN), were represented by less than 1% of the SSR loci.

Polymorphism information content (PIC) value

The PIC value measures the informativeness of a given DNA marker over a set of genotypes. Therefore, the PIC value of SSR markers available in a given window on the consensus map is a good indicator of their potential usefulness. For this reason, we compiled the PIC value available for the SSR markers, from the original studies, in the Table ESM S1. The PIC values are comparable between the different sets of microsatellites because they have been calculated based on similar panels mainly composed of European breeding lines. Overall, the SSR markers that mapped on linkage group 7H had the highest average PIC value (0.59) followed by the markers mapped on linkage groups 2H and 3H. The SSR markers located on 1H had on average the lowest PIC value (0.53). The majority of SSR markers (>54%) for which a PIC value was available had a PIC value of >0.50 and about 16% of the SSR markers had a PIC value of >0.75. The genomic DNA-derived SSR marker loci had a higher PIC value (average 0.61) than the EST/gene-derived SSR loci (average 0.48) (Fig. 2).

In general, the dinucleotide SSRs had the highest PIC value (average 0.58) as compared to mononucleotide (average 0.47), trinucleotide (average 0.46), tetranucleotide (average 0.43), pentanucleotide (average 0.50) or hexanucleotide (0.41) markers. The compound microsatellites had the highest PIC values as 0.59 (average).

The compiled PIC values for microsatellite loci of the SSR consensus map was compared to the PIC values obtained for the same microsatellite loci in other studies and on different panels of barley cultivars (Table 4). The highest correlation coefficient between PIC values ($r = 0.70$) was obtained with a worldwide collection of 953 accessions. About 60% of those 953 accessions are from European origin like the accessions used to calculate the PIC values compiled in our study. Lower correlation coefficients were obtained with the sets of barley breeding lines from other continents. The lowest correlation coefficient ($r = 0.30$)

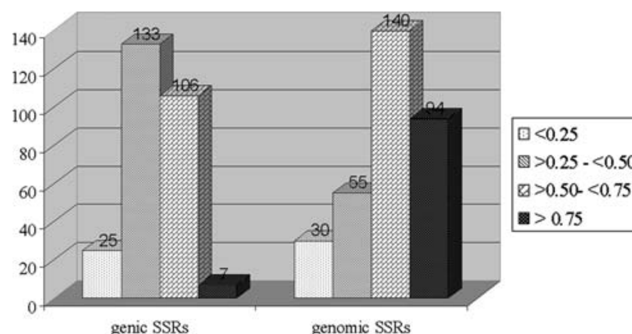

Fig. 2 Distribution of the polymorphism information content (PIC) value of genic and genomic microsatellites. A comparison of PIC value of genic and genomic SSR is shown. The markers are classified into four groups having PIC values, (1) less than 0.25, (2) between 0.25 and 0.50, (3) between 0.50 and 0.75, and (4) more than 0.75

Table 4 Correlation coefficients between the PIC values compiled for the SSR loci of the consensus map, calculated on several sets of European barley cultivars, and the PIC values obtained in previous studies, calculated on different sets of barley accessions

Germplasm description	Common markers[a]	Correlation coefficient[b]	References
953 accessions through the world	44	0.70	Malysheva-Otto et al. (2006)
37 Latvian cultivars	57	0.47	Sjakste et al. (2003)
40 Australian breeding lines	55	0.43	Karakousis et al. (2003)
96 North-American breeding lines	37	0.37	Matus and Hayes (2002)
52 *H. spontaneum* accessions	30	0.31	Ivandic et al. (2003)

[a] Number of microsatellite loci common between the SSR consensus map and the study considered for which PIC values were available

[b] Correlation coefficient between the PIC values compiled for the SSR consensus map and the PIC values obtained for the study considered

was obtained with the set of wild barley accessions (*H. spontaneum*) collected in Israel.

Functional SSR markers

Although SSR markers developed earlier were thought to be associated with retrotransposons, recent analysis on SSRs in genomic and EST sequence data have shown that microsatellite sequences also occur in genes (Morgante et al. 2002). Several gene (EST)-derived SSR markers (= genic SSR markers) have been developed in barley recently. Unlike markers derived from genomic DNA, a putative function can be deduced for gene-/EST-derived markers (Varshney et al. 2005a). Therefore, they represent a functional class of molecular markers (Andersen and Lübberstedt 2003). The functional SSR markers include earlier published genic SSR markers (Saghai Maroof et al. 1994; Becker and Heun 1995; Pillen et al. 2000) and recently developed EST-derived SSR markers (Thiel et al. 2003; Rostoks et al. 2005; Varshney et al. 2006a; Marcel et al. 2007). In total, 44% of the SSR marker loci (339) placed on the consensus map are genic/functional-SSR loci.

Discussion

Since the advent of molecular marker and linkage mapping technologies the number of marker loci placed on genetic maps is increasing exponentially. In crop plant species such as rice, maize and soybean, several high-density genetic maps are available (Phillips and Vasil 2001). Dense genetic maps are very useful for plant breeders to help identify molecular markers closely linked to the genes or QTLs of their interest (Varshney et al. 2006b). Further, dense genetic maps are important to prepare contig-based local or genome wide physical maps, for map-based cloning and for genome sequencing projects. Since microsatellite markers are currently preferred over other molecular markers for a variety of reasons, high density microsatellite maps, such as those developed in rice (McCouch et al. 2002), maize (Sharopova et al. 2002) and wheat (Somers et al. 2004), are very useful.

Features of the barley SSR consensus map

Although a large number of SSR markers are available in barley, they have been developed and mapped in different mapping populations. Ideally, all markers should be mapped in the same mapping population. However, the limited polymorphism in current mapping populations has not allowed all possible SSR markers to be mapped onto a single genetic map. An alternative way to prepare a dense SSR genetic map is to combine the different and available genetic maps by exploiting common bridging markers. Consensus maps including various types of molecular markers have been developed before in several species, e.g. barley (Langridge et al. 1995; Qi et al. 1996; Karakousis et al. 2003), tomato (Haanstra et al. 1999), wheat (Somers et al. 2004), pearl millet (Qi et al. 2004) and potato (Van Os et al. 2006). We have derived the most extensive consensus SSR map of barley so far. The map displays the genetic position of microsatellites at a density (1/1.38 cM) that should enhance their application in both plant breeding and physical mapping. Despite the dense average spacing of the markers, some gaps on the distal ends of linkage groups 5H and 6H occur. These may reflect regions of high recombination. A lack of markers in these regions was observed in other genetic maps of barley (Kleinhofs et al. 1993; Qi et al. 1998; Ramsay et al. 2000).

The consensus SSR map contains almost all types of SSR loci, however, dinucleotide and compound (mainly containing different dinucleotide SSRs) microsatellites (56 and 21%, respectively) occurred in higher proportion than the trinucleotide (16.5%) and other types of microsatellite. The most likely explanation for this observation is that the majority of SSR loci integrated in the consensus map were derived from genomic DNA libraries that had been screened only for dinucleotide SSR probes (Ramsay et al. 2000; Li

et al. 2003). The availability of different types of SSR loci in a given region (chromosome interval) will facilitate selection of the SSR repeat motifs of choice in a particular region of interest.

It is important to note that whenever possible, the primer sequences for the mapped loci were compiled and given in Table ESM S2. Availability of the primer sequences for a total of 580 SSR loci, approximately 75% of all loci integrated in the consensus map, at one place should accelerate the use of SSR markers in barley breeding activities. The primer sequences for 172 SSR loci (170 loci mapped in Varshney et al. 2006a and Marcel et al. 2007; two unpublished loci) have been made available in public domain for the first time. Primer sequences for the remaining 194 SSR loci can be obtained from Andreas Graner (for GBM loci) and Marion Röder (for GBMS loci), as per Material Transfer Agreement (MTA) basis. However, one marker (Bmac0029) is commercialized. The genotyping data made available for all the 775 SSR loci (Table ESM S3) will allow the community to extend the dataset with their own dataset in future.

The majority of the SSR marker loci integrated on the consensus map have high information content. For instance, about 54% of the SSR loci for which the information was available have a PIC value >0.50. The compound and the dinucleotide microsatellite loci had higher PIC values than the trinucleotide and other types of SSR loci. This is probably due to the fact that only 12% of the compound and 37% of the dinucleotide SSR loci were derived from ESTs or genes (Ramsay et al. 2000; Li et al. 2003), while a much larger proportion of the trinucleotide (98.3%), tetranucleotide (90%), pentanucleotide (100%) and hexanucleotide (80%) SSR loci were derived from ESTs or genes (Thiel et al. 2003; Varshney et al. 2006a). Since ESTs or genes represent the transcribed regions of the genome (transcriptome), which are considered more conserved portions of the genome, transcriptome-derived markers generally have a lower polymorphism content (Varshney et al. 2005a). Nevertheless such markers are supposed to be more transferable between related species (Varshney et al. 2005b). Thus, depending on the objective, genomic DNA-derived SSR markers with higher PIC value (for breeding purpose) or EST/gene-derived SSR markers with a lower PIC value (for using across the cereal species) may be selected from the present consensus map. The highest correlation coefficient ($r = 0.70$) obtained with the 953 barley accessions through the world further demonstrates the robustness of the PIC values compiled for microsatellite loci on the consensus map.

Accuracy of the consensus SSR map

Although consensus maps represent the densest possible genetic maps, accuracy and quality of the developed consensus map is very important for its users. In order to construct an as accurate and precise consensus map as possible, a number of improved map construction programmes were used in the present study as compared to earlier studies (Karakousis et al. 2003; Somers et al. 2004; Qi et al. 2004). For instance, the recently developed computer program RECORD (Van Os et al. 2005a) was used for ordering the markers from the six individual linkage data sets and the linkage groups were sorted by graphical genotyping with help of Microsoft® Office Excel 2003. The programme RECORD employs a marker-ordering algorithm based on minimization of the total number of recombination events in any given marker order (Van Os et al. 2005a). To be more accurate, the ordering of markers with RECORD programme was repeated three times for each individual linkage map. During the visual inspection of graphical genotypes, occurrence of singletons and other potential errors in the marker segregation data were identified. Because most singletons are scoring errors, these were replaced by missing values as suggested by Isidore et al. (2003) and Van Os et al. (2005b). The elimination of singletons solves most of the ordering ambiguities during the mapping process, as the risk of cleaning data points that were not erroneous has a very limited effect on the marker ordering. The order of markers as given by RECORD is better than the order of markers as given by traditional linkage mapping software programmes like JoinMap® 3.0 and the simultaneous use of both programmes improves the construction of genetic linkage maps (Vromans et al. 2007).

A bridge marker is more reliable since it has a position on several populations. In case a mistake occurs in the map of one population, the error may be partly corrected by the position on the map of the other population. Therefore, the accurate identification of those bridge markers is of high importance and much attention was placed on assigning identical names to the bridge markers among the data sets. In the sets of marker segregation data obtained for different mapping populations, many inconsistencies especially in naming a particular SSR locus were found. Therefore, we suggested a slight modification in designation of SSR loci (Table ESM S1). We propose to use those designations of SSR loci in future studies in order to achieve a uniform convention.

Subsequently, with the corrected segregation data and with correct bridge markers, the final consensus

map was calculated following the ''neighbours'' map approach described by Cone et al. (2002). In order to allow comparison of this map with other genetic maps, the barley BIN markers also have been integrated (Kleinhofs and Graner 2001; Marcel et al. 2007).

While utmost precautions were taken in preparing the consensus map, there could be some disagreement in the order of closely linked markers between the individual maps within some chromosome intervals. Such a disagreement may be due to the quality as well as the quantity and distribution along the chromosome of the bridge (common) markers used for preparing the consensus map, or to mapping populations, algorithm and stringency criteria of computer programmes. For example, the mapping populations for which the consensus map has been prepared have different numbers and different types of progeny lines. In smaller populations, the chance that informative recombinant progeny lines are present in the population to accurately position markers is lower than in larger populations. Also, the amount of recombination accumulated in RILs exceeds that in DH lines. Further, even for a given mapping population, different markers were mapped using different subsets of progeny lines in different laboratories. Therefore, the users of the consensus SSR map must consider that the marker order is conditioned by several factors like the progeny lines used and the position of crossovers along chromosomes within the progeny lines. The precise fine marker order may differ slightly in other populations and users may need to verify the order of closely linked markers in their mapping and breeding populations. However, we consider the order of the 496 bridge markers used to construct the framework of the consensus map to be highly reliable. The average distance between two consecutive bridge markers is equal to one marker per 2.1 cM, which shows the resolution of the map and the scale to which marker inversion may occur. This resolution is less than half the size of the 5 cM sub-BINs. The sub-BINs are therefore a reliable reference for users of the consensus SSR map to select markers of interest.

About 10% of consecutive pairs of bridge markers are more than 5 cM apart, mostly in the distal parts of the linkage groups. Distances between pairs of consecutive bridge markers are much smaller around the centromeres because of suppressed recombination in the centromeric regions (Künzel et al. 2000). Differences in the order of markers between the SSR consensus map and previously published maps were therefore mostly observed around the centromeres.

Implications of the SSR consensus map

The present SSR consensus map has brought the majority of presently known barley SSR markers together to provide a good estimation of relative order and distance between them. The consensus map integrates already published (Saghai Maroof et al. 1994; Becker and Heun 1995; Liu et al. 1996; Struss and Plieske 1998; Ramsay et al. 2000; Li et al. 2003; Thiel et al. 2003) and very recently developed (mainly GBM and scssr; Rostoks et al. 2005; Varshney et al. 2006a; Marcel et al. 2007) barley SSR markers.

The primary use of the consensus map is in molecular mapping of traits and MAS in plant breeding. The precise marker order over short chromosome intervals (<5 cM) may not be that important to select progenies by marker-assisted approaches. Marker order of stretches of more than 5 cM, the size of a subBIN, is more relevant for that purpose. Here the consensus map provides a large number of markers along the length of each chromosome. This marker density allows a wide selection of markers that can be used to genotype individuals for detection of recombinants, fixation of loci to homozygosity, restoration of a recurrent genetic background or composition of complex genotypes combining several particular alleles (Varshney et al. 2004; Langridge and Chalmers 2004). Further, the information available on PIC value for a large number of markers will help users to select the most polymorphic markers from a region of interest on the genetic map. A putative function associated with genic-SSR loci makes them a useful resource for assaying functional variation in germplasm collections and natural or breeding populations (Varshney et al. 2005a). The integrated genic-SSR loci will not only be useful in barley genetics and breeding, but also for such activities in other cereals, as this class of SSRs are highly transferable among (closely) related species (Varshney et al. 2005a, b).

The integrated SSR map could also help anchor the emerging physical map of barley (http://phymap.ucdavis.edu:8080/barley/). Those SSR markers with known genetic location could be used to screen BAC libraries allowing the positioning of BACs or BAC contig(s) onto the genetic map. Thus, the present consensus SSR map provides an opportunity to correlate genetic and physical maps (Varshney et al. 2006a).

In conclusion, we have brought together the vast majority of mapped barley microsatellite loci into a single consensus genetic map. The map provides molecular breeding strategies with a better choice of genetically located, high quality SSR markers, and, as a result, a higher probability of detecting polymorphic

markers in any target chromosomal interval. In addition, it offers an opportunity to align established genetic and phenotypic maps with the emerging barley physical map and to initiate haplotype diversity and association studies with user friendly and informative molecular markers at a higher than previously possible resolution.

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