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ORIGINAL PAPER

A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics

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Abstract An integrated barley transcript map (consensus map) comprising 1,032 expressed sequence tag (EST)-based markers (total 1,055 loci: 607 RFLP, 190 SSR, and 258 SNP), and 200 anchor markers from previously published data, has been generated by mapping in three doubled haploid (DH) populations. Between 107 and 179 EST-based markers were allocated to the seven individual barley linkage groups. The map covers 1118.3 cM with individual linkage groups ranging from 130 cM (chromosome 4H) to 199 cM (chromosome 3H), yielding an average marker interval distance of 0.9 cM. 475 EST-based markers showed a syntenic organisation to known colinear linkage groups of the rice genome, providing an extended insight into the status of barley/rice genome colinearity as well as ancient genome duplications predating the divergence of rice and barley. The presented barley transcript map is a valuable resource for targeted marker saturation and identification of candidate genes at agronomically important loci. It provides new anchor points for detailed studies in comparative grass genomics and will support future attempts towards the integration of genetic and physical mapping information.

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Introduction

Barley (*Hordeum vulgare* L.) is an important cereal crop species ranking fifth in crop production worldwide after maize, wheat, rice, and soybean (area harvested, FAO 2005, http://www.faostat.fao.org). The barley genome ($n = 7$) comprising more than 5,000 Mb equals approx. 12 times the size of the rice genome and consists of about 80% of repetitive DNA (Flavell et al. 1974). Due to its importance as a staple crop and because of its model character for other Triticeae genomes including wheat, *Triticum aestivum* L. and rye, *Secale cereale* L., comprehensive genetic and genomic resources have been established for barley over the past decades. These include a large number of well-characterized genetic stocks and mutant collections (http://www.untamo.net/cgi-bin/ace/searches/basic) (Caldwell et al. 2004; Lundqvist et al. 1996), various genetic linkage maps (Varshney et al. 2004), large insert bacterial artificial chromosome (BAC) libraries (Isidore et al. 2005; Yu et al. 2000), and a large collection of expressed sequence tag (EST) presently comprising more than 4×10^5 entries in dbEST (dbEST summary July 21st, 2006, http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html)

Expressed sequence tags obtained through cDNA sequencing provide the link to gene information in plant species, which are currently not suitable for whole genome sequencing. In this regard, the available EST collection, representing a large proportion of all barley genes (Zhang et al. 2004), can be exploited for barley in a similar way as has been shown for the construction and anchoring of high-density genetic transcript and physical linkage maps in other plants such as rice and maize (Chen et al. 2002; Davis et al. 1999; Harushima et al. 1998; Kurata et al. 1997; Wu et al. 2002; Zhao et al. 2002). In bread wheat over 6,000 ESTs were allocated by deletion bin mapping to more than 18,000 loci distributed across its allo-hexaploid genome (http://wheat.pw.usda.gov/NSF/progress_mapping.html, Sorrells et al. 2003). This dataset provides a starting point for the systematic analysis of gene/trait associations, candidate gene identification and comparative genome analysis in grass species, keeping in mind the limitation of low genetic and physical resolution provided by the employed 159 cytogenetic mapping bins (Qi et al. 2003).

Several approaches have been pursued for detecting sequence polymorphisms in barley relying on hybridisation- (Restriction Fragment Length Polymorphisms, RFLPs; e.g., Graner et al. 1991), or PCR-based molecular marker systems like RAPD (Randomly Amplified Polymorphic DNA; e.g., Weyen et al. 1996), simple sequence repeats (SSRs or microsatellites; e.g., Pillen et al. 2000), amplified fragment length polymorphisms(AFLPs; e.g., Waugh et al. 1997) and single nucleotide polymorphisms (SNPs; e.g., Kota et al. 2001b). Prior to the availability of PCR-based marker techniques RFLPs have been most widely used because of their simple development and their reproducibility. Several detailed RFLP maps have been constructed in barley comprising together more than 1,000 different markers (Kleinhofs and Graner 2001). RFLPs are usually inherited in a codominant way and bear the potential of parallel or subsequent multilocus mapping due to cross hybridisation to independent gene family members. Due to this feature, RFLP markers or their derivatives facilitate an efficient screening of BAC libraries and provided the basis for the discovery of syntenous relationships between plant genomes (Devos 2005; Hulbert et al. 1990). In case of cereals this facilitated access to the fully sequenced genome of rice. However, their detection is laborious and requires large amounts of DNA especially in species with large genome size. Hence, PCR-based SSR and SNP markers became the preferred marker type in the past decade. SSRs are stretches of DNA consisting of tandemly repeated short units of 1–6 bp in length (Tautz 1989). Their polymorphic character arises due to variation in the number of repeat units. They are multi-allelic and co-dominant in nature and thus very informative (Powell et al. 1996). EST databases can be mined for SSR containing ESTs (for review see Varshney et al. 2005) allowing to obtain markers at reduced cost for mapping of genes. On the other hand SNPs are the most abundant form of genetic variation and are less prone to mutations than SSRs (Giordano et al. 1999). At genome-wide scale SNPs can be expected at a frequency of 1/200–240 bp in barley (Kota et al. 2001a; Rostoks et al. 2005). Computational algorithms have been developed for querying EST databases for the presence of SNPs (Kota et al. 2003), facilitating the systematic development of SNP markers, for which innumerous assays have been developed (Rafalski 2002; Wang et al. 1998).

High density genetic maps of gene-based markers represent a powerful resource for enhanced genome analysis. They are essential for linking genetic and physical mapping information and allow for a detailed comparative genome analysis across both closely related and distantly related grass species. Moreover, gene-based markers, also termed "functional markers," can be regarded as candidate genes in trait mapping experiments. As a first step towards a comprehensive transcript map of barley, more than 330 EST-derived SNP markers were placed on a consensus map derived from three mapping populations (Rostoks et al. 2005).

The aim of the present study was to further extend the resource of mapped EST markers by developing a high-density transcript map of the barley genome. To maximize the potential of detecting polymorphisms, different marker technologies were employed, and genetic mapping was performed in a genetically diverse set of doubled haploid populations (Kota et al. 2001a). Here, we report a genetic map of 1,055 loci detected by 1,032 EST-based markers. It provides a resource for trait/gene association, candidate gene identification, marker saturation at independent target trait loci, and represents a high density grid of entry points to the genomes of rice and other grass species allowing a refined view onto grass genome colinearity and comparative genome organisation between rice and barley.

Materials and methods

Plant material

Three previously described doubled haploid (DH) mapping populations were used in this study. Of these, the population Igri × Franka (I/F) (Graner et al. 1991) was represented by 71 genotypes and the populations Steptoe × Morex (S/M) (Kleinhofs et al. 1993) and Oregon Wolfe Dom × Oregon Wolfe Rec (D/R) (Costa et al. 2001), were represented by 94 genotypes each. A comprehensive set of public marker data is available (http://wheat.pw.usda.gov/ggpages/map_summary.html) for all three populations providing anchor points for map integration and landmarks for map comparisons within barley and to other grass species.

DNA markers

Expressed sequence tag sequences were obtained from random sequencing of cDNA libraries developed from a diverse set of tissues and developmental stages (Michalek et al. 2002; Zhang et al. 2004) (CR-EST database: http://pgrc.ipk-gatersleben.de/cr-est, Kuenne et al. 2005) and served as source for EST-based marker development (RFLPs, SSRs, SNPs). A tentative unigene set was defined by iterative clustering analysis (project ID = g00−g02 including between 13,000 and 111,000 ESTs; http://pgrc.ipk-gatersleben.de/cr-est) using the software package StackPACK v2.1.1 (SANBI, South Africa). For genetic mapping, either a singleton or a representative EST/cDNA-clone per sequence contig was selected to avoid redundant mapping of genes. RFLP and SNP markers were randomly selected from the EST collection, except of a subset comprising about 60 SNP-markers, which were developed based on the identification of SNPs present in the public EST resource, which is derived from different genotypes (Kota et al. 2003). The development of SSR-markers was based on pre-selecting ESTs containing the corresponding repeat motifs (Thiel et al. 2003). The developed markers were designated as GBR, GBM and GBS (Gatersleben barley RFLP, microsatellite and SNP) followed by a unique 4-digit numerical identifier. All mapped GB-markers were finally cross-checked (BlastN, Altschul et al. 1990) for previously unobserved redundancies against a unigene dataset comprising over 370,000 publicly available EST sequences (TIGR barley gene index release 9.0, 2004, http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species = barley).

Marker analysis

DNA extraction and Southern analysis were carried out as described earlier (Graner et al. 1991) utilising a set of six restriction enzymes (*Bam*HI, *Hin*dIII, *Eco*RI, *Eco*RV, *Xba*I and *Dra*I). Autoradiography was performed by exposure of hybridised blots to imaging plates (Fuji Photo Film, Japan) and subsequent signal detection on a phosphoimager (FLA-3000, Fuji, Japan). cDNA inserts were amplified by utilising standard sequencing primers, purified (Qiaex; Qiagen, Hilden, Germany), radioactively labelled (according to manufacturers instructions: Megaprime labelling system; Amersham Biosystems, Freiburg, Germany) and utilised as RFLP probes according to Graner et al. (1991). The development and analysis of EST-based SNP and SSR markers followed previously published protocols (Kota et al. 2001b; Thiel et al. 2003). Detailed information (NCBI Genbank accession number of underlying EST, chromosome location, consensus map position, primer sequences in case of PCR-markers) is provided as Electronic Supplementary Material (ESM) Table 1. Primer info for markers GBM1001-1076 is available based on an MTA upon request to the corresponding author.

Linkage analysis and map construction

Genotyping information was recorded for each marker by entering segregation data into population files utilising the software MAPMANAGER QTX v0.30 (Manly et al. 2001). These files included previously published marker data (see below) thus allowing to fit new marker data into the seven barley linkage groups using the command "Distribute" (LOD 3.0 for I/F, and LOD 4.0 for S/M and D/R). JOINMAP V3.0 (Kyazma, The

Netherlands) was used for grouping of markers (LOD score = 4.0) and subsequent determination of marker order (minimum LOD score = 1.0, recombination threshold 0.4, ripple value = 1, jump threshold = 5). The Kosambi mapping function (Kosambi 1944) was applied for converting recombination units into genetic distances. Graphical genotypes of the resulting individual chromosome maps were visually inspected for consistency. In order to avoid a contradictory placement of loci (i.e., new double crossing-over introduced due to false marker order) that occurred occasionally, individual maps were recalculated by setting individual loci at 'fixed order'. Map integration (consensus map) was performed with JOINMAP V3.0 under the conditions/settings as described above applying the Kosambi mapping function (Kosambi 1944) for converting recombination units into map distances. The marker order of the consensus chromosome maps was compared to the original order in the individual population maps. In six cases blocks of markers spanning at maximum 3 cM displayed an inverted order compared to the map of the individual population thus violating the original graphical genotype. Here the consensus map was hand-curated to conform to the marker order supported by experimental evidence.

Mapping data of 200 previously published markers (ESM Table 2) was utilised as a framework for building the consensus map. These markers originated from various laboratories and included apart from morphological and isozyme loci a majority of DNA-based molecular markers originating from cDNA (BCD and CDO, Heun et al. 1991; cMWG, Graner et al. 1991; ABC, Kleinhofs et al. 1993; Bmac, Ramsay et al. 2000) or genomic clones (MWG, Graner et al. 1991; ABG, Kleinhofs et al. 1993; WG, Heun et al. 1991; and Ksu, Gill et al. 1991), or miscellaneous clones (ABA, Kleinhofs et al. 1993). The approximate position of the centromeres was determined according to Kuenzel et al. (2000). Final chromosome maps were drawn with the graphical package MAPCHART (Voorrips 2002).

All mapping data (individual maps, consensus map, comparative map) can be visualized through internet (http://pgrc.ipk-gatersleben.de/transcript_map) by utilizing the visualisation tool MoMaVis.

Identification of orthologous genes in the rice genome

Expressed sequence tags of the 1,032 experimentally mapped barley cDNAs were aligned with the publicly available rice genome sequence (TIGR, http://www.tigr.org/tdb/e2k1/osa1/, version 3, February 18, 2005) by BlastN ($E \leq 1E$-10). The genetic map positions of the barley genes were plotted against the physical coordinates of their best homologs (putative orthologs) from rice (Fig. 4) in order to determine the subset of syntenic genes between barley and rice.

Inferring barley duplications

Barley chromosomes 2H and 6H carry colinear regions to rice chromosomes Os04 and Os02, which were involved in an ancient whole genome duplication in rice (Yu et al. 2005) predating the species divergence of barley and rice. Syntenic regions are based on finding the putative ortholog for a mapped barley EST, which is defined as the rice gene with the BlastN alignment with the lowest E value ($E \leq 1E$-10). In order to find putative paralogs between barley chromosomes 2H and 6H and rice chromosomes Os02 and Os04, respectively, second-best rice homologs were additionally extracted. To examine whether second-best BlastN hits were significantly accumulated in these syntenic regions, a one-sided Fisher's exact test was used to test the null hypothesis of no association between the variables "located on rice chromosome x" and "located on barley chromosome y". The distribution of best and second-best rice homologs was studied with the same test on the null hypothesis assuming no correlation between ESTs from barley chromosome 2H and chromosome 6H ESTs and the distribution of their corresponding best and second-best rice homologs across the rice chromosomes Os02 and Os04. In both cases the null hypothesis was rejected if $P \leq 0.05$.

Results

Analysis of RFLP markers

An overview of the overall number and characteristics of all newly derived EST-based RFLP, SSR and -SNP markers is provided in Table 1 and 2. If compared across all three populations, SNP-markers represented the most polymorphic class of markers: 57% detected a polymorphism as compared to 51 and 38% for RFLPs and SSRs, respectively (Table 1).

For the development of RFLP-markers cDNA clones were selected based on their corresponding EST sequence and tentative unigene information. Overall, 1,539 out of 1,578 clones (97.5%) showed clear and useful hybridisation signals among the parents of the employed mapping populations (Table 1). 539 RFLP markers out of 782 polymorphic probes were finally mapped detecting 555 loci. Together with previously characterized cDNA markers (Graner et al. 1991) a total of 584 EST-based RFLP-markers detecting 607

Table 1 Aggregated information on polymorphism for different types of EST-based markers

Assay employed	RFLP[a]	SNP[b]	SSR[c]	
Screened ESTs	1,578	710	759	
Potential candidates	1,539	436	532	
Polymorphism detected	782 (51%)	264 (57%)	201 (38%)	
Polymorphic in I/F	246 (16%)	74 (17%)	58 (11%)	
Polymorphic in S/M	452 (29%)	158 (36%)	107 (20%)	
Polymorphic in D/R	518 (34%)	193 (45%)	155 (30%)	Total
Mapped in I/F	114	18	23	156
Mapped in S/M	162	92	47	302
Mapped in D/R	282	154	136	572
Total non-redundant	539[d]	258[d]	190[d]	990[d]

[a] Excluding cMWG markers

[b] R. Kota et al., unpublished data

[c] Varshney et al. (2006)

[d] 17, 6, and 16 RFLP-, SNP-, SSR-markers were mapped in two populations, respectively. One multi-locus RFLP-marker was mapped at one locus in two and at a second locus in all three populations

Table 2 Summary of EST-based marker loci for the individual maps and the integrated consensus map

Chromosome	Population/map																			
	I/F					S/M					D/R					Integrated map[a]				
	No. of loci				Map length	No. of loci				Map length	No. of loci				Map length	No. of loci				Map length
	RFLP	SNP	SSR	Total	(cM)	RFLP	SNP	SSR	Total	(cM)	RFLP	SNP	SSR	Total	(cM)	RFLP	SNP	SSR	Total	(cM)
1H	22	2	5	29	132.5	28	9	5	42	122.3	38	17	20	75	133.0	84	27	27	138	134.3
2H	24	1	1	26	133.7	35	18	8	61	146.7	56	21	25	102	174.5	107	39	33	179	165.1
3H	27	7	3	37	137.6	32	14	11	57	157.6	48	24	24	96	210.3	101	43	35	179	199.3
4H	9	0	1	10	137.8	9	10	7	26	129.7	36	20	20	76	130.5	53	28	26	107	129.8
5H	23	2	2	27	187.1	29	12	4	45	153.8	50	35	18	103	222.3	98	49	23	170	197.2
6H	22	3	7	32	129.2	17	12	5	34	108.9	25	17	17	59	143.0	60	32	25	117	149.7
7H	41	3	4	48	168.3	22	17	7	46	133.5	42	20	12	74	150.4	104	40	21	165	142.9
Total	169	18	23	210	1,026.2	173	92	47	312	952.5	296	154	136	586	1,164.0	607[b]	258	190	1,055[c]	1,118.3

[a] Number of redundant EST mapped either by RFLP (including cMWG), SSR and SNP

[b] 18 RFLP detected 23 secondary or tertiary loci (607 loci—23) = 584 EST-RFLP probes used

[c] Represented by 1,032 non-redundant markers

loci were included for map construction. Altogether, between 53 and 107 RFLP loci could be assigned to each of the seven barley chromosomes. Out of the 607 loci, 168, 172, and 295 were mapped in the populations I/F, S/M, and D/R, respectively (Table 2) with 13 markers detecting either two and 5 markers detecting three polymorphic loci, respectively (ESM Table 3).

Analysis of SSR and SNP markers

A set of 190 EST-SSR markers (including 185 previously published; Varshney et al. 2006) as well as 258 SNP markers (including 221 to be published elsewhere) were analysed as described before (Kota et al. 2001b; Thiel et al. 2003; Varshney et al. 2006) and the results were integrated together with the RFLP data into a combined barley transcript map (Fig. 1).

Construction of a transcript map

Individual genetic maps were calculated for each of the three DH populations (I/F, S/M and D/R) preceding the integrated map construction. 585 loci were mapped in D/R, 311 in S/M and 209 in I/F (Table 2). Furthermore, segregation data of 200 published markers (Costa et al. 2001; Graner et al. 1991; Kleinhofs et al. 1993; http://wheat.pw.usda.gov/ggpages/map_summary.html) was included (ESM Table 2) to provide a framework for the construction of the consensus map and to serve as points of reference to previously published maps. The observed order of anchor markers in the computed individual maps was in accordance with previously published maps.

Subsequently, a consensus transcript map was calculated (Fig. 1) comprising 1,032 EST-based marker

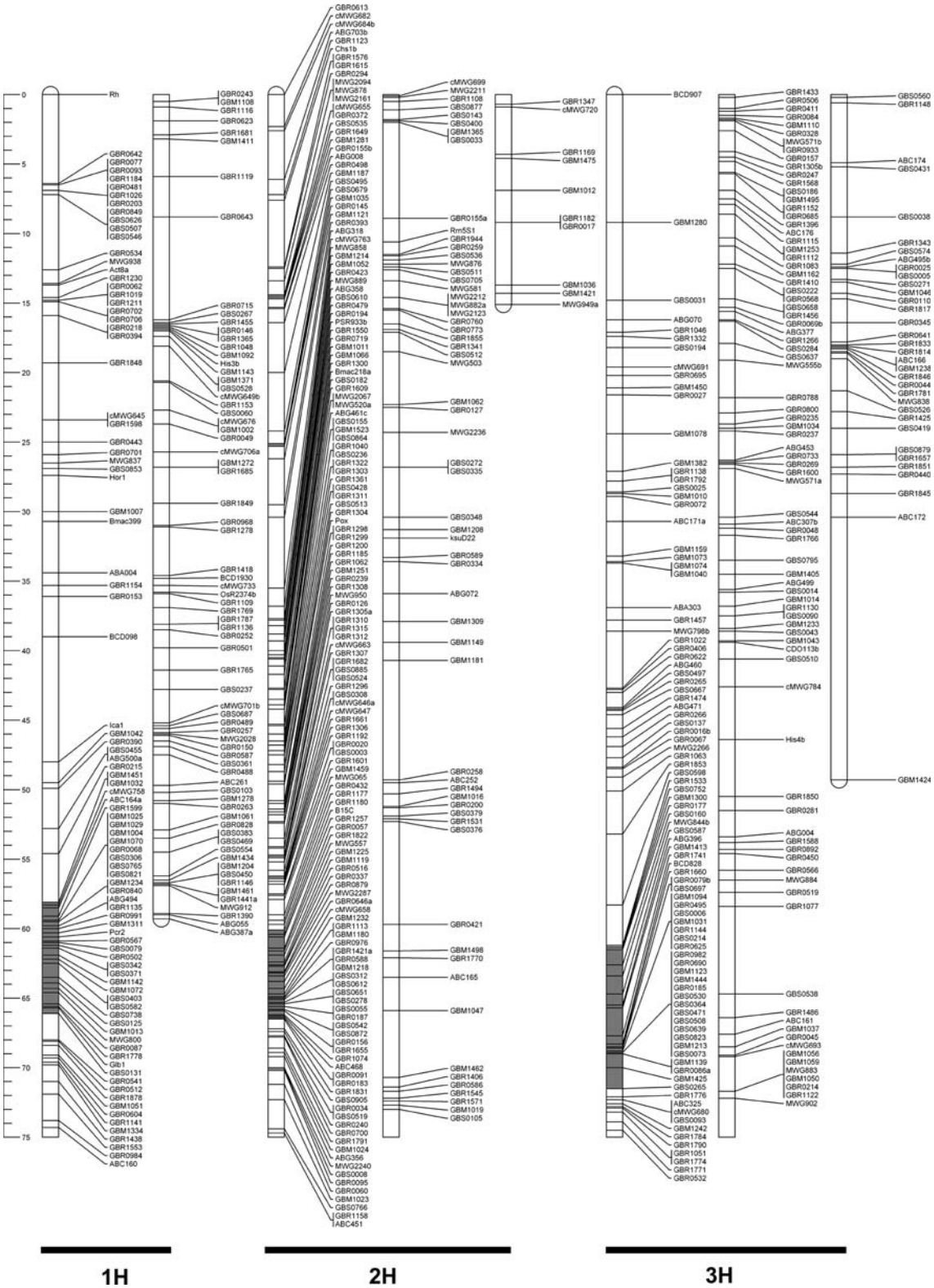


Fig. 1 Consensus transcript map of the barley genome. All seven barley chromosomes are visualized carrying in total 1,255 loci including new GBM, GBR, and GBS markers, along with previously published, EST-based, cMWG and 200 additional anchor and reference markers. Chromosomes are represented with the short arms pointing to the *top*. For better representation individual chromosome maps were cut at 75 cM and the remaining fragments were placed to the *right*. The relative position of the centromeres is indicated by *black bars* according to Kuenzel et al. (2000). Detailed views of the consensus maps and those derived from the individual populations can be obtained via visualization in MoMaVis (U. Scholz et al., unpublished, http://pgrc.ipk-gatersleben.de/transcript_map/momavis.php; see also Fig. 2)

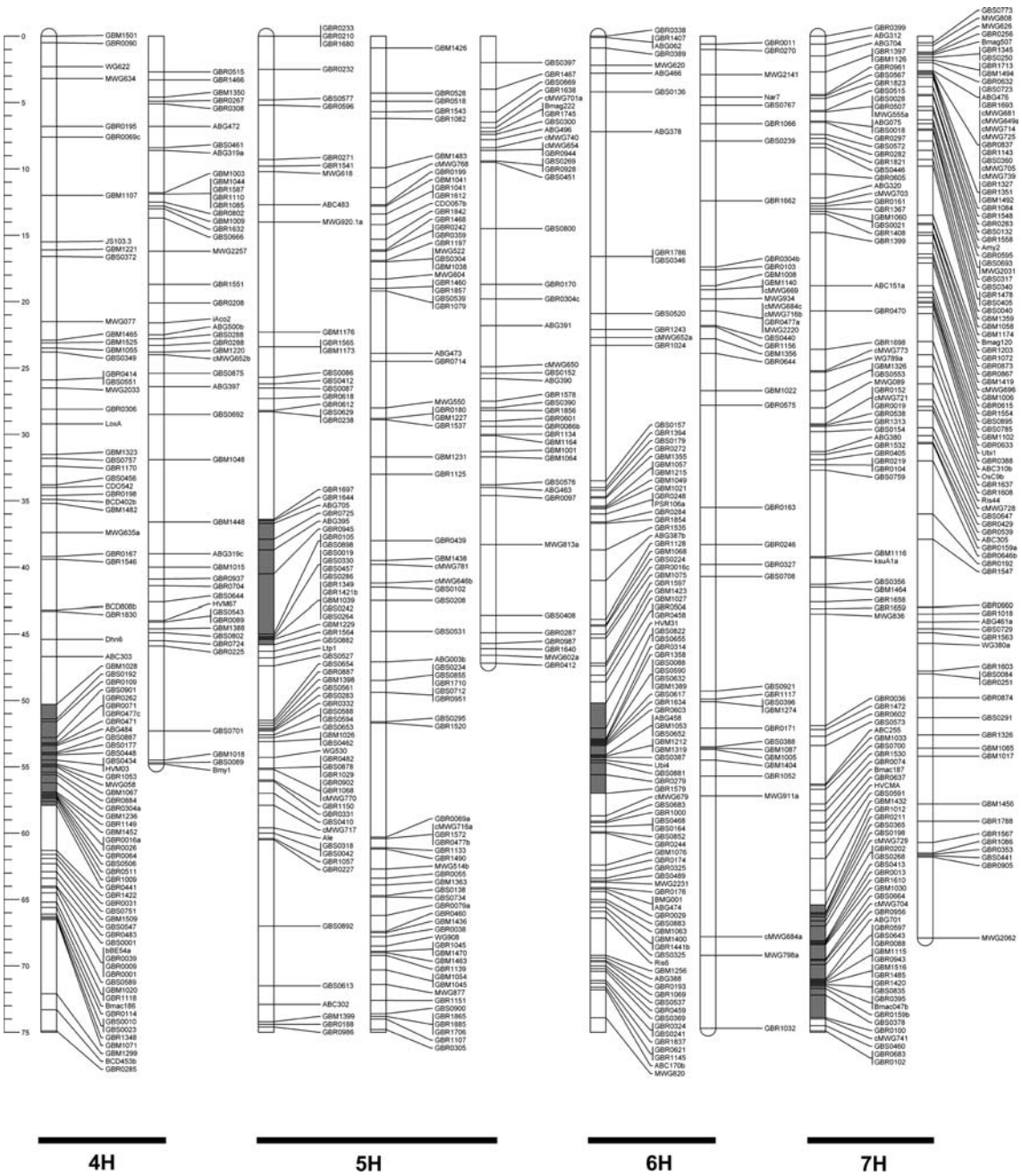


Fig. 1 continued

(RFLP, SSR, SNP) detecting 1,055 loci (total of 1,232 markers/1,255 loci including all anchor and reference markers = non-GB and non-cMWG markers) (Table 2, ESM Table 2). The length of the individual consensus linkage groups ranged from 134.3 cM (1H) to 199.3 cM (3H) with an average of 159.7 cM. The whole consensus transcript map comprised a genetic length of 1,118.3 cM with an average interval length of 0.9 cM between neighbouring loci. All individual maps and the consensus map can be visualised directly via the software MoMaVis (U. Scholz et al., unpublished data, http://pgrc.ipk-gatersleben.de/transcript_map/momavis.php) (Fig. 2).

Nineteen EST/tentative unigenes were mapped redundantly by at least two of the three marker systems - providing confirmation of genetic map position (ESM Table 4). For a further 28 tentative unigenes mapped by two out of the three marker systems non-coinciding map positions were detected. Here, in all but one case an RFLP probe generated a multiple fragment pattern detecting also non-polymorphic loci, which is pointing to a complex or multigene organisa-

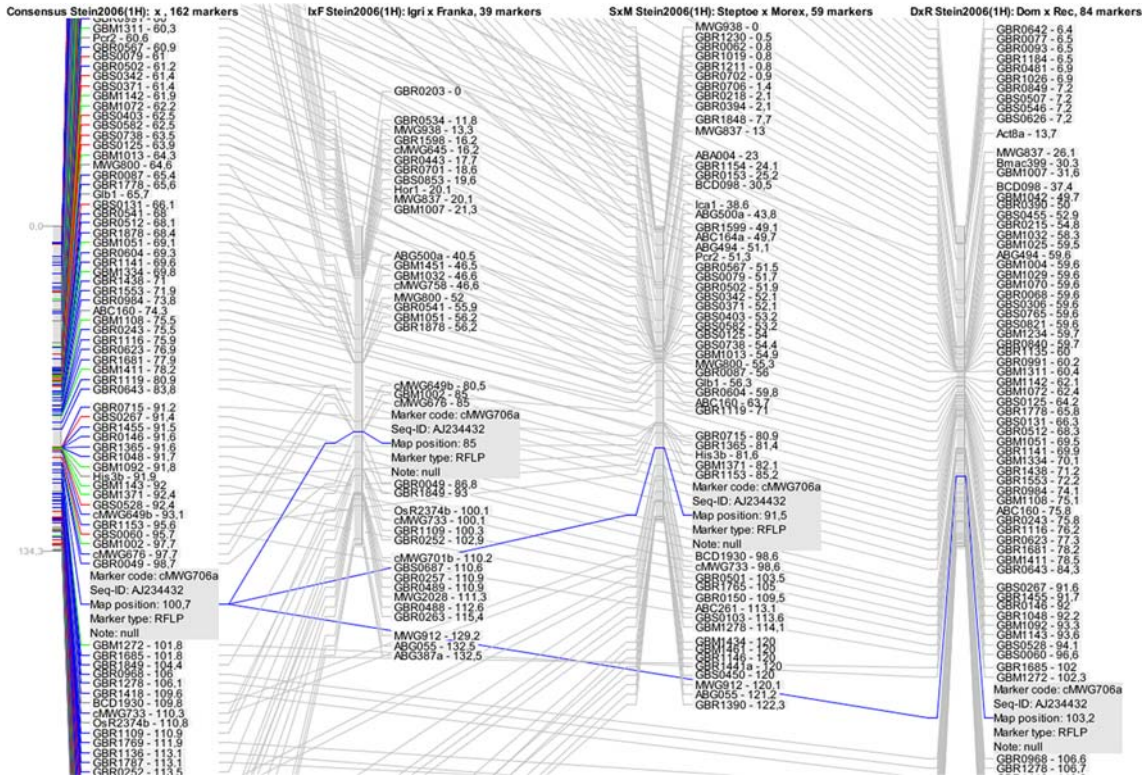


Fig. 2 Visualisation of mapping information for barley chromosome 1H by "MoMaVis". All individual and consensus maps can be visualised and compared to each other and to rice chromosomes by utilising MoMaVis: http://pgrc.ipk-gatersleben.de/transcript_map/momavis.php (U. Scholz et al., unpublished). An example is given for the comparison of barley 1H of the consensus map versus the I/F, S/M, and D/R maps. A single "mouse klick" on markers opens a dialog box providing more detailed marker information; a double "mouse klick" will provide EST information for the respective GB-markers by direct link to the EST-database "CR-EST" (http://pgrc.ipk-gatersleben.de/est/index.php, Kuenne et al. 2005), respectively NCBI Genbank (http://www.ncbi.nlm.nih.gov)

tion with different paralogs being detected by RFLP and PCR-based assays, respectively. In a single case, two closely linked loci were detected by an SSR and a SNP marker (GBM1482, GBS0456). These amplify the 5′ and 3′ region of the same EST-consensus sequence (TC131060, TIGR HvGI.1004), possibly indicating the occurrence of intragenic recombination.

Integrating the individual maps required the availability of markers commonly mapped between populations—so-called framework or anchor markers. Out of the 200 markers included from published data, 70 had been previously mapped in at least two of the three mapping populations. Moreover, 41 of the newly derived EST-based 'GB'-markers and 5 EST-based cMWG markers were mapped in two or all three populations, respectively, giving a total of 116 anchor markers (in total 119 loci: the three markers ABG500, GBR0086, MWG555 were mapped at two loci each; ESM Tables 5 and 6). Thus, approximately 10% of all 1,232 markers were anchored in at least two of the individual mapping populations.

Distorted segregation

Most markers segregated at the allele frequency of 1:1 as can be expected for doubled haploid populations. However, in each population this ratio deviated significantly ($P < 0.05$) from the expected allele frequency for a certain proportion of markers. In I/F this applied to 143 out of all 306 loci (47%) with 44 loci being skewed towards the parental genotype 'Franka' and 101 towards 'Igri', respectively. In contrast to this relatively high frequency in the I/F population, only 8.4% (37 loci) and 19.4% (125 loci) of the loci exhibited distorted segregation in the S/M and D/R, population. Regarding the former, 22 of 37 loci were skewed towards 'Steptoe' and 15 towards 'Morex', while in the latter 77 of 125 loci were skewed towards 'Rec' and 48 towards 'Dom'. Loci exhibiting distorted segregation occurred in clusters and were not randomly distributed (ESM Table 7). Furthermore, clustering of markers was observed around the centromeres, except for chromosomes 4H and 6H.

Rearranged marker orders

After consensus map calculation, the marker order was reconfirmed for consistency with the individual maps. Occasionally, a flipped marker order was observed affecting in most cases intervals of less than 1–2 cM distance. These mainly included markers that did not show recombination in their original individual mapping population but exhibited different numbers of missing data points. In a few cases blocks of markers extending over larger intervals (>2 cM) were affected (i.e., 1H, MWG938-GBR1848; 1H, GBS0267-GBS0528; 2H, GBR1576-ABG008; 7H, GBR0399-MWG555a). The marker order of these intervals was manually curated according to the graphical genotypes of the individual chromosome maps eliminating obvious contradictions in the marker order of the consensus map. A few individual markers swapped over larger distances of >2 cM. This applied exclusively to anchor markers, especially those located in or near regions of distorted segregation (i.e., 2H, MWG950 cons. map vs. I/F; 6H, ABG458 cons. map vs. D/R).

In silicio comparative EST mapping between barley and rice

Extensive colinearity of genes/markers has been documented for large chromosomal regions of barley, rice, and other grass genomes by comparative mapping of markers. In order to determine the total number of tentative barley/rice orthologs represented among the 1,032 genetically mapped barley ESTs, BlastN alignments were computed for the identification of the coordinates of the best matching homologous rice sequences (ESM Table 8). A subset of 763 (74%) of these barley ESTs matched to a homologous sequence ($E \leq 1E\text{-}10$) (Table 3). These markers exhibited a more or less even distribution along the barley chromosomes except for some significant clustering due to decay of recombination frequency around the genetic region of the centromeres (ESM Figure 1). Markers representing EST that failed to match the above selection criterion followed a similar distribution along the genetic maps of the barley chromosomes (ESM Figure 1). Eventually, 475 (46%) barley ESTs were assigned to syntenic linkage groups of rice according to the commonly accepted circular model of grass genome colinearity (for review see: Devos 2005; Moore et al. 1995). An intuitive and comprehensive overview of the colinear organization of both genomes was obtained by visualisation of barley markers along with their homologous rice genes in a dot plot matrix (Fig. 3). In all cases, the highest numbers of markers found their best homologous rice sequence in the known colinear linkage group (Table 3). We tested whether the remaining non-colinear barley markers would follow a random distribution across the rice genome. Taking into consideration that individual rice chromosomes differ in their gene content, less gene-rich chromosomes would have a smaller chance to exhibit matches to mapped barley markers. After normalization for gene content of the respective rice chromosome a significant (P value ≤ 0.05) deviation from a random distribution of Blast hits was observed only for chromosomes 1H and 5H. However, for none of these two barley chromosomes the order of the putative orthologs followed an

Table 3 Distribution of markers with best BlastN[a] hit to individual rice chromosomes

Barley chromosomes	Rice chromosomes[b]												Total	Distribution of BlastN-hits to the rice genome excluding syntenic barley genes[c]		
	Os01	Os02	Os03	Os04	Os05	Os06	Os07	Os08	Os09	Os10	Os11	Os12		χ^2	*df*	*P* value
1H	15	2	2	6	*36*	5	3	2	2	*15*	3	2	93	18.8*	9	0.0274
2H	8	6	9	*52*	1	1	*49*	1	3	3	4	6	143	11.7	9	0.2297
3H	*91*	4	6	3	5	3	4	2	3	0	7	3	131	8.6	10	0.5685
4H	5	5	*43*		4	1	4	1	3	2	3	3	75	5.9	10	0.8219
5H	13	7	*30*	1 1	3	0	6	6	*26*	6	0	*19*	117	21.6**	8	0.0056
6H	5	*50*	9	4	6	3	3	3	1	1	0	1	86	12.9	10	0.2290
7H	9	12	6	3	6	*43*	3	*21*	5	5	1	4	118	10.8	9	0.2929
Total	147	86	105	70	61	57	72	36	44	32	18	38	763			

[a] Altschul et al. (1990)

[b] Numbers in italics indicate marker BlastN hits to known colinear rice chromosomes

[c] After masking of markers with best match to known colinear rice chromosomes (numbers in italics, according to Moore et al. 1995), a chi^2 test was performed to test whether the remaining marker hits are randomly distributed across all rice chromosomes: Normalisation for predicted gene content was perfomed considering only non-TE genes/loci and excluding small gene models based on TIGR Rice Pseudomolecules v.3.0 http://www.tigr.org/tdb/e2k1/osa1/pseudomolecules/info.shtml)

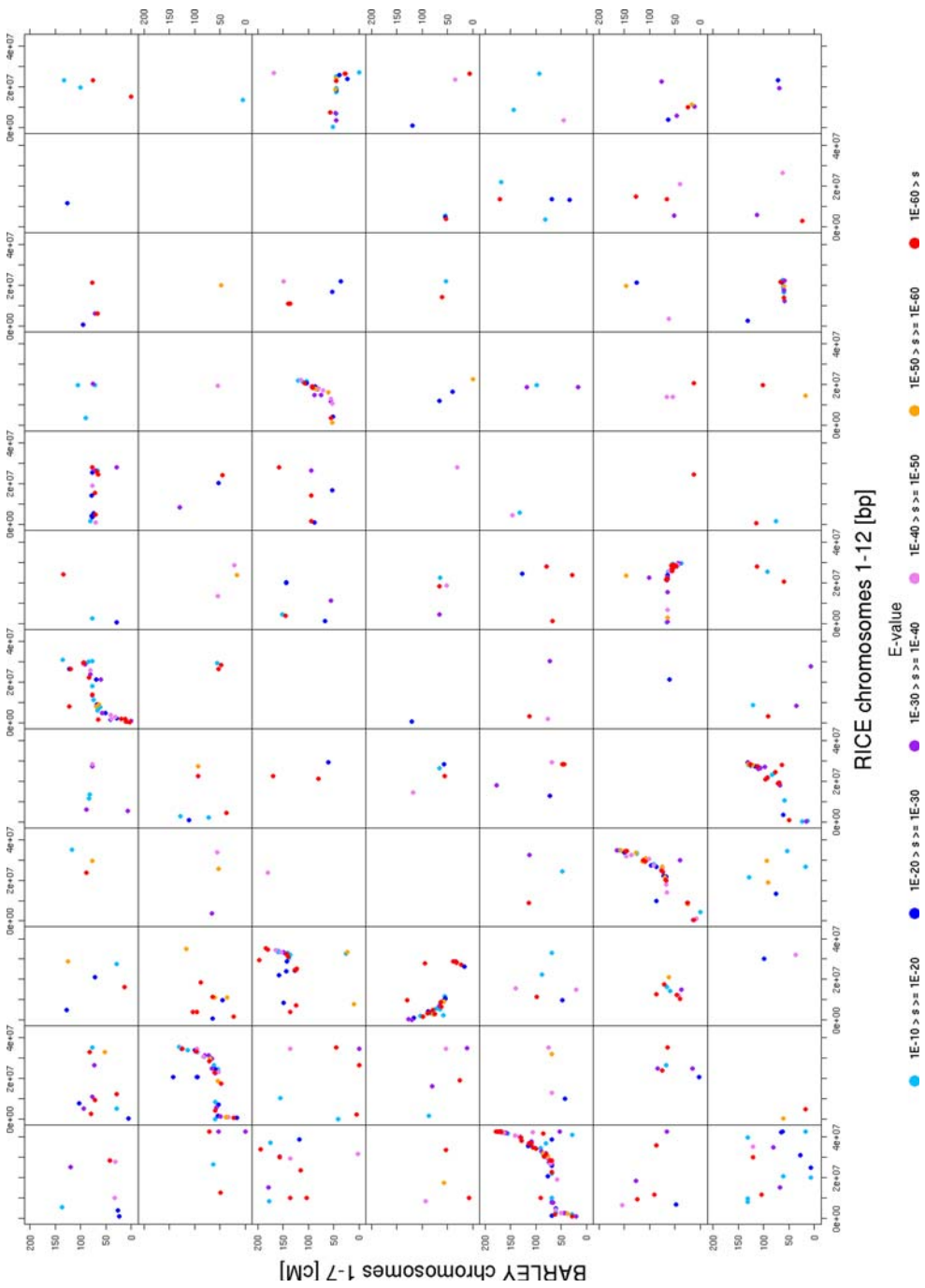
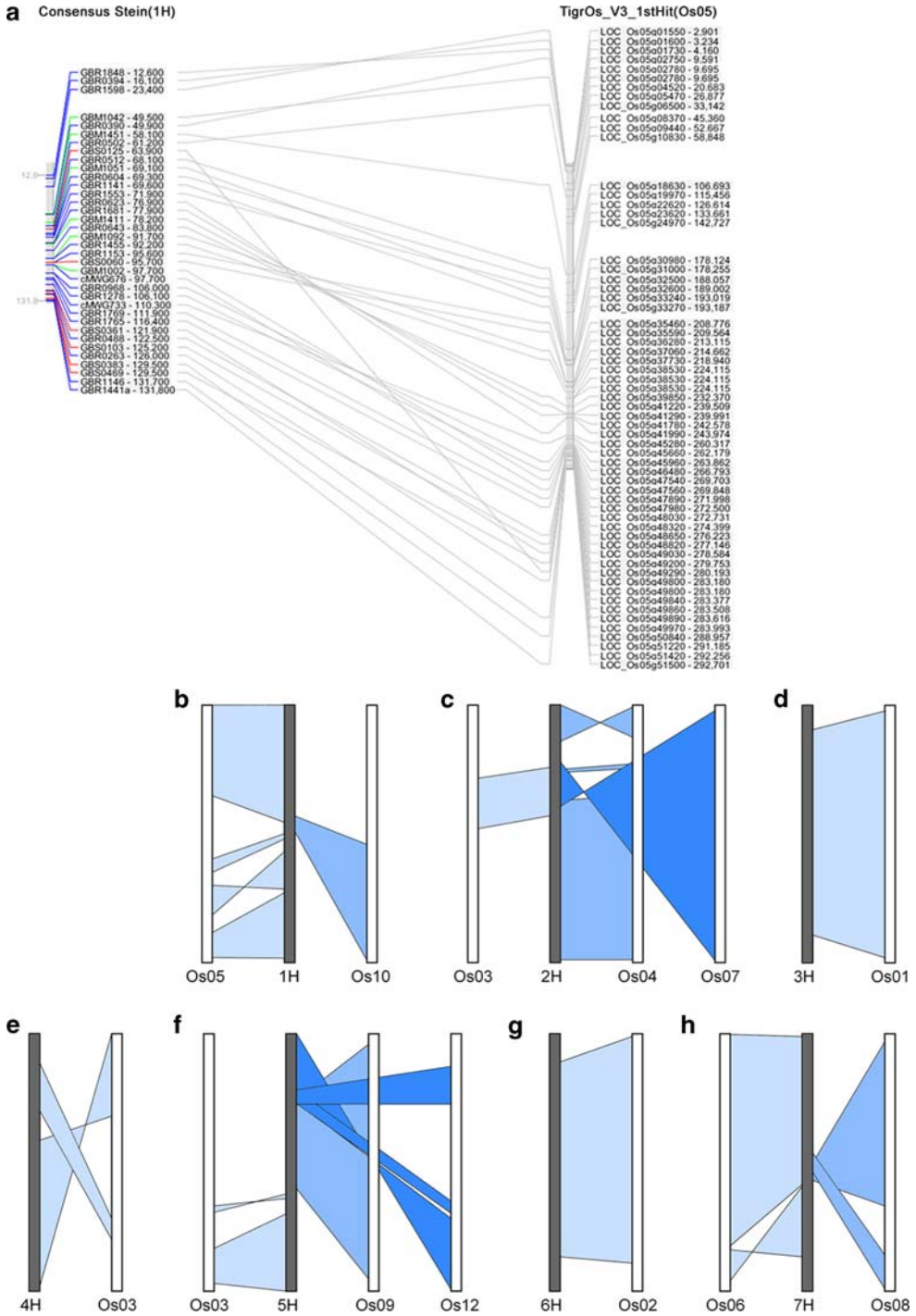


Fig. 3 Comprehensive view on barley/rice genome colinearity. Genetic positions of barley EST-based markers (cM) were plotted against the physical position (bp) of their best putative ortholog within the TIGR annotated rice genome (version 3, http://www.tigr.org/tdb/e2k1/osa1/) after BlastN analysis ($E \leq 1E$-10). Colinear relationships are characterized by continuous stretches of *aligned dots*. The *sigmoidal shape of these curves* is due to the nature of the two underlying maps (genetic vs. physical distances)

Fig. 4 Schematic visualisation of colinearity information between the barley consensus map and rice chromosomes. Details about barley/rice colinear linkage groups can be visualised by help of the software MoMaVis (U. Scholz et al., unpublished, http://pgrc.ipk-gatersleben.de/transcript_map/momavis.php). **a** An example is given for the comparison of barley chromosome 1H and rice chromosome Os05 providing insights into the extent of the colinear arrangement of markers and the plain belonging to overall syntenic linkage groups. The main regions of colinear marker arrangements to rice, which are represented by the current barley marker data set, are given as schematic illustrations for each barley chromosome consensus map after confirming colinearity of marker order in the individual maps **b–h** Barley genetic chromosome maps are shown as *dark grey bars* whereas rice physical chromosome maps are given as *open bars*. All barley/rice chromosomes are normalised to the same size and the extension of genetic/physical intervals of colinearity was deduced as percentage of total length based on cM/bp intervals of the original maps. Regions of colinearity are indicated as shadings between rice/barley chromosomes. A previously unobserved putative region of colinearity between barley 2H and rice Os03 is represented by six true colinear markers, which comprise a 31 cM interval in the barley consensus map (107 Mbp interval in rice). The seeming lack of colinearity to rice at telomeric ends of some barley chromosomes is basically an effect of low marker density combined with large genetic distances in these map regions rather than a true proof of lack of synteny ▶



obvious colinear pattern in the corresponding rice chromosomes (Fig. 3). By contrast, of the markers from chromosome 2H, which did not show a significant deviation from random distribution on rice chromosomes other than Os04 and Os07 (which are known to be syntenic), six markers covering a 31 cM interval in barley were colinear to a central part of rice chromosome Os03 (Figs. 3, 4c), indicating a hitherto unknown colinear region in the two genomes.

Detailed views on the level of colinearity between individual barley and rice chromosome pairs or triplets can be obtained via visualising maps in the application MoMaVis (U. Scholz et al., unpublished, http://pgrc.ipk-gatersleben.de/transcript_map/momavis.php, Fig. 4a).

A schematic overview summarising the relevant relationships (Fig. 4b–h) illustrates that larger syntenic regions in several cases include areas of considerable reorganization of gene blocks between otherwise colinear rice and barley chromosomes, i.e., 1HL/Os05L (Fig. 4b).

Inferring barley genome duplications from rice/barley genome colinearity

Approximately 66% of the rice genome can be assigned to duplicated segments (Yu et al. 2005) that arose to a large extent from a whole-genome duplication predating the divergence of rice and the Triticeae species. This suggests that ancestral patterns of genome duplication may be conserved also in the genomes of the Triticeae. In order to investigate the utility of the present transcript map for the identification of such ancient duplications in the barley genome, chromosomes 2H and 6H were analyzed in more detail. Both chromosomes are (at least partially) colinear to regions duplicated between rice chromosomes Os04 and Os02, respectively. Best homologs (putative orthologs) of ESTs located on barley chromosomes 2H and 6H were found on rice chromosomes Os04 and Os02 (Fig. 5), whereas the second-best homologs (putative paralogs) were mostly found on the corresponding duplication in rice, i.e., on rice Os02 in case of barley chromosome 2H and on Os04 in case of 6H. The second-best homologs were accumulated significantly in these syntenic "paralogous" regions according to Fisher's exact test (Table 4) yielding $P = 5.0\text{E-}03$ for chromosome 2H and $P = 9.7E\text{-}04$ for chromosome 6H. In addition, the separation of best and second-best hits for barley chromosome 2H ($P = 1, 1E\text{-}03$) and chromosome 6H ($P = 1, 15E\text{-}02$) ESTs into rice chromosomes Os02 and Os04 (Table 5) was significant. Thus the observed distribution of BlastN hits was not random but indicative of the presence of a duplicated region in the barley genome (Fig. 5).

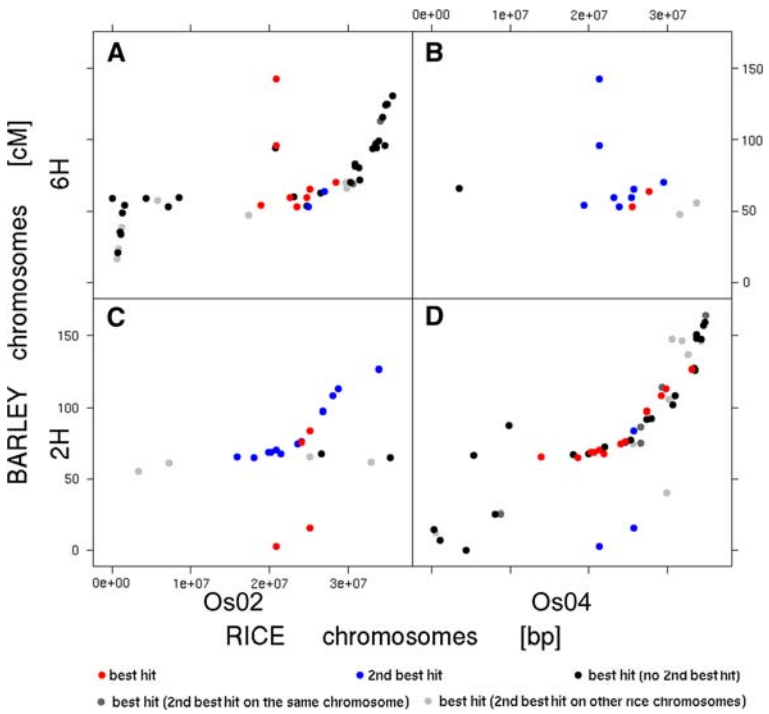


Fig. 5 Inferring barley genome duplications via rice/barley genome colinearity. The coordinates of the best and second-best rice homologs of barley EST markers located on chromosomes 2H and 6H determined by BlastN analysis were plotted into a *dot matrix*. Barley ESTs with best and second-best homologs on rice chromosomes Os02 and Os04 were visualised as *red dots* (best homolog = putative ortholog) and *blue dots* (second-best homolog = putative paralog). ESTs with one and only one homolog in the rice genome were visualised with *black dots*. Dots were coloured *dark grey* if second-best homologs were found in close proximity to the best hit on the same rice chromosome or shown in *light grey* if additional homologs were found on other rice chromosomes than Os02 and Os04. Corresponding best and second-best homologs of barley chromosomes 6H and 2H were arranged in colinearity to the two rice chromosomes Os02 and Os04, which are known to be derived from an ancestral duplication event predating the rice/barley divergence (Yu et al. 2005) and thus inferring a segmental duplication of barley chromosomes 2H and 6H

Table 4 2 × 2 contingency table for the distribution of second-best rice homologs in syntenic regions

	Second-best homolog on rice Os02 (Os04)	Second-best homolog not on rice Os02 (Os04)	Total
Barley EST on 2H (6H)	18 (9)	60 (31)	78 (40)
Barley EST not on 2H (6H)	25 (24)	262 (301)	287 (325)
Total	43 (33)	322 (332)	365 (365)

Table 5 2 × 2 contingency table for the distribution of best and second-best rice homologs of barley 2H (6H) markers

	Best homolog	Second-best homolog	Total
Located on rice Os02	4 (8)	14 (2)	18 (10)
Located on rice Os04	14 (2)	4 (8)	18 (10)
Total	18 (10)	18 (10)	36 (20)

Discussion

A new resource for Triticeae genomics

A transcript map of the barley genome comprising more than 1,000-gene loci at a genetic resolution of approximately 1 cM was developed providing an important resource for structural genomics research in barley and other Triticeae species. It provides new information for targeted marker saturation at agronomically important loci and new anchor points for an improved evaluation of genome colinearity among grass species.

Several comprehensive genetic maps with similar or even higher marker densities have been developed for barley. Initially these were mainly constructed using markers derived from genomic DNA and were based on RFLP (Kleinhofs and Graner 2001), AFLP (Hori et al. 2003) or array-based technology (DArT, Wenzl et al. 2004). More recently, the availability of comprehensive EST data facilitated the access to Triticeae gene sequence information, which in turn resulted in the development of gene-based marker maps. As to barley, first functional maps were developed using SSR- (185 markers, Varshney et al. 2006) or SNP-markers (333 markers, Rostoks et al. 2005). A large set comprising more than 1,700 genes was recently assigned to individual barley chromosomes by probing the Affymetrix barley1 gene chip with RNA from wheat-barley addition lines that carry individual barley chromosomes in a hexaploid wheat genomic background (Cho et al. 2006). The same source of cytogenetic stocks was employed for assigning 701 ESTs to barley chromosomes based on barley-specific amplification (Nasuda et al. 2005). In the closely related allohexaploid bread wheat (*T. aestivum*), which shares a colinear organisation to barley for most parts of its genome, 6,426 ESTs (18,785 loci, status: February 2, 2004, http://wheat.pw.usda.gov/NSF/progress_mapping.html) have been allocated to 159 so-called chromosome bins represented mainly by nulli-tetrasomic and chromosomal deletion stocks (Qi et al. 2003, 2004). The latter attempts were, however, limited in their physical resolution, which averaged out at 7.6 bins per chromosome (Qi et al. 2004). On a genome-wide level the genetic resolution of the present barley consensus transcript map is approximately 1 cM and thus provides a dense grid of genetically ordered genes in barley.

Integrating the consensus transcript map

The main goal of the study was to create a high density transcript map serving as a resource for gene-based marker saturation in mapping experiments. We followed the strategy of generating a consensus map in order to take advantage of a higher combined level of polymorphism, which can be achieved by utilizing populations derived from diverse, non-related germplasm—a concept followed in barley for more than a decade (Langridge et al. 1995). In the present study, this provided a 50% probability of mapping any gene by at least one out of the three employed marker technologies, compared to 15–36% chance if relying on individual populations only. Similarly to other consensus maps of barley (Kleinhofs and Graner 2001; Langridge et al. 1995; Rostoks et al. 2005; Wenzl et al. 2006), the overall marker order was well in agreement with the individual maps. Locally, consensus map resolution was slightly compromised by occasional flips of marker groups covering about 1–3 cM and swaps of individual markers over even longer distances. Such features are frequently observed for integrated maps and can be the result of significant differences of local recombination frequencies (map length) between populations (Doligez et al. 2006; Maliepaard et al. 1998; Wenzl et al. 2006). Another factor influencing the reliability of marker order and resolution is the overall number of commonly mapped markers (anchor markers) used for building the framework of the integrated map. For constructing the present consensus map, 116

anchor markers were employed representing about 10 % of all markers. Increasing this number of evenly distributed anchor markers across all three populations would help to further increase the confidence in the obtained marker order. This will be considered for future expansion of the present resource.

New insights into barley/rice colinearity

Because of the high level of sequence conservation within genes, EST-based genetic maps offer the opportunity of comparing conserved genome organisation between related species like barley and rice, which have diverged already about 50 million years ago (Gaut 2002). Initial observations of genome colinearity among grass species detected via mapping RFLP-probes in related species (Ahn and Tanksley 1993; Hulbert et al. 1990; Van Deynze et al. 1995) led to the development of the LEGO model of grass genome evolution (Moore et al. 1995) postulating that the genomes of *Poaceae* consist of conserved linkage blocks that evolved from a progenitor of the rice genome. Subsequent refinement of the model led to the conclusion that the gross chromosomal organization of the *Poaceae* genomes can be traced back over more than 70 million years of evolution and that any extant grass genome can be reconstituted from 30 linkage blocks of the rice genome (Devos and Gale 2000).

In the present study, over 1,000 genetically mapped gene loci provide a large dataset to compare the genomes of barley and rice. Known relationships of extensive colinearity or plain syntenic organisation could basically be confirmed. Individual chromosomal pairs such as barley 3H/rice Os01 and 6H/Os02 show a colinear organisation over almost their entire length with few exceptions, whereas other barley chromosomes resemble a mosaic of individual rice chromosomes (i.e. 5H/Os03, Os09, and Os12). In addition, linear rearrangements characterised by inverted blocks of marker order between both species were uncovered between otherwise colinear barley and rice chromosomes (i.e., 1H/Os05). Despite the high level of local colinearity, only 46% of the mapped barley genes found their homologous sequence at a syntenic position in the rice genome (referring to the accepted model of colinear linkage groups between barley and rice). This is less than the 50% synteny reported previously for RFLP probes while comparing Triticeae/rice colinearity (Gaut 2002). However, among genes featuring a sequence homology to rice at $E \leq 1E\text{-}10$, more than every second gene (62%) was present at a syntenic position reflecting the influence of the BlastN threshold (results not shown).

Patterns of ancient genome duplication

In some instances, the distribution of best homologous gene pairs along the barley and rice chromosomes implied the presence of previously unobserved regions of genome conservation. For barley chromosomes 1H and 5H a significant number of best BlastN hits to rice genes located on chromosome Os01 was observed. However, the corresponding marker pairs were distributed more or less randomly along the chromosomes without evidence for a colinear organisation. In case of the 1H/Os01 relationship, the observed clustering may be attributable to the evolutionary origin of these linkage groups. Rice Os01 and Os05 (representing the best conserved colinear group to barley 1H) have likely originated from an ancient duplication event (Guyot and Keller 2004; Wang et al. 2005; Yu et al. 2005), predating the divergence of the cereal lineages. The presence of this duplication was predicted to be retained in the Triticeae genomes, and traces of this ancient event involving rice Os01 and Os05 have recently been reported. After fine mapping the *Eps-A^{m}1* region on the short arm of *T. monococcum* chromosome 1A^m, at least 19 duplicated genes could be determined between chromosomes 1H–3H/Os01–Os05 in a 35 cM interval in wheat (Valarik et al. 2006). The increased number of BlastN matches between barley 5H and rice Os01, however, cannot be explained by the same principle. None of the accepted 5H-colinear linkage groups of rice (Os03, Os09, Os12) are known to be involved in segmental duplications to rice chromosome Os01 (Guyot and Keller 2004; Yu et al. 2005).

In another case, six markers delimiting a relatively small (35 cM) interval of barley chromosome 2H matched in linear arrangement to rice Os03. This chromosome is not known to represent a colinear linkage group to barley 2H. The region detected on rice Os03, however, is known to be involved in a duplication to part of chromosome Os07 (Wang et al. 2005; Yu et al. 2005), which in turn is representing one of the previously accepted orthologous linkage groups to this barley chromosome.

Additional support for the presence of ancient duplications in the barley genome was obtained by using an alternative approach; instead of examining only the best BlastN hit of a barley EST to rice, the second-best BlastN hit was also taken into consideration. Exemplarily, this strategy has been validated for ESTs mapped to barley chromosomes 2H and 6H. Both chromosomes represent linkage groups colinear to rice chromosomes Os04 and Os02 (Moore et al. 1995), respectively, that are known to be involved in an ancient genome duplication predating the species

divergence of rice an barley (Yu et al. 2005). Thus, the best BlastN matches between barley 2H and 6H markers to rice chromosome Os04 and Os02 genes, respectively, highlighted most likely orthologous gene pairs, whereas the weaker matches to the corresponding duplicated rice chromosome segments indicated the presence of duplicated (paralogous) rice genes. The reciprocal pattern of putative orthology/paralogy between barley chromosomes 2H and rice chromosomes Os04/Os02 on one hand and between 6H and rice chromosomes Os02/Os04 on the other hand, implies that segmental duplication in the rice genome is also present in the barley genome.

Transcript mapping opens a new perspective in cross-Triticeae genomics

Mapping of gene-based markers does not only allow comparisons to the sequenced rice genome but also allows anchoring of transcript maps obtained from related Triticeae species. A rough comparison of mapped genes present in this dataset and the wheat EST-deletion bin map (Qi et al. 2004), on the basis of sequence similarities of marker ESTs versus the wheat and barley unigene indices at TIGR (The Institute of Genome Research, http://www.tigr.org/tdb/tgi/plant.shtml), indicated approximately 200 putatively orthologous markers in the maps of barley and wheat (data not shown). Mapped orthologs would provide the basis for developing a cross-Triticeae consensus transcript map including at current state a redundant set of ~10,000 genes genetically and physically mapped in barley (Cho et al. 2006; Nasuda et al. 2005; Rostoks et al. 2005; Varshney et al. 2006), wheat (Qi et al. 2004), and rye (Hackauf and Wehling 2002). Despite the limited genetic and physical resolution of such a map it would represent the most comprehensive dataset for evaluating Triticeae—rice genome relationships and could be exploited for predicting colinear genes between rice and the Triticeae for targeted marker saturation and gene isolation.

In conclusion, the presented 1,000 loci transcript map of barley represents a valuable resource for targeted marker saturation and identification of candidate genes at agronomically important loci, as a grid of anchor points for detailed studies in comparative grass genomics, and as a foundation for linking genetic map information to a future physical map of the barley genome.

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