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High-density mapping and comparative analysis of agronomically important traits on wheat chromosome *3A*

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Abstract

Bread wheat chromosome *3A* has been shown to contain genes/QTLs controlling grain yield and other agronomic traits. The objectives of this study were to generate high-density physical and genetic-linkage maps of wheat homoeologous group *3* chromosomes and reveal the physical locations of genes/QTLs controlling yield and its component traits, as well as agronomic traits, to obtain a precise estimate of recombination for the corresponding regions and to enrich the QTL-containing regions with markers. Physical mapping was accomplished by 179 DNA markers mostly representing expressed genes using 41 single-break deletion lines. Polymorphism survey of cultivars Cheyenne (CNN) and Wichita (WI), and a substitution line of CNN carrying chromosome *3A* from WI [CNN(WI3A)], with 142 RFLP probes and 55 SSR markers revealed that the extent of polymorphism is different among various group *3* chromosomal regions as well as among the homoeologs. A genetic-linkage map for chromosome *3A* was developed by mapping 17 QTLs for seven agronomic traits relative to 26 RFLP and 15 SSR chromosome *3A*-specific markers on 95 single-chromosome recombinant inbred lines. Comparison of the physical maps with the *3A* genetic-linkage map localized the QTLs to gene-containing regions and accounted for only about 36% of the chromosome. Two chromosomal regions containing 9 of the 17 QTLs encompassed less than 10% of chromosome *3A* but accounted for almost all of the arm recombination. To identify rice chromosomal regions corresponding to the particular QTL-containing wheat regions, 650 physically mapped wheat group *3* sequences were compared with rice genomic sequences. At an *E* value of $E \leq 10^{-5}$, 82% of the wheat group *3* sequences identified rice homologs, of which 54% were on rice chromosome 1. The rice chromosome 1 region collinear with the two wheat regions that contained 9 QTLs was about 6.5 Mb.

Keywords: Wheat chromosome *3A*; Grain yield; QTLs; Deletion mapping; Genetic-linkage mapping; RFLP; SSR; Wheat–rice comparison

Grain yield (GYLD) is perhaps the most commonly studied and poorly understood trait related to agronomic performance of wheat (*Triticum aestivum* L. em. Thell.). GYLD is controlled by a number of genes and is manifested via a complex relationship among the yield component traits such as 1000-kernel weight (TKWT), kernels per spike (KPS), spikes per square meter (SPSM), and kernels per square meter (KPSM) [1–4]. Some other agronomic traits, including anthesis date, plant height (PHT), and grain volume weight (GVWT), also have an effect on GYLD.

Detailed analysis of intervarietal chromosome substitution lines between the two winter wheat cultivars "Wichita" (WI) and "Cheyenne" (CNN) identified chromosome *3A* accounting for the major part of the yield advantage of WI over CNN [5–8]. Consistently detected across more than 15 different location-year environments, chromosome *3A* from WI, when presented in the CNN background, increased GYLD by 15–20% [1–4]. This effect of chromosome *3A* seems independent of the background, at least between these two cultivars, as the

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reciprocal substitution line carrying CNN chromosome *3A* in the WI background showed a 15–20% decrease in GYLD. Detailed field and DNA marker analyses of 95 single-chromosome recombinant inbred lines (RICLs) involving chromosome *3A* of WI and CNN in the CNN background identified quantitative trait loci (QTLs) for GYLD, TKWT, KPS, SPSM, KPSM, PHT and GVWT [1–4]. The Physical locations of these QTLs and accurate estimates of kb/cM ratios for the harboring chromosomal regions are, however, not known.

It is critical to know the physical locations of genes and QTLs, particularly in wheat, because the distribution of both genes and recombination is highly uneven on chromosomes [9–15]. More than 85% of the wheat genes are present in 48 gene-rich regions (GRRs) with varying sizes and densities, encompassing less than 29% of the wheat genome [10]. The remaining 71% of the genome is very gene-poor, consisting of large blocks of repeated DNA interspersed with very few genes. Recombination occurs mainly in the GRRs but various GRRs differ as much as 140-fold in recombination rate [10]. Of the 252 phenotypically characterized useful wheat genes, 241 were physically localized in GRRs [10,16]. Twenty-one of these genes were located on wheat homoeologous group *3* chromosomes.

Genomic information from model systems has been used for isolation of genes from larger genomes via comparative analysis [17–26]. For wheat, the rice (*Oryza sativa*) genome is of particular interest as rice and wheat belong to the same family, Poaceae. Based on comparisons of common markers on genetic linkage maps, wheat and rice genomes were reported to be very collinear, but detailed analyses at the micro level show many exceptions [19,27–31]. Comparison of 4485 physically mapped wheat ESTs with the rice genomic sequence confirmed that the wheat–rice collinearity is frequently interrupted by segments from the other chromosomes [30,32,33]. Wheat homoeologous group *3* chromosomes are collinear with rice chromosome 1 and this is perhaps the best among all chromosomes of wheat–rice comparisons [30,32]. Conserved blocks are present on syntenic chromosomes; however, several rearrangements are also apparent, particularly around the centromeric region [32]. Along with many discontinuities, the exact size, order, and distribution of the rice segments orthologous to gene-containing regions on wheat group *3* chromosomes are, however, not known. The objectives of this study, therefore, are to reveal the precise genetic as well as physical location of genes controlling agronomic traits such as yield and yield component traits relative to DNA markers and to enrich the corresponding regions with additional markers by using the available genomic resources of both wheat and rice.

Results

Physical mapping

Gel-blot analysis results for 179 DNA probes on wheat group *3* nullisomic–tetrasomic (NT) and chromosome deletion lines are given in Table 1. Except for 6, all probes identified wheat group *3*-specific bands. The probes TAM32, TAM63, and KSUG59 detected a smear pattern, and none of the fragment bands detected by the probes BCD1802, CDO1396, and CDO389 were missing in group *3* NT lines. The 173 probes detected 924 fragment bands, of which 520 (56%) mapped to group *3* chromosomes. The remaining 402 (44%) fragment bands were either not resolved by the restriction enzyme used or specific for other wheat chromosomes. The 520 group *3*-specific bands corresponded to 415 unique loci, of which 141 mapped on *3A*, 142 on *3B*, and 132 on chromosome *3D* (Fig. 1). About 55% (94/172) of the probes detected loci on all three homoeologs, 27% on two, and 18% on only one of the homoeologs (Table 1; Fig. 1). On average, each probe detected 2.4 loci. Seven of the probes detected paralogous loci, as the fragment bands for probes FBB271, FBA133, KSUE2, KSUH7, UNL130, BE444148, and BE483203 showed more than one location on each of the group *3* chromosomes (Fig. 1).

Consensus physical map

Physical mapping data from the three homoeologs were combined to generate a consensus map (Fig. 2). There were 43 breakpoints on the consensus map corresponding to the 41 group *3* deletion lines. The consensus map contains 170 loci corresponding to 167 of the 173 physically mapped markers (Fig. 2, left). The remaining 6 markers were not placed on the consensus map because their location either was inconsistent among homoeologs (*XksuG62*) or had insufficient mapping data (*Xbcd1127, Xbe497524, Xbe637850, Xfbb366*, and *XksuF34*). Location of markers *Xbcd1380, Xpsr56, Xunl150, Xbe500083*, and *Xbe607045* was not considered discrepant among the homoeologs and thus they were placed between fraction length (FL) 0.3 bracketed by deletion lines 3DL-1 (0.23) and 3BL-9 (0.38) on the consensus map (Fig. 2, left). These markers were present between FL 0.28 and 0.42 on *3A*, between 0.07 and 0.22 on *3B*, and between 0.23 and 0.27 on *3D* (Figs. 1 and 2). This difference among homoeologs was within the range of a 5% error rate that is observed for the FL value estimations [45].

Distribution of the markers was highly uneven on the consensus map. Forty-three breakpoints on the homoeologous group *3* chromosomes resulted in 14 chromosomal regions on the consensus map. Of these regions, 7 contained about 91% of the mapped loci but physically spanned only about 36% of the chromosomes. The remaining 9% of the loci were present in marker-poor regions containing markers *Xbcd102, XksuI32, Xbcd127*, and *Xpsr909* on the short arm and markers *Xbcd134, Xcdo920, XksuE2.2, XksuH7.2, Xpsr394, Xtag609*, and *Xwg222* on the long arm (Fig. 2).

The seven marker-rich regions differed significantly in their size and marker density. Of the seven marker-rich regions three were present on the short arm and four on the long arm. The size of these regions ranged from 1.5 (3L0.8) to 8.5% (3S0.9) of the chromosome and their number of loci ranged from 13

Table 1
Gel-blot DNA analysis of the homoeologous group *3* chromosome markers using aneuploid stocks

Probe	Total No. of fragments	Score No. of fragments			Enzyme
		A	*B*	*D*	
ABC161	5	1	2	1	*Eco*RI
ABC171	3	1	1	1	*Eco*RI
ABC174	5	1	2	0	*Eco*RI
ABG389	4	2	1	1	*Eco*RI
ABG396	2	1	1	0	*Eco*RI
ABG460	8	1	2	1	*Eco*RI
BCD102	7	1	1	1	*Eco*RI
BCD1127	3	0	0	1	*Eco*RI
BCD1142	3	1	0	0	*Eco*RI
BCD1145	3	1	1	0	*Eco*RI
BCD127	3	1	1	1	*Eco*RI
BCD1278	2	1	1	0	*Eco*RI
BCD131	3	0	1	0	*Eco*RI
BCD134	5	1	1	1	*Eco*RI
BCD1380	9	1	1	1	*Eco*RI
BCD141	3	1	1	1	*Eco*RI
BCD1418	11	1	1	1	*Eco*RI
BCD1426	1	0	1	0	*Eco*RI
BCD15	5	0	1	1	*Eco*RI
BCD1532	4	2	1	1	*Eco*RI
BCD1555	5	1	1	1	*Eco*RI
BCD1661	3	0	1	1	*Eco*RI
BCD1823	5	1	1	1	*Eco*RI
BCD2044	9	1	0	1	*Eco*RI
BCD22	6	0	1	1	*Eco*RI
BCD263	2	1	1	0	*Eco*RI
BCD358	5	1	1	1	*Eco*RI
BCD361	4	1	1	0	*Eco*RI
BCD366	5	1	2	1	*Eco*RI
BCD372	5	1	1	1	*Eco*RI
BCD451	6	1	1	2	*Eco*RI
BCD452	4	1	2	1	*Eco*RI
BCD515	3	1	1	0	*Eco*RI
BCD706	5	1	2	1	*Eco*RI
BCD809	3	1	1	1	*Eco*RI
BCD927	3	1	1	1	*Eco*RI
CDO105	5	0	1	1	*Eco*RI
CDO113	4	1	1	1	*Eco*RI
CDO1174	3	0	2	0	*Eco*RI
CDO1345	3	1	1	1	*Eco*RI
CDO1435	6	2	2	2	*Eco*RI
CDO1523	6	1	0	1	*Eco*RI
CDO281	4	1	0	0	*Eco*RI
CDO328	3	1	1	1	*Eco*RI
CDO395	7	4	2	1	*Eco*RI
CDO407	3	0	1	1	*Eco*RI
CDO460	3	1	1	1	*Eco*RI
CDO480	7	0	0	1	*Eco*RI
CDO482	5	2	1	1	*Eco*RI
CDO534	6	0	1	0	*Eco*RI
CDO54	4	1	1	1	*Eco*RI
CDO549	6	1	1	1	*Eco*RI
CDO635	3	1	1	1	*Eco*RI
CDO638	5	1	1	0	*Eco*RI
CDO681	4	1	1	1	*Eco*RI
CDO920	6	1	1	1	*Eco*RI
FBA127	7	0	1	0	*Eco*RI
FBA133	10	1	5	2	*Eco*RI
FBA159	12	1	0	1	*Eco*RI
FBA167	8	1	3	1	*Eco*RI

Table 1 (*continued*)

Probe	Total No. of fragments	Score No. of fragments			Enzyme
		A	*B*	*D*	
FBA171	9	1	1	0	*Eco*RI
FBA190	5	1	0	1	*Eco*RI
FBA213	3	1	1	1	*Eco*RI
FBA214	5	1	1	1	*Eco*RI
FBA217	1	0	1	0	*Eco*RI
FBA27	6	2	0	1	*Eco*RI
FBA347	3	1	1	1	*Eco*RI
FBA91	2	1	0	1	*Eco*RI
FBB142	5	0	1	0	*Eco*RI
FBB250	2	1	0	1	*Eco*RI
FBB271	6	2	0	0	*Eco*RI
FBB277	5	0	1	1	*Eco*RI
FBB315	5	0	2	0	*Eco*RI
FBB316	1	0	1	0	*Eco*RI
FBB366	2	1	1	0	*Eco*RI
KSUD19	6	2	0	2	*Eco*RI
MWG2266	5	1	1	1	*Eco*RI
MWG2273	3	0	0	1	*Eco*RI
MWG582	2	1	1	0	*Eco*RI
MWG680	4	1	2	1	*Eco*RI
MWG802	8	1	1	1	*Eco*RI
MWG803	7	2	1	1	*Eco*RI
MWG973	5	1	1	2	*Eco*RI
PSR1077	6	1	1	1	*Eco*RI
PSR1196	5	1	1	1	*Eco*RI
PSR1203	3	1	1	1	*Eco*RI
PSR1205	6	1	0	0	*Eco*RI
PSR394	3	1	1	0	*Eco*RI
PSR56	6	1	1	1	*Eco*RI
PSR578	3	1	1	1	*Eco*RI
PSR598	6	2	1	2	*Eco*RI
PSR78	6	0	1	0	*Eco*RI
TAG637	5	1	2	0	*Eco*RI
TAG80	3	1	1	1	*Eco*RI
TAM12	3	1	1	1	*Eco*RI
TAM33	6	1	0	1	*Eco*RI
TAM5	3	1	1	1	*Eco*RI
TAM55	11	3	5	1	*Eco*RI
TAM56	16	1	1	1	*Eco*RI
TAM61	10	2	4	1	*Eco*RI
WG110	2	0	1	0	*Eco*RI
WG177	2	1	0	1	*Eco*RI
WG178	3	1	0	0	*Eco*RI
WG222	8	2	1	1	*Eco*RI
WG889	10	0	0	1	*Eco*RI
BE499910	2	1	1	0	*Eco*RI
BE442854	14	1	0	1	*Eco*RI
BE499177	3	1	1	1	*Eco*RI
BE492937	8	1	1	1	*Eco*RI
BE607045	3	1	1	1	*Eco*RI
BE591154	2	1	1	0	*Eco*RI
BE637850	5	1	0	0	*Eco*RI
BE483203	5	1	2	0	*Eco*RI
BE444148	2	1	1	0	*Eco*RI
BE405711	3	1	1	1	*Eco*RI
BE500083	6	2	1	1	*Eco*RI
BE499834	15	1	0	1	*Eco*RI
BE496983	8	0	4	1	*Eco*RI
BE517923	3	1	1	0	*Eco*RI
BE518446	3	1	0	1	*Eco*RI
BE497177	6	2	0	1	*Eco*RI
BF200008	9	1	1	1	*Eco*RI

Table 1 (*continued*)

Probe	Total No. of fragments	Score No. of fragments			Enzyme
		A	*B*	*D*	
BF482366	5	1	1	1	*Eco*RI
BF474092	7	0	1	0	*Eco*RI
BF474158	8	1	0	2	*Eco*RI
BF200697	4	2	1	1	*Eco*RI
BF200700	7	1	1	1	*Eco*RI
BF200549	2	0	1	0	*Eco*RI
BF473786	13	1	7	1	*Eco*RI
BG607212	4	1	1	1	*Eco*RI
UNL112	6	0	1	2	*Eco*RI
UNL130	8	0	2	1	*Eco*RI
UNL132	8	2	2	1	*Eco*RI
UNL150	3	1	1	1	*Eco*RI
UNL153	5	1	1	1	*Eco*RI
UNL167	4	1	1	1	*Eco*RI
UNL180	6	0	1	2	*Eco*RI
BG274134	5	2	1	0	*Eco*RI
BE425222	9	3	2	2	*Eco*RI
BF203138	3	1	1	1	*Eco*RI
BE497524	6	2	0	1	*Eco*RI
MWG2149	3	1	1	1	*Eco*RI
BCD907	6	1	1	1	*Hin*dIII
KSUA6	6	1	3	1	*Hin*dIII
KSUE14	9	0	0	1	*Hin*dIII
KSUF34	11	0	0	1	*Hin*dIII
KSUG13	7	1	2	1	*Hin*dIII
KSUG36	4	0	2	1	*Hin*dIII
KSUG48	5	0	1	1	*Hin*dIII
KSUG53	2	1	0	1	*Hin*dIII
KSUG62	15	3	4	5	*Hin*dIII
KSUH15	9	2	4	3	*Hin*dIII
KSUH2	4	1	1	1	*Hin*dIII
KSUH7	6	1	3	1	*Hin*dIII
KSUE2	6	1	3	1	*Hin*dIII
KSUI19	2	0	0	1	*Hin*dIII
KSUI32	9	1	0	2	*Hin*dIII
PSR116	3	0	1	0	*Hin*dIII
PSR123	3	1	1	1	*Hin*dIII
PSR156	3	1	1	1	*Hin*dIII
PSR170	6	1	1	1	*Hin*dIII
PSR689	3	1	1	1	*Hin*dIII
PSR703	5	1	1	2	*Hin*dIII
PSR74	3	1	1	1	HindIII
PSR902	9	2	3	1	*Hin*dIII
PSR909	4	1	1	2	*Hin*dIII
PSR910	4	1	1	1	*Hin*dIII
PSR926	3	1	1	1	*Hin*dIII
PSR931	3	1	1	1	*Hin*dIII
TAG609	3	1	0	0	*Hin*dIII
TAG683	2	0	2	0	*Hin*dIII
TAG718	3	1	1	1	*Hin*dIII
TAG724	6	2	2	2	*Hin*dIII
BCD1802	5	0	0	0	*Hin*dIII
CDO1396	2	0	0	0	*Eco*RI
CDO389	4	0	0	0	*Hin*dIII
KSUG59	Smear				*Hin*dIII
TAM32	Smear				*Hin*dIII
TAM63	Smear				*Hin*dIII

(3S0.5) to 34 (3L0.9). The regions with higher density were concentrated mainly on the distal ends of the chromosome. About 81% of the short arm loci mapped to less than 30% of the arm. Similarly, 52% of the long arm loci mapped to less than 22% of the arm.

Linkage analysis of markers and QTLs

One-hundred forty-two physically mapped group *3*-specific DNA probes were used for a polymorphism survey of WI, CNN, and CNN(WI3A) lines with 11 different restriction enzymes (see Material and methods and Supplemental Table 1). Of the 142 probes used, 68 detected polymorphism between WI and CNN with one or more restriction enzymes and 29 detected polymorphism only between CNN and CNN (WI3A). The remaining probes detected polymorphism for either *3B* or *3D* chromosomes. Overall, the *BglI* enzyme detected the highest level of polymorphism between CNN and WI cultivars (25 probes) followed by *Hind*III and *Eco*RI (17 and 15 probes, respectively). The level of polymorphism was the lowest with *Eco*RV (2 probes). Between CNN and CNN (WI3A), however, enzyme *Sca*I detected polymorphism for the highest number of probes among the 7 enzymes tested. The enzymes *Bam*HI and *Sac*I failed to detect polymorphism between CNN and CNN(WI3A) for any of the probes. Of the *55* SSR markers that were screened for polymorphism between CNN and CNN(WI3A), 18 detected polymorphism (Supplemental Table 2).

Fig. 3 shows the level of polymorphism for various major and minor physical regions of wheat homoeologous group *3* chromosomes. Both the total number and the percentage of probes detecting polymorphism between CNN and WI, and between CNN and CNN(WI3A), were dramatically different among chromosomal regions. In general, the probes present in the proximal regions of the chromosomes detected less polymorphism compared to the probes present in the distal regions (Fig. 3a). About 72% of the nonpolymorphic markers between the two parents physically mapped in the proximal half of the chromosome, and the remaining mapped in the distal half. Differences were observed both between chromosome arms and among various regions for the extent of polymorphism. On the short arm, probes from the S1 and S2 regions detected essentially no polymorphism. More than 40% of the probes from the regions 3S0.5 and 3S0.8 detected polymorphism between CNN and WI (Fig. 3a). For chromosome *3A*, however, these probes did not detect much polymorphism. The most distal region (3S0.9) on the short arm had the highest number of polymorphic probes. On the long arm, however, the region with the highest number of polymorphic probes between CNN and WI was 3L0.5 but none of these probes detected polymorphism for chromosome *3A*. Similarly, the region 3L0.9 had a higher number of markers detecting polymorphism between CNN and WI but the extent of polymorphism was lower for chromosome *3A* (Fig. 3b). Probes present in regions 3L0.3 and 3L0.8 detected the highest level of polymorphism for chromosome *3A* (Fig. 3 and Supplemental Table 3).

Of 142 RFLP probes, 29 were polymorphic for group *3* and only 14 were polymorphic for *3A*. Additionally, 15 of the 18 microsatellite markers detecting chromosome *3A*-specific

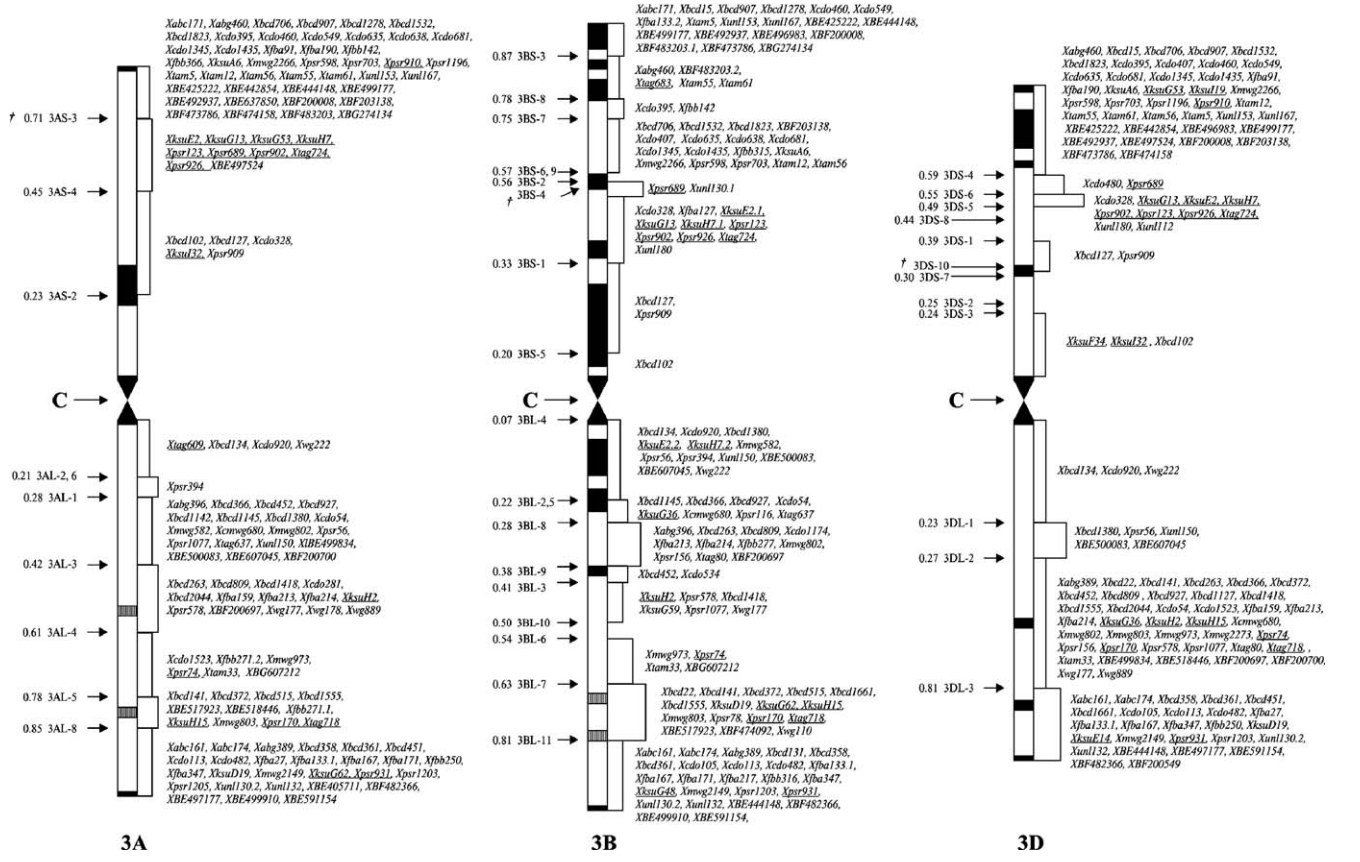

Fig. 1. Physical maps of wheat homoeologous group *3* chromosomes. The chromosome length, arm ratio, C-banding pattern (shown as solid black boxes for major bands and hatched boxes for the minor bands), and deletion breakpoints are all drawn to scale following previously published measurements [45]. The centromeres are marked by "C". The deletion line breakpoints and fraction lengths (FL) of the retained arms are marked by arrows on the left of the chromosomes. The FL value of the deletion lines marked with "†" is unconfirmed. Locations of the DNA marker loci are given on the right. Mapping information for the underlined marker loci on some of the deletions was also given in a previous map [42].

polymorphism were mapped on the RICL population. The previous map using the same population contained 12 RFLP markers [1,4]. Data for the 41 markers and eight phenotypic traits were analyzed as described under Material and methods. Total genetic length of chromosome *3A* was 213.8 cM with an average distance among markers being 5.2 cM and with a range of 0.5 to 22.3 cM. Number of crossovers per meiotic event can be accurately estimated using these mapping data for chromosome *3A*, because each RICL line represents a gamete resulting from recombination in F_1. Total length of 213.8 cM resulted from an average 4.8 crossovers per chromosome 3A per meiotic cycle. These crossovers are visible (as a switch from the CNN-type band to the WI band) from the data if arranged in the same order of markers as appears on the chromosome (data not shown).

QTL analyses using the data of 41 chromosome *3A*-specific DNA markers along with phenotypic data on seven agronomic traits identified 17 QTLs located in seven physical regions on chromosome *3A*. A composite interval mapping QTL scans for GYLD, KPSM, SPSM, PHT, GVWT, and KPS along chromosome *3A* is also given in Fig. 4. Two of the seven regions bracketed by markers *Xbcd907* and *Xbe425222* (Region 1) and *Xbarc86* and *Xbcd366* (Region 2) contained 9 of these QTLs. The QTLs for GYLD, KPSM, SPSM, PHT, GVWT, and KPS detected in Regions 1 and 2 explained 14–21% of the total variation for the corresponding traits and were highly significant across environments (Table 2). The remaining QTLs mapped in the regions flanked by markers *Xabc171* and *Xgwm218* (GYLD and TKWT), *Xbcd927* and *Xpsr56* (GVWT), *Xcdo1523* and *Xcfa2193* (SPSM and GVWT), *Xgwm155* and *Xcfa2183* (GVWT), and *Xbarc102* and *Xmwg802* (KPS) (Figs. 2 and 4). The QTLs located in regions other than Regions 1 and 2 represent minor effect QTLs for the corresponding traits (Table 2). The WI allele provided a higher value for each of the QTLs located in Regions 1 and 2, except for PHT, for which the WI allele provided a lower value. The WI alleles provided higher values for other QTLs also except for a TKWT and a KPS QTL, for which the WI alleles provided lower values for a single environment.

Physical mapping of recombination and the QTLs

With the objectives of physically mapping QTLs and accurately estimating recombination for each of the group *3* regions, the consensus physical map was constructed and compared with the *3A* genetic linkage map via 24 common markers (Fig. 2). The marker order was the same between the

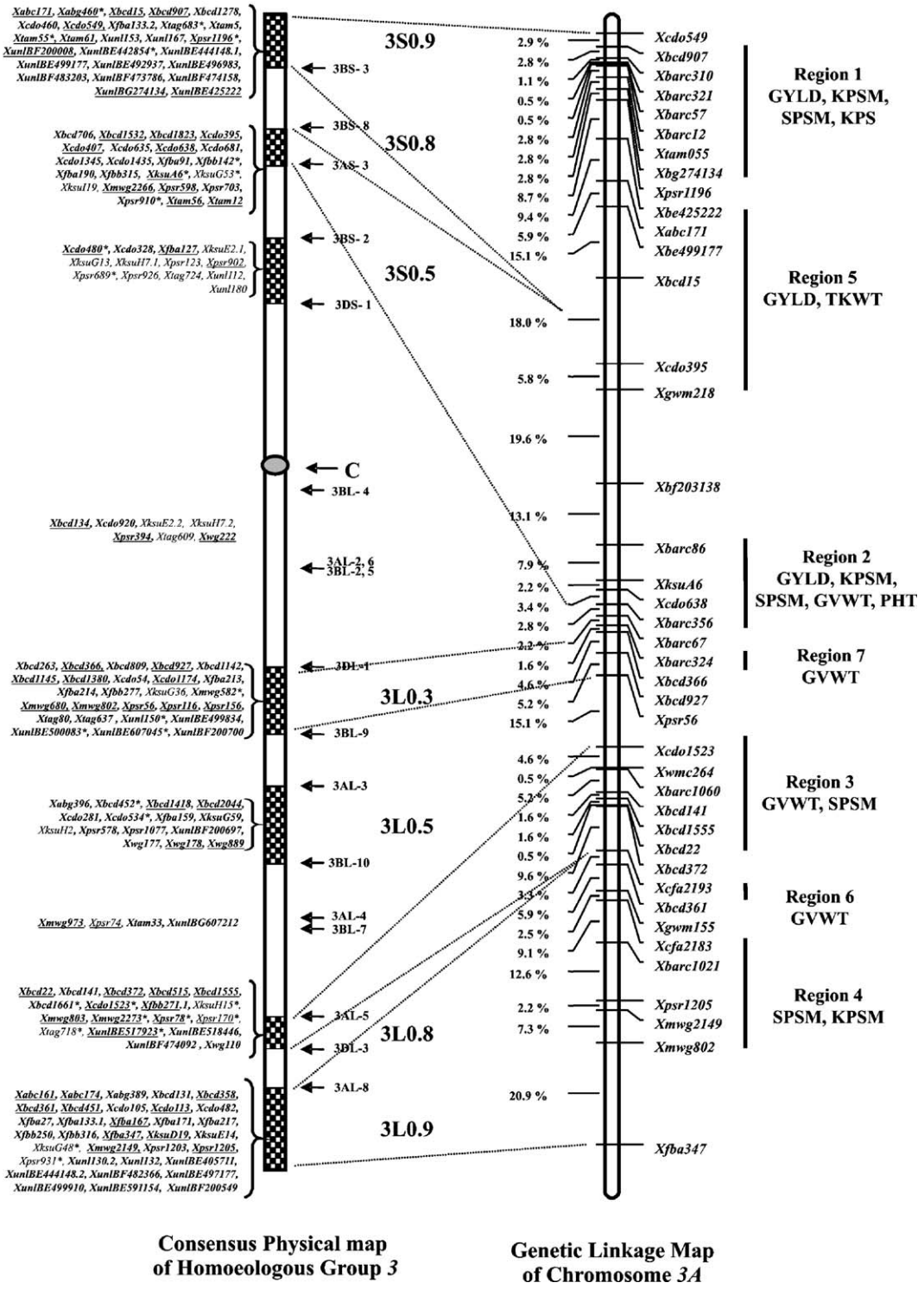

Fig. 2. Comparison of a consensus physical map with the genetic linkage map of wheat chromosome *3A*. The regions with the checkered boxes on the physical map are drawn to scale and indicate GRRs. Dotted lines connect the common markers between the two maps. The deletion lines bracketing the GRRs and the names of the GRRs are given on the right of the physical map. The recombination fraction distances among markers on the genetic linkage map are given on the left of the map. The QTL-containing regions along with the QTLs are given on the right of the genetic linkage map.

two maps except for *Xmwg802*. Physically, the marker *Xmwg802* mapped in the proximal region between FL 0.28 and 0.38 of the long arm but mapped in a very distal region on the genetic linkage map (Figs. 1 and 2).

Comparison of the consensus physical and genetic linkage map revealed the distribution of recombination on the *3A* chromosome. About 80% of the *3A* recombination occurred in the five GRRs. The remaining 20% of the recombination

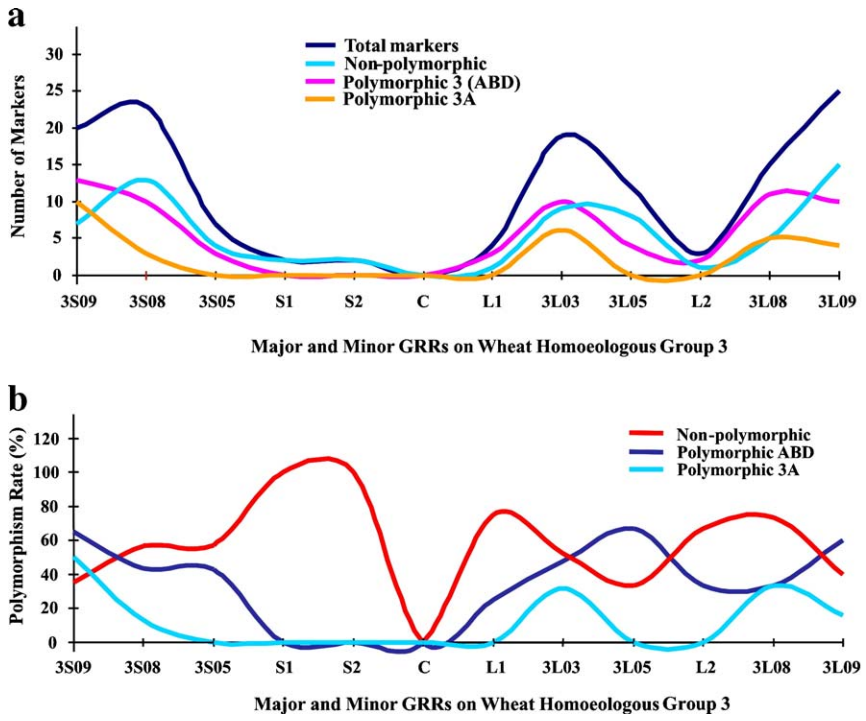

Fig. 3. Distribution of polymorphism for total, polymorphic, polymorphic for *3A*, and nonpolymorphic markers in the GRRs of wheat homeologous group *3*. Lines were drawn based on (a) the number of markers and (b) the ratio (%) within the particular GRRs.

occurred in two regions (3S0.5 and 3L0.5) and in gene-poor regions (Fig. 2). Furthermore, about 66% of the recombination occurred in the three distal regions 3S0.9, 3S0.8, and 3L0.9 that spanned about 13% of the chromosome (Fig. 2). Recombination rate varied dramatically even among other GRRs. Maximum recombination was observed in the 3L0.9 region that was about three times higher than in the 3L0.8 region (Fig. 2).

Comparison of the chromosome *3A* linkage map with the consensus physical map revealed the physical location of the QTLs. The QTLs for GYLD, KPSM, SPSM, and KPS were mapped to the 3S0.9 region of the short arm as both markers *Xbcd907* and *Xbe425222*, which flank the QTLs, physically map in this region (Fig. 2). Similarly, the GYLD and KPSM QTLs flanked by *Xbarc86* and *Xbcd366* markers were mapped to the 3S0.8 region. Among the long-arm QTLs, GVWT was

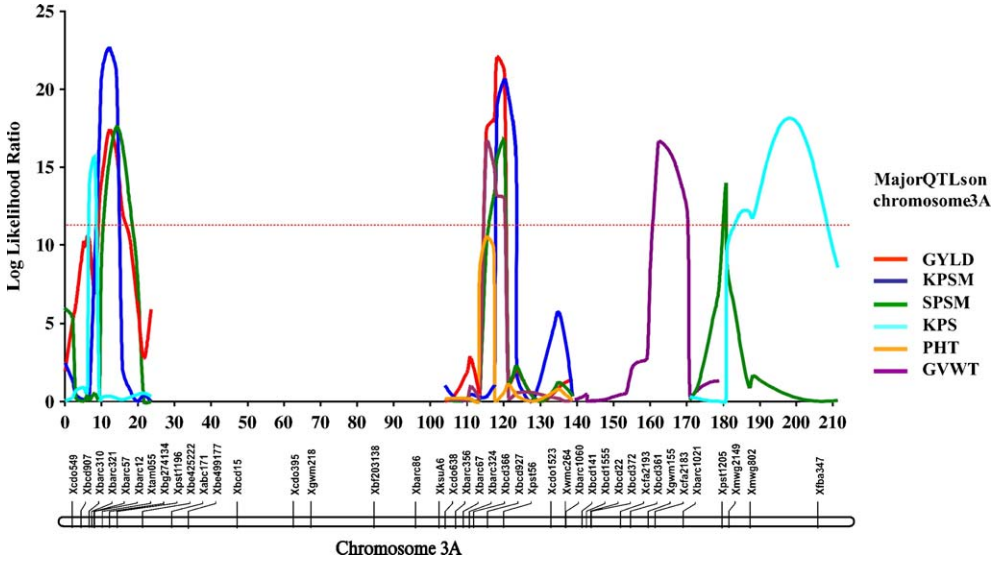

Fig. 4. Locations of major grain yield- and yield-related trait QTLs on wheat chromosome *3A*. The QTL locations on the genetic linkage map were according to the analysis described under Material and methods. Each agronomic trait is represented by a different color and the height of the peak is according to the log likelihood ratio. The red dotted line marks the average significance threshold (LOD = 10.88).

Table 2
The markers flanking the QTL regions identified by composite interval mapping for seven agronomic traits in seven environments and combined across environments

Trait	QTL	Lincoln 99	Lincoln 00	Lincoln 01	Sidney 00	Sidney 01	Mead 00	Mead 01	Combined	R^2 %[a]
GYLD	*QGyld.unl-3A.2*	—	BARC86–BCD366	BARC86–BCD366	—	—	—	**BARC86–BCD366**[b]	BARC86–BCD366	21
	QGyld.unl-3A.1	BCD907–BE425222	BCD907–BE455222	—	**BCD907–BE455222**[b]	—	—	—	BCD907–BE455222	19
	QGyld.unl-3A.5	—	—	—	ABC171–GWM218	**ABC171–GWM218**[b]	—	—	—	17
KPSM	*QKspm.unl-3A.1*	BCD907–BE455222	BCD907–BE455222	BCD907–BE455222	**BCD907–BE455222**[b]	—	—	BCD907–BE455222	BCD907–BE455222[b]	21
	QKspm.unl-3A.2	—	BARC86–BCD366	—	—	—	—	**BARC86–BCD366**[b]	BARC86–BCD366[b]	21
TKWT	*QTwkt.unl-3A.5*	ABC171–GWM218	—	**ABC171–GWM218**[b]	—	ABC171–GWM218	—	—	—	10
SPSM	*QSpsm.unl-3A.1*	BCD907–BE455222[b]	BCD907–BE455222	—	**BCD907–BE455222**[b]	—	—	—	—	17
	QSpsm.unl-3A.2	—	—	—	—	—	—	**BARC86–BCD366**[b]	—	16
	QSpsm.unl-3A.3	—	CDO1523–CFA2193	CDO1523–CFA2193	—	**CDO1523–CFA2193**[b]	—	—	CDO1523–CFA2193	9
	QSpsm.unl-3A.4	—	BARC1021–MWG802	BARC1021–MWG802[b]	**BARC1021–MWG802**[b]	—	—	—	BARC1021–MWG802	11
KPS	*QKps.unl-3A.1*	—	—	—	—	**BCD907–BE455222**[b]	—	BCD907–BE455222	BCD907–BE455222[b]	14
	QKps.unl-3A.4	—	BARC1021–MWG802	BARC1021–MWG802[b]	—	—	—	—	BARC1021–MWG802	30
GVWT	*QGvwt.unl-3A.2*	—	—	—	—	BARC86–BCD366[b]	—	—	**BARC86–BCD366**[b]	14
	QGvwt.unl-3A.3	**CDO1523–CFA2193**[b]	—	CDO1523–CFA2193	CDO1523–CFA2193[b]	—	—	—	—	16
	QGvwt.unl-3A.7	BCD927–PSR56	—	BCD927–PSR56	—	—	**BCD927–PSR56**[b]	—	—	11
	QGvwt.unl-3A.6	—	GWM155–CFA2183	**GWM155–CFA2183**[b]	—	—	—	—	—	14
PHT	*QPht.unl-3A.2*	—	BARC86–BCD366	BARC86–BCD366	BARC86–BCD366	BARC86–BCD366[b]	BARC86–BCD366[b]	—	BARC86–BCD366[b]	17

GYLD, grain yield; KPSM, kernels per square meter; TKWT, 1000-kernel weight; SPSM, spikes per square meter; KPS, kernels per spike; GVWT, grain volume weight; and PHT, plant height. —,:no QTL detected.
[a] R^2 represents the amount of variation explained by the QTL/location combination (in bold) with the largest effect for each QTL.
[b] QTL with significance threshold while other without significance threshold.

physically mapped to the 3L0.3 region and the KPS was mapped to 3L0.8 (Fig. 2). The QTLs SPSM and KPSM were localized to the 3L0.9 region. Recombination was high for all of the QTL-containing regions. The three regions that contained the grain yield and agronomic trait QTLs accounted for about 70% of the *3A* recombination.

Wheat–rice comparisons

To identify wheat–rice syntenic genomic regions containing the GYLD and other agronomic trait QTLs, 650 wheat sequences mapping to homoeologous group *3* were compared with the available rice genome sequence using BLASTN analysis (see Material and methods). Of the 650 sequences, 100 were for the markers physically mapped in the current study using 41 group *3* deletion lines and 550 were other ESTs physically mapped on only 14 deletion lines (http://wheat.pw.usda.gov/NSF). Of the 650 sequences, 491 (75%) detected a high level of sequence homology ($E \leq 10^{-15}$ to $E \leq 10^{-133}$) with rice and 45 (7%) detected a low level of homology ($E \leq 10^{-5}$) (Supplemental Table 3 and Table 3). The remaining 114 (18%) sequences, however, did not identify any homolog in the rice genome. Sequences corresponding to 187 (29%) of the 650 sequences were present in more than one copy in the rice genome (Table 3).

The wheat homoeologous group *3* chromosomes showed maximum homology to rice chromosome 1, as 352 of the 536 (65%) sequences identified their homologs on rice chromosome 1 (Table 3). The *E* values for 280 of the 352 sequences were between $E \leq 10^{-30}$ and $E \leq 10^{-133}$ with a sequence match of >80%. For the remaining 72 sequences, the *E* values ranged between $E \leq 10^{-30}$ and $E \leq 10^{-5}$. Homologs for 10 additional sequences with low *E* values ($E \leq 10^{-10}$ to $E \leq 10^{-5}$) were also placed on rice chromosome 1 because those were the only homologs found in the rice genome. Homologs for the remaining 174 of the 536 wheat sequences were present on the other 11 rice chromosomes (Table 3). Rice chromosome 2 had the maximum number (36 sequences) of homologs after chromosome 1, and chromosome 12 had the minimum (only 1 sequence). The wheat sequences identified

Table 3
Homology assignment of wheat group *3* sequences on rice chromosomes

Wheat group 3 sequence	Rice chromosome												
	1	2	3	4	5	6	7	8	9	10	11	12	Total
High level of homology[a]	342	32	15	20	10	18	14	20	2	14	3	1	491
Low level of homology[b]	10	4	7	4	2	3	3	7	—	3	2	—	45
No homology													114
Duplication[c]	33	14	17	12	45	25	7	10	2	13	1	8	—
Total													650

[a] Based on $E \leq 10^{-10}$ or lower and/or >85% sequence identity.
[b] $E \leq 10^{-10}$ to $E \leq 10^{-5}$ and >80% nucleotide sequence identity.
[c] $E \leq 10^{-5}$ or lower and 80% nucleotide sequence identity.

either a single BAC/PAC on other rice chromosomes or small and interspersed blocks that consisted of two to three BAC/PACs (Table 3).

Distribution of the 352 wheat sequences on rice chromosome 1 is shown in Fig. 5. The wheat sequences were color coded according to their physical location on wheat group *3* chromosomes (Fig. 5a). The wheat sequences from each of the GRRs identified blocks of rice chromosome 1 ranging in size from 110 kb to 2 Mb, as 1 to 15 contiguous rice BAC/PACs. Each of the rice BAC/PACs was identified with 1 to 6 wheat sequences with an average of 2.3 (Fig. 5c). In general, rice BACs/PACs mapping in the distal regions of chromosome 1 were detected by more than two ESTs compared to the ones present in the proximal regions that were generally identified with only 1 wheat sequence (Fig. 5c). There were eight larger conserved blocks (R_1 to R_8) between wheat group *3* and rice chromosome 1, of which three (R_8, R_6, and R_5) were with no interruption. The approximate lengths of these regions were 1.6 (~3 cM), 1.3 (~4 cM), and 1.8 Mb (~15 cM), respectively (Fig. 5c, Supplemental Table 4). The remaining five blocks consisted of contiguous BAC/PACs with either 1 or 2 missing BAC/PACs in the corresponding regions. Nonhit areas, however, interrupted these blocks.

Rice homologs corresponding to each of the wheat GRRs were highly interspersed by homologs from other rice chromosomes. For example, 16 wheat EST sequences that were physically localized in the proximal 10% of the consensus wheat chromosome *3* were dispersed over about 75% of the rice chromosome 1 (Figs. 5a and 5c; brown-colored sequences). Each of these 16 sequences detected a single rice BAC/PAC. Wheat GRRs differed significantly in the dispersion pattern of their homologs on the rice genome. Rice homologs for wheat 3S0.5 and 3L0.3 regions were scattered over 30% of the rice chromosome 1 (Fig. 5c; light green coded). On the other hand, homologs for wheat regions 3S0.9, 3L0.8, and 3L0.9 were together on rice chromosome 1 with only a few interruptions (Fig. 5c).

With the objective of identifying rice chromosomal regions corresponding to the wheat QTLs, the order of the markers present on the *3A* genetic linkage map was compared with the rice BAC/PAC sequences (Fig. 5). The QTL-containing distal wheat regions 3S0.9, 3S0.8, 3L0.8, and 3L0.9 identified their homologs on rice chromosome 1 regions marked as R_1, R_2, R_7, and R_8, respectively. Wheat–rice sequence similarity was very high (between $E \leq 10^{-22}$ and $E \leq 10^{-87}$) for these regions. The order of the markers surrounding the GYLD, KPS, SPSM, KPSM, and TKWT QTLs was very similar on the rice chromosome except for the markers *Xabg460* and *Xabc161*. The marker *Xabg460* physically mapped to the 3S0.9 region in wheat but identified a homolog in R_2 instead of R_1 in rice. The marker *Xabc161* physically mapped to the 3L0.9 region of wheat; however, it identified a homolog in R_7 instead of R_8 (Fig. 5).

Discussion

The present study reports a comprehensive map of wheat homoeologous group *3* chromosomes that was generated by comparison of a detailed high-density, deletion line-based physical map with a genetic linkage map developed using unique RICL lines. This study also revealed better physical and genetic locations of 514 marker loci and 17 QTLs for grain yield and other agronomic traits. The physical map developed using 44 group *3*-specific aneuploid stocks (nulli-tetras and deletion lines) was of much higher resolution compared to the available physical map for which only 14 group *3* deletion lines were used [32]. For the genetic linkage map, a polymorphism survey using 152 RFLP probes and 55 SSR markers revealed a novel distribution of polymorphism on group *3* chromosomes. Furthermore, significant differences were observed for the extent of polymorphism among various group *3* chromosomal regions as well as among the three homoeologs.

The distribution of markers and genes on chromosomes matched the previously published pattern [10]. About 91% of the group *3* genes localized to seven regions encompassing about 36% of the chromosome (Fig. 2). These numbers matched very well with the previous results, in which 96% of the group *3* genes were localized to 36% of the chromosomal region [10]. Comparison of the physical map with the genetic maps revealed that about 80% of the *3A* recombination occurred in the five GRRs. The recombination rate in the remaining two regions (3S0.5 and 3L0.5) was insignificant (Fig. 2). Almost all of the short-arm recombination occurred in the two GRRs, whereas about 71% of the long-arm recombination occurred in the three GRRs. Previously 100% of the long-arm recombination was reported to be present in the GRRs [10].

Detailed genetic analysis using agronomic trait data in individual environments and combined across environments

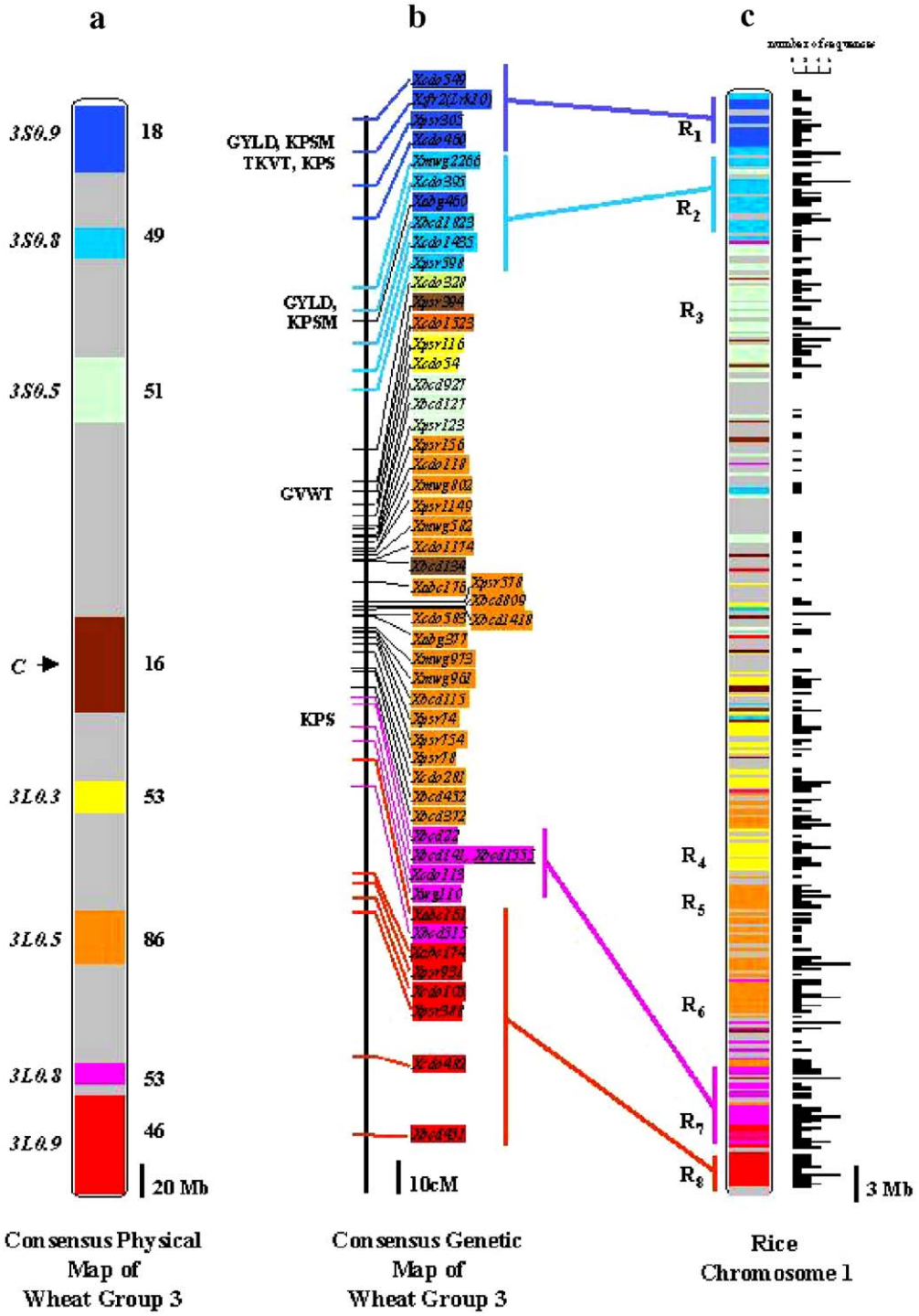

Fig. 5. Comparative analysis of the (a) consensus physical map of wheat group *3,* (b) genetic linkage, and (c) rice chromosome 1 BAC/PACs. Wheat GRRs are color coded such that markers present in a GRR are shown by a unique color and the same color was given to markers mapping to regions. The number of markers located within each GRR is given on the right side and the names of the GRRs are given on the left side of the wheat consensus physical map. Gray on the consensus physical map shows the gene-poor regions and on the rice chromosome, it shows lack of homology to the wheat group *3* chromosomes that could simply be because of the type and number of sequences used for the comparison.

identified 17 QTLs localized to seven regions, of which three mapped on the short arm and four on the long arm. Ten of the 17 QTLs were highly significant. Previous QTL analyses using a 16-marker linkage map placed these QTLs to four regions on the genetic linkage map of chromosome *3A* [1,2]. Addition of 29 new RFLP and SSR markers in the current analysis increased precision of QTL location on the genetic linkage map. Compared to previous analyses [1–4], QTL analyses using the current and more comprehensive linkage map localized agronomic trait QTLs to seven regions by splitting three of the previously identified four regions into two separate regions each (Fig. 2).

Comparison of the *3A* genetic linkage maps with the consensus physical map revealed the physical location of the QTLs. Ten of the 17 QTLs mapped on the short arm and seven on the long arm. All 10 short-arm QTLs mapped to the two major GRRs (3S0.8 and 3S0.9). The long-arm QTLs were scattered over three of the four long-arm GRRs (Fig. 2). Since the two QTL-containing short-arm regions accounted for almost all of the arm's recombination, these QTLs appear to be amenable to map-based cloning approaches. Similarly the three QTLs present in 3L0.9 GRR of the long arm are good targets for map-based cloning because of a higher recombination rate for the region. The recombination rate in the long-arm GRRs 3L0.3 and 3L0.8, however, is not very high; therefore, these three QTLs are not good targets for map-based cloning approaches.

It has been reported that the wheat sequences near centromeres are more conserved compared to those present toward the telomeres [46]. A linear relationship was predicted between distance from centromere and sequence divergence due to the distribution of recombination. By plotting polymorphism survey results relative to the location of the probes on the chromosome (Fig. 3), the present study clearly shows that, in general, polymorphism is much scarcer for the sequences present around centromeres. However, the rate of polymorphism is not necessarily related to the distance from the centromere, especially in the distal parts of the chromosome. For example, probes present in 3L0.3 detected a much higher level of polymorphism compared to those present in the 3L0.5 and S2 regions. Similarly, the probes from the short-arm region 3S0.8 showed a higher level of polymorphism compared to that present in the 3S0.9 region (Fig. 3).

For some chromosomal regions, dramatic differences were observed among homoeologs for the level of polymorphism detected by the same probes. For example, probes from the 3S0.5 region detected a high level of polymorphism for chromosome 3B or 3D but essentially none of these probes detected polymorphism for chromosome 3A. Similar differences were observed for other regions as well (Fig. 3).

Significantly different and sometimes conflicting reports have been published about the gene synteny conservation between the wheat and the rice genomes [30,46–48]. Gene conservation between wheat homoeologous group *3* and rice chromosome 1 has been reported to be more prevalent than any other wheat–rice chromosome combination [30,32,46]. The most comprehensive comparison, in which 537 wheat ESTs were compared with the rice genome, showed that only 58% of the wheat group *3* sequences found homologs in the rice genome, of which 43% were on rice chromosome 1 [32].

Many significant and novel observations were made during the current analysis about the wheat–rice synteny. First, use of 650 wheat sequences (mostly ESTs) revealed about 76% sequence match between wheat and rice. About 54% of the total sequences were also present on rice chromosome 1. The main reasons to obtain much higher match rates from the previous studies would be due to the use of more sequences and, perhaps, better precision of threshold values used for sequence comparisons. Second, since the precise physical location of the wheat sequences was known, it was possible to make a region-by-region comparison between wheat and rice (Fig. 5a). Finally, use of 53 both genetically and physically mapped sequences for wheat–rice comparison allowed us to identify the collinearity at the micro level. The order of the wheat chromosome *3A* homologs on rice chromosome 1 is not always conserved and the extent of differences varied among the wheat GRRs (Fig. 5). The homologs for the four GRRs (3S0.9, 3S0.8, 3L0.8, and 3L0.9) were adjacent to one another compared to those for the remaining three GRRs (3S0.5, 3L0.3, and 3L0.5), for which the homologs were scattered all over rice chromosome 1 (Fig. 5). In general, homologs for the distal wheat regions were closer to each other on rice chromosome 1 compared to that from the proximal regions (Fig. 5; Supplemental Table 3). Very few wheat homologs were found in the centromeric region of rice chromosome 1 probably because of the presence of repetitive sequences.

Results from the present study show that although about 82% of the wheat genes have homologs in the rice genome the gene order is conserved only at very small chromosomal regions (Fig. 5). The average size of the rice chromosomal blocks in which the gene order was conserved with that of wheat was about 1 Mb, with a range of 200 kb to 2 Mb. However, sizes of the homolog-containing blocks on the other rice chromosomes were about 300 kb with a range of 150 to 450 kb. Similar results were obtained for wheat groups *2* and *6* sequence comparisons with rice [29,33]. The extent of similarity in the gene order between wheat and rice even within these small syntenic blocks is, however, not known.

Considering rice chromosome 1, eight major blocks (R_1 to R_8) homologous to wheat group *3* chromosomes were identified. Four of these regions corresponded to wheat regions (3S0.9, 3S0.8, 3L0.9, and 3L0.8) where grain yield and other agronomic trait QTLs mapped (Fig. 5). No major rice chromosome block was identified for the 3L0.3 region containing the QTL for GVWT, and the homologs were scattered all over rice chromosome 1. Except for 3L0.3, therefore, the regions that are collinear between wheat and rice can further be utilized for extensive analysis of the grain yield-related traits/genes in both genomes.

In summary, both a high-density physical map of wheat homoeologous group *3* and a chromosome-specific (*3A*) genetic map containing 17 QTLs identified for seven agronomic traits were generated. Comparison of the physical and genetic linkage maps revealed the physical location of the grain yield-related QTLs and provided an accurate estimate of the recombination rate for each of the chromosome *3A* regions. Nine of the QTLs were localized to 13% of chromosome *3A* and are present in high-recombination regions. Therefore, these QTLs should be amenable to map-based approaches. By comparing 650 wheat sequences present on chromosome *3A* to the rice genomic sequences, we identified various chromosome *3A* regions significantly differing from the rice genome and showed that wheat–rice synteny holds only at small blocks of varying-sized sequences. Our comparison is perhaps the most extensive and accurate that has been accomplished region by region. This analysis accurately identified rice genomic regions corresponding to the major wheat regions containing QTLs.

Along with the wheat physical and genetic map information, the rice information will assist in identifying rice homologs for the major QTLs for grain yield and other agronomic traits.

Material and methods

Plant material

Genetic linkage analysis for the chromosome *3A* (RICLs-3A) was performed using a set of 95 single-chromosome recombinant inbred lines. Construction of this population has already been described [34]. Briefly, reciprocal substitution lines for chromosome *3A* between hard red winter wheat cultivars CNN and WI were first developed [5,6]. Cultivar CNN was crossed with its substitution line carrying chromosome *3A* from the cultivar WI [CNN(WI3A)]. Recombinant *3A* chromosomes were individually recovered as separate lines allowing only a single recombination cycle, by crossing the F_1 to monosomic *3A* in cultivar CNN. The 41-chromosome progeny were identified by root-tip chromosome analysis and were allowed to self-pollinate to recover the recombinant chromosome in the homozygous condition.

Deletion mapping was performed using several aneuploid stocks. Chromosome arm location of the DNA markers was revealed using NT and ditelosomic (DT) lines [35]. For sub-arm localization of the markers, 41 single-break deletion lines for wheat homoeologous group *3* chromosomes were used [36]. All the aneuploid stocks for the study were kindly provided by Dr. Bikram Gill at the Wheat Genetic Resource Center (Manhattan, KS, USA).

Markers and DNA analysis

The DNA marker analysis for the project was carried out using 179 RFLP markers specific for wheat homoeologous group *3* chromosomes and 55 simple-sequence repeat (SSR) markers. The 179 RFLP probes included 36 EST singletons (http://wheat.pw.usda.gov) and 143 cDNA and *Pst*I genomic probes from *Aegilops tauschii*, barley, oat, and wheat developed by the North American Barley Genome Project (ABC and ABG), Cornell University, USA (BCD, CDO, and WG), Kansas State University, USA (KSU), the University of Nebraska at Lincoln, USA (UNL), the Institute for Resistance Genetics, Germany (MWG and cMWG), and the John Innes Centre, UK (PSR) (Table 1). Among the 55 SSR markers, 13 were GWM [37], 8 were CFA and CFD [38,39], 6 were WMC [40], and 28 were BARCs [41].

Physical mapping

Using a previously described method of gel-blot analysis [12], the probes were first mapped on the NT and the DT lines for interchromosomal and arm mapping unless that information was already available. Confirmed group *3*-specific probes were then physically mapped on 41 group *3*-specific deletion lines using either *Eco*RI or *Hin*dIII restriction enzymes. Thirty-four of the 41 deletion lines were homozygous and 7 were heterozygous (one deletion and one normal chromosome). Additional information about the deletion lines is available at http://www.ksu.edu/wgrc/Germplasm/Deletions/group3.html.

Data scoring for deletion mapping was previously described [13]. The homozygous deletion lines were scored for the presence or absence of fragment bands and the heterozygous deletion lines were scored for band intensity. Each fragment band was mapped to a chromosomal region flanked by the breakpoints of the smallest deletion lacking the fragment band and the largest deletion possessing it. Multiple fragment bands mapping on a chromosome arm were scored as nonallelic (shown by a letter at the end of the probe name) if the bands mapped to different chromosomal regions.

The deletion mapping information from the three homoeologs (*A, B*, and *D*) was combined on a consensus physical map to localize the probes to the smallest possible physical regions, and the details of the method have previously been described [13]. Briefly, the breakpoints of all group *3* deletions were placed on a hypothetical chromosome drawn to scale based on the average length of the three chromosomes. Each probe was then placed on the shortest possible interval. In the case of a discrepancy, location consistent with two homoeologs was used. Other publicly available physical mapping information was also integrated into the map [42].

Genetic linkage analysis of markers and QTLs

A chromosome *3A*-specific genetic linkage map was developed using a population of 95 RICLs-*3A*. This population has been extensively evaluated for seven agronomic traits (PHT, GYLD, TKWT, KPS, SPSM, KPSM, and GVWT) in replicated field trials across seven environments located in Nebraska [1,2]. For the RFLP markers, a polymorphism survey of the parents was performed using 11 (*Eco*RI, *Hin*dIII, *Bam*HI, *Xho*I, *Eco*RV, *Kpn*I, *Dra*I, *Sac*I, *Sca*I, *Apa*I, and *Bgl*I) restriction enzymes. Additionally, 55 SSR markers were screened for polymorphism between CNN and CNN(WI3A) using the Licor genetic analyzer. Linkage map construction was performed using MAPMAKER/EXP 3.0 [43] with the distance unit set to recombination fractions. The GROUP command with a lod threshold of 5.0 and an independent distance of 50 cM was used to assign markers to a linkage group. Repetitive use of the RIPPLE command identified the best order of markers within a linkage group. Windows QTL Cartographer 2.00 software [44] was used to identify QTLs for single-marker analysis and composite interval mapping (CIM) based on the adjusted LSMEANS trait values for each RICL. Three hundred permutation tests ($p < 0.05$) determined the appropriate significance threshold for CIM analyses, and 2-cM intervals were scanned for the presence of QTLs using a 1-cM window of no more than five markers to control for background effects. Background markers were selected by means of forward–backward step-wise regression with thresholds of $p < 0.1$ for entering and remaining in the model. This analysis was performed both for individual environments and across environments to detect the main effect of chromosomal regions associated with the agronomic traits measured.

Wheat–rice comparison

With the objective of identifying the rice chromosomal segments corresponding to various wheat group *3* regions, 650 wheat homoeologous group *3*-specific sequences were compared with the rice genomic sequences using various comparison tools publicly available at http://wheat.pw.usda.gov, http://ncbi.nlm.nih.gov, http://www.rgp.dna.affrc.go.jp, and http://www.tigr.org, where wheat and rice sequences are stored and sequence comparisons can be made by Blasting wheat group *3* sequences on the rice BAC/PACs using the BLASTN option. We initially Blasted rice chromosome 1 BAC/PAC sequences on the wheat EST database to ensure the overlapping order of BAC/PACs on rice chromosome 1. The wheat sequences included in this study were 499 ESTs and 151 other cDNA or *Pst*I genomic clones. For the latter 151 clones, the physical location was revealed using 41 single-break deletion lines. The physical location of the remaining 499 ESTs was revealed using 14 group *3* deletions (http://wheat.pw.usda.gov/NSF).

Of the 151 cDNA or *Pst*I genomic clones, 53 were mapped both genetically and physically. The sequences of these 53 DNA markers were used to study microcollinearity between rice chromosome 1 and the consensus genetic map of wheat group *3* chromosomes. To find the optimum cut-off value, two different approaches were taken. First, the randomly selected cloned wheat genes were Blasted with rice genomic sequences. This analysis revealed that the wheat–rice homology could be as low as 72% sequence match and as low as 75 bases of sequence identity. Second, the rice genomic probes were hybridized with several wheat ESTs selected based on different levels of sequence similarity and varying *E* values (data not shown). For the sake of a conservative analysis, a cut-off value of $E \leq 10^{-5}$, with more than 80% sequence identity for longer than 100 bp, was used for wheat–rice comparison. However, 45 wheat EST sequences showing $E \leq 10^{-5}$ and less than 80% sequence identity were also included in the analysis because these sequences had homology to only rice chromosome 1, which is the most syntenic rice chromosome to wheat homoeologous group *3*.

After the BLAST searches, we manually singled out false positive sequence matches such as matches with less than 80% identity (for more than 100 bases) or false positive *E* values $\geq 10^{-5}$ with the rice genome. This criterion was used as the resulting homoeologs produced reliable band patterns for gel-blot analysis across species. The size of the rice blocks was calculated based on the total lengths of corresponding BAC/PACs. Marker distribution and size of the GRRs on wheat chromosomes were calculated as previously described [10].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygeno.2006.02.001.

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