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Identification and Physical Localization of Useful Genes and Markers to a Major Gene-Rich Region on Wheat Group *1S* Chromosomes

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ABSTRACT

The short arm of Triticeae homeologous group *1* chromosomes is known to contain many agronomically important genes. The objectives of this study were to physically localize gene-containing regions of the group *1* short arm, enrich these regions with markers, and study the distribution of genes and recombination. We focused on the major gene-rich region ("1S0.8 region") and identified 75 useful genes along with 93 RFLP markers by comparing 35 different maps of Poaceae species. The RFLP markers were tested by gel blot DNA analysis of wheat group *1* nullisomic-tetrasomic lines, ditelosomic lines, and four single-break deletion lines for chromosome arm *1BS*. Seventy-three of the 93 markers mapped to group *1* and detected 91 loci on chromosome *1B*. Fifty-one of these markers mapped to two major gene-rich regions physically encompassing 14% of the short arm. Forty-one marker loci mapped to the 1S0.8 region and 10 to 1S0.5 region. Two cDNA markers mapped in the centromeric region and the remaining 24 loci were on the long arm. About 82% of short arm recombination was observed in the 1S0.8 region and 17% in the 1S0.5 region. Less than 1% recombination was observed for the remaining 85% of the physical arm length.

COMMON wheat (*Triticum aestivum* L. em Thell, $2n = 42$, AABBDD) has a large genome, ~16 million kb/haploid cell. The wheat genome is ~35 times larger than that of rice and ~110 times that of Arabidopsis (Bennett and Smith 1976). The gene-containing fraction of the wheat genome should therefore be <2.7%. Since only a small fraction of the wheat genome is expected to represent genes, identification and marking of the gene-containing regions is invaluable for their characterization.

A strategy to identify and preferentially map the gene-containing regions of the wheat genome was proposed (Gill and Gill 1994) and demonstrated (Gill *et al.* 1996a,b). The strategy involved physical mapping of protein, DNA, and morphological markers on single-break chromosome deletion lines. The physical maps thus generated were compared with various genetic linkage maps of Triticeae via common markers. A total of 436 deletion lines involving all 21 wheat chromosomes was isolated using the gametocidal chromosome of *T. cylindricum* (Endo and Gill 1996) and used to generate composite maps of all wheat chromosomes (Werner *et al.* 1992; Gill *et al.* 1993, 1996a,b; Kota *et al.* 1993; Delaney *et al.* 1995a,b; Mickelson-Young *et al.* 1995; Weng *et al.* 2000). The resulting composite maps revealed the distribution of genes and recombination on the chromosomes. High-density composite maps have revealed that >85% of the wheat genes are present in gene-rich regions, physically spanning only 5–10% of the chromosomal region (Gill *et al.* 1996a,b). The gene-rich regions are interspersed with blocks of repetitive DNA sequences visualized as regions of low gene density. About two to four major gene-rich regions were observed per chromosome arm. Gene-rich regions were mainly observed in the distal regions of chromosomes.

Division of higher organism genomes into gene-rich and gene-poor compartments may be a common feature (see Sumner *et al.* 1993 for review). Among animal systems, gene distribution in the human genome is best studied. The conclusion based on the *in situ* hybridization of a random pool of mRNA (Yunis *et al.* 1977) or with the G + C-richest isochore H3 (Saccone *et al.* 1992), and by DNAase hypersensitivity (Weintraub and Groudine 1976; Elgin 1988), was that genes in the human genome are localized in R-bands and are more concentrated in T-bands, which are terminal R-bands (Sumner *et al.* 1993). In all well-studied higher organisms, a major part of the genome is composed of repetitive DNA, most of which is made up of transposons (Hake and Walbot 1980; Mouchiroud *et al.* 1991; Sumner *et al.* 1993; Carels *et al.* 1995; Barakat *et al.* 1997, 1999). A partial sequence of the region near the Adh-F gene of maize showed that genes are present in clusters and are interspersed with long stretches of repeat units of retrotransposons (Bennetzen *et al.* 1998). This gene-rich region is also conserved in sorghum, where homologues of the maize genes were present in a colinear order (Bennetzen *et al.* 1998).

In wheat and many other organisms, a strong correlation was observed between the distribution of genes and

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recombination (Thuriaux 1977; Civardi *et al.* 1994; Zhang *et al.* 1994; Gill *et al.* 1996a,b; Buschges *et al.* 1997; Kunzel *et al.* 2000). The actual ratio of physical to genetic distance for the regions around genes of most plants ranges from 14 kb/cM in maize (Brown and Sundaresan 1991) to 90 kb/cM in tomato (Ganal *et al.* 1989), which is significantly smaller than the estimates made from total genome size and length of genetic linkage maps. These results clearly show that recombination is unevenly distributed along chromosomal lengths of most organisms and gene-containing regions are its preferred sites. This pattern of gene distribution and its relationship with that of recombination appears to be similar in vertebrates, where the genomes are organized into large domains of uniform (G + C) content (Bernardi 1993). Most genes and chiasmata are found in regions richest in (G + C) content (Ikemura and Wada 1991). This explains why gene-rich regions are higher in recombination.

Two major gene-rich regions at fraction length (FL) 0.8 (1S0.8 region) and FL 0.5 (1S0.5 region), were identified on the short arm of homeologous group *1* chromosomes (Gill *et al.* 1996b). The 1S0.8 region is one of the largest gene-rich regions of wheat. On chromosome *1B*, the region is present in the middle satellite part of the chromosome and is bracketed by the breakpoints of deletions 1BS-4 (FL 0.54) and 1BS-19 (FL 0.31). This region is only 23% of the satellite. Fourteen agronomically important genes, including genes conferring resistance to diseases [leaf rust (*Lr, Lr21*), stem rust (*Sr33*), powdery mildew (*Pm3*)], preharvest sprouting (*Qphs.cnl*), fertility restoration gene (*Rf3*), and seed storage proteins, and 11 DNA markers were also localized to the gene-rich region (Gill *et al.* 1996b).

Gene synteny is conserved among living organisms and the extent of conservation is proportional to the evolutionary distances involved. Wheat belongs to the grass family Poaceae (Gramineae) that includes other major cereal crops such as barley (*Hordeum vulgare*), oat (*Avena sativa*), rye (*Secale cereale*), maize (*Zea mays*), and rice (*Oryza sativa*). Gene synteny is conserved among the genomes of the tribe Triticeae (Hart 1987). Conserved linkage blocks exist even among genomes of wheat, rice, and maize (Ahn *et al.* 1993; Ahn and Tanksley 1993; Bennetzen and Freeling 1993; Van Deynze *et al.* 1995; Gallego *et al.* 1998). The number of restriction fragment length polymorphism (RFLP) markers mapped on one or more of the Triticeae species is >2800. As RFLP markers can be used across maps, markers from different maps can be selected by comparative mapping and can be used on deletion lines to enrich a particular region in the wheat genome.

The objectives of this study were to identify and physically localize useful genes to the wheat homeologous group *1* short arm, enrich the gene containing regions with markers, and study the relationship between distribution of genes and recombination.

MATERIALS AND METHODS

Plant material: Various chromosome, arm, and subarm aneuploid stocks were used to physically map DNA markers to their respective chromosomal regions. Wheat homeologous group *1* nullisomic-tetrasomic lines (missing a pair of chromosomes, the deficiency of which is compensated for by a pair of homeologous chromosomes) and ditelosomic lines (missing a pair of chromosome arms; Sears 1954) were used to assign DNA restriction fragments to their respective chromosomal arms. Four single-break deletion lines with their breakpoints flanking two previously known gene-rich regions of wheat homeologous group *1* short arm were used for subarm localization of DNA markers. Among these, breakpoints of deletions 1BS-4 and 1BS-19 bracket the 1S0.8 region (Figure 1; Gill *et al.* 1996b). Between these, 1BS-4 is the smaller deletion with only 46% of the satellite region deleted compared to 69% in 1BS-19 (Endo and Gill 1996). The breakpoints of deletion lines 1BS-9 and 1BS-20 flank the 1S0.5 region. In addition to the satellite, the deletion line 1BS-9 has 16% (FL 0.84) of the short arm missing compared to 28% in 1BS-20.

Comparative mapping: Eleven markers that were previously mapped in the 1S0.8 region (Gill *et al.* 1996b) were used for comparative mapping across Poaceae maps to identify agronomically important genes and additional markers present in the gene-rich region. The published Poaceae maps and Graingenes database were compared with each other and with the consensus physical map of wheat (Chao *et al.* 1989; Lagudah and Appels 1991; Kohler *et al.* 1992; Wang *et al.* 1992; Laurie *et al.* 1995; Bezant *et al.* 1996; Gill *et al.* 1996b; Devos *et al.* 1998; Korzun *et al.* 1998; Ward *et al.* 1998). A total of 11 wheat, 11 barley, 6 rye, 3 rice, 2 oat, and 2 Triticeae consensus maps were used for the comparative mapping. In the first phase of comparative mapping, markers present in the 1S0.8 region in the existing physical map were used as anchor markers on genetic linkage maps. All the markers on any genetic linkage map, present in between two anchor markers, were selected. In the second phase of comparative mapping, the markers selected in the first phase were used as anchor markers on other genetic linkage maps to select more markers. Some markers that were not flanked by two anchor markers but were tightly linked to one of the anchor marker were also included.

Probes: The cDNA and genomic DNA probes used to construct the physical map were derived from wheat (CS, KSU, WG, PSR, NOR, TAM), barley (ABC, ABG, BCD, MWG, and cMWG), oat (CDO), and rice (RZ). The RFLP probes were described by the following authors. BCD, CDO, WG: Heun *et al.* (1991); RZ: Causse *et al.* (1994); KSU: Gill *et al.* (1991b); MWG and cMWG: Graner *et al.* (1991); ABC, ABG: Kleinhofs *et al.* (1993); PSR: Sharp *et al.* (1989); and TAM: Devey and Hart (1993). LRK10 was kindly supplied by Dr. C. Feuillet, University of Zurich.

DNA analysis: Genomic DNA from various plant materials was isolated following a method described elsewhere (Anderson *et al.* 1992). For each sample, 15 μg of genomic DNA was digested with a restriction enzyme and electrophoretically separated on 0.8% agarose gel as previously described (Gill *et al.* 1993). Two restriction enzymes (*Eco*RI and *Hin*dIII) were used for physical mapping. Southern blotting onto nylon membrane (Micron Separations Inc., Westborough, MA), DNA immobilization, and hybridization were performed following manufacturer's recommendations.

Probe preparation, hybridization, and autoradiography: Approximately 30 ng of probe DNA was labeled with 30 μCi of [^{32}P]dCTP in a 15-μl reaction volume, following random primer labeling technique (Feinberg and Vogelstein 1983). Hybridization was performed in 35 × 300-mm glass bottles

containing 10 ml of hybridization buffer (5% dextran sulfate, 6× SSPE, 5% Denhardt's solution, 0.5% SDS), incubated at 65° for 16–18 hr in a hybridization rotisserie oven (Hybaid, Inc.). Blots were washed at 65° in 2× SSPE, 0.5% SDS for 30–50 min and exposed for 3–7 days at −80°.

RESULTS

Comparative mapping: A total of 195 markers were identified for the 1S0.8 region by comparing 35 different maps of Poaceae. Seventy-five of these were useful genes, 93 were RFLPs, 15 were simple sequence repeats, 7 were sequence-tagged sites, and 5 were amplified fragment length polymorphism markers. Among the agronomically important genes were several resistance genes including 6 leaf rust (*Lr*), 5 yellow rust (*Yr*), 4 stem rust (*Sr*), 1 barley rust (*Pa*), and 10 powdery mildew (*Mla*, *Mlk*, *Mlnn*, *Mlra*, *Pm*) genes, and a suppressor of powdery mildew (*Su-Pm*); genes for seed storage proteins such as gliadin (*Gli*), glutenin (*Glu*), triticin (*Tri*), and Hordein (*Hor*); and some other interesting genes such as preharvest sprouting resistance (*Qphs.cnl*), the restorer for cytoplasmic male sterility (*Vi* and *Rf*), and a tiller-inhibitor gene (*Tin*) (Table 1). Of the 93 RFLPs, 3 were wheat cDNA (PSR and *Nor*), 16 were wheat genomic (PSR, WG, TAM, and LRK), 22 were barley cDNA (ABC, BCD, and cMWG), 31 were barley genomic (ABG and MWG), 4 were *Pst*I genomic clones from *T. tauschii* (KSU), 15 were oat cDNA (CDO) and 2 were rice cDNA clones (RZ). A total of 42 (45%) probes were cDNA and 51 (55%) probes were genomic.

Physical mapping: The 93 putative RFLP probes for the gene-rich regions were physically mapped by gel blot DNA analysis of wheat homeologous group *1* nullisomic-tetrasomic lines, ditelosomic lines, and the deletion lines 1BS-4, 1BS-19, 1BS-9, and 1BS-20. Restriction enzymes *Eco*RI and *Hin*dIII were used for the analysis. Of the 93 RFLP probes, 73 mapped on wheat homeologous group *1*. Twenty-eight of 73 probes were specific to group *1* and 45 detected bands on other chromosome groups also. These 73 probes detected 223 loci on group *1* (Table 2). Three probes detected fragments for only one of the three homeologous group *1* chromosomes, 8 detected fragments for two and the remaining probes detected fragments for all three homeologous chromosomes (Table 2).

Seventy-three group *1* probes detected 91 loci on chromosome *1B*. Of the 73 group *1* probes, 39 mapped in the 1S0.8 region and detected 41 marker loci on chromosome *1B* (Figure 1). These 39 probes detected 121 loci on three homeologous chromosomes. Three probes detected fragments for only one of the three homeologous group *1* chromosomes, 6 detected fragments for two, and 33 probes detected fragments for all three homeologous chromosomes. Three marker loci (*Xbcd98*, *Xcdo99*, and *Xcdo580b*) showed *1B* specific fragment band missing in 1BS-4 and 1BS-19, mapping them distal to the breakpoint of 1BS-4. Seven markers mapped just proximal to the breakpoint of 1BS-19 and detected 20 loci on the three homeologous chromosomes. One marker detected fragments for one of the homeologous groups and six markers detected fragments for all three homeologous chromosomes. Ten markers mapped in the 1S0.5 region and detected 32 loci on the three homeologous chromosomes. All 10 markers detected fragments for all three homeologous chromosomes. Four markers mapped proximal to the breakpoint of 1BS-20. For two marker loci (*Xbcd1072* and *Xpsr161*) the B fragment band was present in both of the ditelosomic lines, mapping them in the centromere. The remaining 24 marker loci mapped to the long arm.

Twelve of the 1S0.8 region probes (CDO388, CDO580, CMWG645, MWG835, MWG837, MWG913, MWG938, MWG2021, MWG2048, MWG2056, PSR381, PSR688) detected a second locus on chromosome *1B* and two probes (KSUD14, MWG2148) detected three loci each on the same chromosome. All three loci for KSUD14 were present in the 1S0.8 region, whereas two loci for MWG2148 were present on chromosome *1BL*. A second locus for probe CDO580 was present distal to the breakpoint of 1BS-4 and a second locus of MWG913 was present proximal to the breakpoint of 1BS-20. Two of 1S0.5 region probes (BCD762, CDO127) detected a second locus on the same chromosome. A second locus of BCD762 was present on the long arm, whereas a second locus of CDO127 was present just proximal to the breakpoint of 1BS-19.

All the three markers that mapped distal to the breakpoint of 1BS-4 were cDNA. Among the 41 marker loci mapped to the 1S0.8 region, 12 were cDNA and 29 were genomic. Of the 14 1S0.8 region markers, which had more than one locus on chromosome *1B*, 3 were cDNA and 11 were genomic clones. Four marker loci of the 7 that mapped just proximal to the breakpoint of 1BS-19 were cDNA and 3 were genomic. Of the 10 marker loci that mapped to the 1S0.5 region, 6 were cDNA and 4 were genomic. Two marker loci of four that mapped proximal to the breakpoint of 1BS-20 were cDNA and 2 were genomic. Both markers present at the centromere were cDNA. Eight of the 24 loci that mapped to the long arm were cDNA and 16 were genomic DNA clones.

Distribution of genes/markers: Physical mapping revealed that the distribution of markers on the chromosomes was not uniform (Figure 1). Seventy-eight percent (51/65) of the marker loci present on the *1BS* arm were present in two major gene-rich regions. Deletion lines 1BS-4 and 1BS-19 bracket ~6% of the total arm (23% of satellite) and 41 marker loci mapped in this small region. The region encompassed by 1S0.5 region was ~8% of chromosome *1BS* and 10 marker loci were located in this region. Only 14 markers mapped in the remaining 86% of the arm. Two cDNA marker loci

TABLE 1

List of useful genes present in the 1S0.8 region on the short arm of group *1* chromosomes in Triticeae

Gene Symbol	Gene	Chromosome location[a]	Source
Act8	Actin	*1HS*	Langridge *et al.* (1995)
Act8a	Actin	*1HS*	Langridge *et al.* (1995)
Aga6	ADPgluco phosphatase	*1HS*	Langridge *et al.* (1995)
amo1	High amylose	*1HS*	Schondelmaier *et al.* (1992)
Bg	Black glume color	*1AS*	Van Deynze *et al.* (1995)
Chs	Chalcone synthase	*1AS*, *1HS*	Langridge *et al.* (1995)
Clh[b]	Curled leaf dwarf	*1HS*	Jensen (1996)
fch3(*f3*)[b]	Chlorina seedling 3	*1HS*	Jensen (1996)
fst2(*fs2*)[b]	Fragile stem	*1HS*	Jensen (1999)
Gle1	Glossy spikes	*1HS*	Jensen (1996)
Gli1	Gliadins 1	*1AS*, *1BS*, *1DS*	Dubcovsky *et al.* (1995)
Gli3	Gliadins 3	*1AS*, *1BS*, *1DS*	Dubcovsky *et al.* (1995)
Gli5	Gliadins 5	*1AS*, *1BS*	Dubcovsky *et al.* (1997)
Glo1	Salt soluble globulins	*1AS*, *1BS*, *1DS*	Gomez *et al.* (1988)
Glu2	Glutenins 2	*1BS*	Dubcovsky *et al.* (1997)
Glu3	Glutenins 3	*1AS*, *1BS*, *1DS*	Dubcovsky *et al.* (1995)
Gpi1	Glucose phosphate isomerase	*1AS*, *1BS*, *1DS*, *1HS*, *1RS*	Van Deynze *et al.* (1995)
Gpt1[b]	Glutamate pyruvate transaminase	*1AS*, *1BS*, *1DS*, *1H*	Sun and Dvorak (1992)
H5[b]	Reaction with *Maytiola destructor*	*1AS*	Roberts and Gallun (1984)
Hex1	Hexokinase	*1HS*	Jensen (1999)
Hg	Hairy glume	*1AS*	Van Deynze *et al.* (1995)
Hor1	C-hordeins	*1HS*	Langridge *et al.* (1995)
Hor2	B-hordeins	*1HS*	Langridge *et al.* (1995)
Hor4	Hordeins	*1HS*	Jensen (1996)
Hor5	g-Hordeins	*1HS*	Langridge *et al.* (1995)
Ica1	Chymotrypsin inhibitor	*1HS*	Jensen (1996)
Lr10	Reaction to *Puccinia recondita*	*1AS*	Howes (1986)
Lr21	Reaction to *P. recondita*	*1DS*	Van Deynze *et al.* (1995)
Lr26[b]	Reaction to *P. recondita*	*1BS*	Hartl *et al.* (1993)
Lr39[b]	Reaction to *P. recondita*	*1DS*	Cox *et al.* (1993)
Lr40[b]	Reaction to *P. recondita*	*1DS*	Cox *et al.* (1993)
Lr41[b]	Reaction to *P. recondita*	*1DS*	Cox *et al.* (1993)
Lys4	High lysine	*1HS*	Jensen (1996)
Mdh1	Malate dehydrogenase	*1HS*	Jensen (1996)
Mla	Reaction to *Erysiphe graminis hordei*	*1HS*	Jensen (1999)
Mla6	Reaction to *E. graminis hordei 6*	*1HS*	Langridge *et al.* (1995)
Mla12	Reaction to *E. graminis hordei* 12	*1HS*	Giese *et al.* (1981)
Mla13	Reaction to *E. graminis hordei* 13	*1HS*	DeScenzo *et al.* (1996)
Mla14	Reaction to *E. graminis hordei* 14	*1HS*	DeScenzo *et al.* (1996)
Mlk(*Reg4*)	Reaction to *E. graminis hordei* 4	*1HS*	Jensen (1996)
Mlnn	Reaction to *E. graminis hordei*	*1HS*	Jensen (1999)
Mlra	Reaction to *E. graminis hordei*	*1HS*	Jensen (1999)
msg4[b]	Male sterile genetic 4	*1HS*	Jensen (1996)
msg31[b]	Male sterile genetic 31	*1HS*	Jensen (1996)
Ndh3	NADH dehydrogenase 3	*1HS*	Jensen (1996)
Ndh5	NADH dehydrogenase 5	*1HS*	Jensen (1996)
Nor1	Nucleolor organizer region	*1AS*, *1BS*	Van Deynze *et al.* (1995)
Pa4(*Rph4*)	Reaction to *P. hordei* 4	*1HS*	Jensen (1996)
Per1	Peroxidase 1	*1BS*, *1DS*	Gale *et al.* (1995)
Pm3	Reaction to *E. graminis*	*1AS*	Van Deynze *et al.* (1995)
Pm8	Reaction to *E. graminis*	*1BS*	Hartl *et al.* (1993)
Qphs.cn13	Resistance to preharvest sprouting	*1HS*	Van Deynze *et al.* (1995)
Rf1[b]	Restorer of cytoplasmic male sterility	*1AS*	Maan (1992)
Rf3	Restorer of cytoplasmic male sterility	*1BS*	Van Deynze *et al.* (1995)
Rg1	Red glume	*1BS*	Hartl *et al.* (1993)
Rg2	Red glume	*1DS*	Jones *et al.* (1990)
Rg3	Red glume	*1AS*	Van Deynze *et al.* (1995)

(*continued*)

TABLE 1

(Continued)

Gene Symbol	Gene	Chromosome location[a]	Source
Sex76	Shrunken endosperm	*1HS*	Jensen (1999)
Si2	Subtilisin inhibitor 2	*1BS*, *1DS*, *1HS*, *1RS*	Koebner (1990)
Sr21	Reaction to *P. graminis*	*1DS*	Hartl *et al.* (1993)
Sr31	Reaction to *P. graminis*	*1BS*	Hartl *et al.* (1993)
Sr33	Reaction to *P. graminis*	*1DS*	Van Deynze *et al.* (1995)
Sr45[b]	Reaction to *P. graminis*	*1DS*	McIntosh *et al.* (1995)
5S-Rrna1	5S-ribosomal DNA	*1AS*, *1BS*, *1DS*, *1RS*	Dvorak *et al.* (1989)
Sls[b]	Small lateral spikelet	*1HS*	Jensen (1996)
Su-Pm8	Supressor of powdery mildew resistance	*1AS*	Ren *et al.* (1996)
Tin[b]	Tiller inhibitor	*1AS*	McIntosh *et al.* (1995)
Tri	Triticin protein	*1AS*	Van Deynze *et al.* (1995)
Tsc1	Resistance to chlorosis induction	*1AS*	McIntosh *et al.* (1995)
Vi[b]	Restorer of CMS: *T. longissimum* cytoplasm	*1BS*	Anderson and Maan (1995)
Yr4	Reaction to *P. striiformis*	*1HS*	Jensen (1996)
Yr9	Reaction to *P. striiformis*	*1BS*	Hartl *et al.* (1993)
Yr10	Reaction to *P. striiformis*	*1BS*	Metzger and Silbaugh (1970)
Yr15	Reaction to *P. striiformis*	*1BS*	Hartl *et al.* (1993)
Yr24[b]	Reaction to *P. striiformis*	*1BS*	McIntosh *et al.* (1995)

[a] Published Poaceae maps and Graingenes database were compared with each other and with the consensus physical map of wheat. All the genes that were flanked by two gene-rich region markers in one or more of the Triticeae maps were localized to 1S0.8 region.

[b] The gene is not bracketed with two 1S0.8 region markers but is tightly linked to one of the gene-rich region markers and the genetic distance between the markers and the genes is manyfold less than the total genetic length of the gene-rich region.

(*Xbcd1072* and *Xpsr161*) were present at the centromere of *1B* chromosome.

Genetic *vs.* physical maps: The genetic linkage map of chromosome *1B* in the Synthetic × Opata population and the consensus genetic linkage map of Triticeae homeologous group *1* (Van Deynze *et al.* 1995) were used for a comparison with the physical map. The consensus genetic linkage map of Triticeae homeologous group *1* was constructed using the mapping information from >13 different genetic linkage maps of wheat, *T. tauschii*, *T. monococcum*, barley, and oat. Of the markers present on the physical map, 30 were common with the consensus map and 21 with the genetic linkage map of *T. aestivum* (Figure 1). The consensus genetic linkage map for the Triticeae showed higher marker density around the centromere. The trend was similar for the genetic linkage maps of *T. aestivum*. The order of markers was fairly consistent between the consensus genetic linkage map and physical map, but there were a few inconsistencies between physical and genetic linkage maps of *T. aestivum*. Most of these inconsistencies were for the markers that were mapped at LOD < 3.0 (Figure 1, probes in parentheses). This means that accuracy in mapping these probes was lower compared to other probes. There are some markers for which there were two loci in the physical map and there was only one locus in the genetic linkage map of *T. aestivum*, which may have affected the accuracy of the genetic map. For example, probe CDO580 detected two loci (*Xcdo580a* and *Xcdo580b*) on the physical map, whereas it detected only one locus on genetic linkage maps. Two marker loci (*Xbcd98* and *Xcdo99*) mapped on the distal end on the physical map, whereas, on all genetic maps these loci are present in the proximal regions (Figure 1). We do not have any valid explanation for this inconsistency. Upon comparison with the physical map, it became apparent that the markers present in the 1S0.8 region were scattered within a 45-cM distance on the consensus genetic linkage map and within a 25-cM distance on the genetic linkage map for *T. aestivum*. The 1S0.8 region, which is only 6% of the total chromosome arm, showed ~82% of the arm's recombination. About 17% of recombination occurred in the 1S0.5 region, which is physically ~8% of the *1BS* arm. Less than 1% of the short arm's recombination occurred in the remaining 86% of the arm.

DISCUSSION

The grass family Poaceae includes major crop plants such as wheat, barley, oat, rice, and maize. Triticeae is one of the tribes containing >15 genera and 300 species including wheat and barley. Gene order and synteny are highly conserved among the species of Triticeae and moderately conserved among various tribes of the family (Feuillet and Keller 1999). There are now >2800 DNA markers present on one or more of the Triticeae genetic maps and the number is more than double for the Poaceae family (GrainGenes, http://wheat.pw.usda.gov/; Barley database, http://barleygenomics.wsu.edu/;

TABLE 2

Physical mapping results of RFLP markers selected for the 1S0.8 region using comparative mapping

Locus	cDNA (C)-genomic (G)	No. of fragments								Location
		*Eco*RI				*Hin*dIII				
		A	B	D	Others	A	B	D	Others	
Xabc151	C	0	1	1	3	0	1	1	3	Long arm
Xabc156	C	0	0	1	1	1	1	1	1	1S0.8 region
Xabg53	G	0	0	0	1	1	1	1	1	1S0.8 region
Xabg59	G	0	1	1	2	0	1	1	0	1S0.8 region
Xabg74	G	—	—	—	—	1	1	1	1	1S0.8 region
Xabg452	G	1	1	1	0	1	1	1	0	Long arm
Xabg494	G	1	1	1	0	1	1	1	0	1S0.5 region
Xabg500	G	0	0	0	1	1	1	1	1	1S0.8 region
Xbcd22	C	1	1	1	3	0	0	0	5	Long arm
Xbcd98	C	1	0	0	3	1	1	1	3	Distal to 1S0.8 region
Xbcd249	C	1	1	1	1	1	2	1	0	1S0.8 region
Xbcd340	C	0	1	0	5	0	1	0	5	Proximal to 1BS-19
Xbcd372	C	0	0	1	4	0	1	0	4	Long arm
Xbcd762a	C	3	1	1	4	2	2	2	2	1S0.5 region
Xbcd762b	C	0	1	0	0	0	2	0	0	Long arm
Xbcd921	C	1	2	1	0	1	1	1	1	Long arm
Xbcd1072	C	1	4	1	2	1	1	1	0	Centromere
Xbcd1124	C	0	1	1	0	1	1	1	0	Proximal to 1BS-19
Xbcd1340	C	1	1	1	0	1	1	1	1	1S0.8 region
Xbcd1434	C	1	1	1	0	—	—	—	—	1S0.8 region
Xbcd1796	C	1	0	1	7	1	1	1	1	1S0.5 region
Xcdo99	C	1	0	0	3	1	1	1	3	Distal to 1S0.8 region
Xcdo127a	C	2	1	0	3	0	1	1	3	1S0.5 region
Xcdo127b	C	0	1	0	0	0	1	0	0	Proximal to 1BS-19
Xcdo388a	C	0	1	1	11	0	0	0	16	1S0.8 region
Xcdo388b	C	0	1	0	0	0	0	0	0	Long arm
Xcdo442	C	2	1	1	1	1	1	1	1	1S0.8 region
Xcdo534	C	0	1	0	8	—	—	—	—	1S0.8 region
Xcdo580a	C	1	4	3	2	0	1	3	3	1S0.8 region
Xcdo580b	C	0	1	0	0	0	0	0	0	Distal to 1S0.8 region
Xcdo618	C	1	1	1	0	—	—	—	—	1S0.5 region
Xcdo658	C	1	1	1	0	1	1	1	0	Proximal to 1BS-20
Xcdo1173	C	1	1	1	0	—	—	—	—	1S0.5 region
Xcdo1188	C	1	3	1	1	1	1	1	0	1S0.5 region
Xcdo1340	C	1	1	2	0	1	1	1	0	Proximal to 1BS-19
Xcdo1423	C	1	1	1	1	2	1	3	1	1S0.8 region
Xcmwg645a	C	0	1	2	10	1	3	2	8	1S0.8 region
Xcmwg645b	C	0	2	0	0	0	1	0	0	Long arm
Xcmwg758	C	1	1	1	2	1	1	1	0	Long arm
XksuD14a	G	0	2	3	4	0	1	1	2	1S0.8 region
XksuD14b	G	0	2	1	0	0	1	1	0	1S0.8 region
XksuD14c	G	0	2	0	0	0	1	0	0	1S0.8 region
XksuE18	G	—	2	2	8	—	2	2	9	1S0.8 region
XksuE19	G	2	6	2	1	2	8	3	2	Long arm
XksuF43	G	1	1	1	2	—	—	—	—	1S0.8 region
XLrk10	G	1	2	3	3	1	3	3	1	1S0.8 region
Xmwg36	G	1	1	1	0	—	—	—	—	1S0.8 region
Xmwg60	G	2	2	1	1	1	3	2	1	1S0.8 region
Xmwg68	G	1	1	0	0	1	1	1	1	1S0.8 region
Xmwg539	G	1	1	1	11	0	0	0	4	Long arm
Xmwg584	G	1	1	1	7	2	1	2	7	Long arm
Xmwg835a	G	3	1	3	6	1	1	1	8	1S0.8 region
Xmwg835b	G	0	1	0	0	0	0	0	0	Long arm
Xmwg837a	G	1	2	5	4	2	3	3	4	1S0.8 region
Xmwg837b	G	0	0	0	0	0	1	0	0	Long arm

(*continued*)

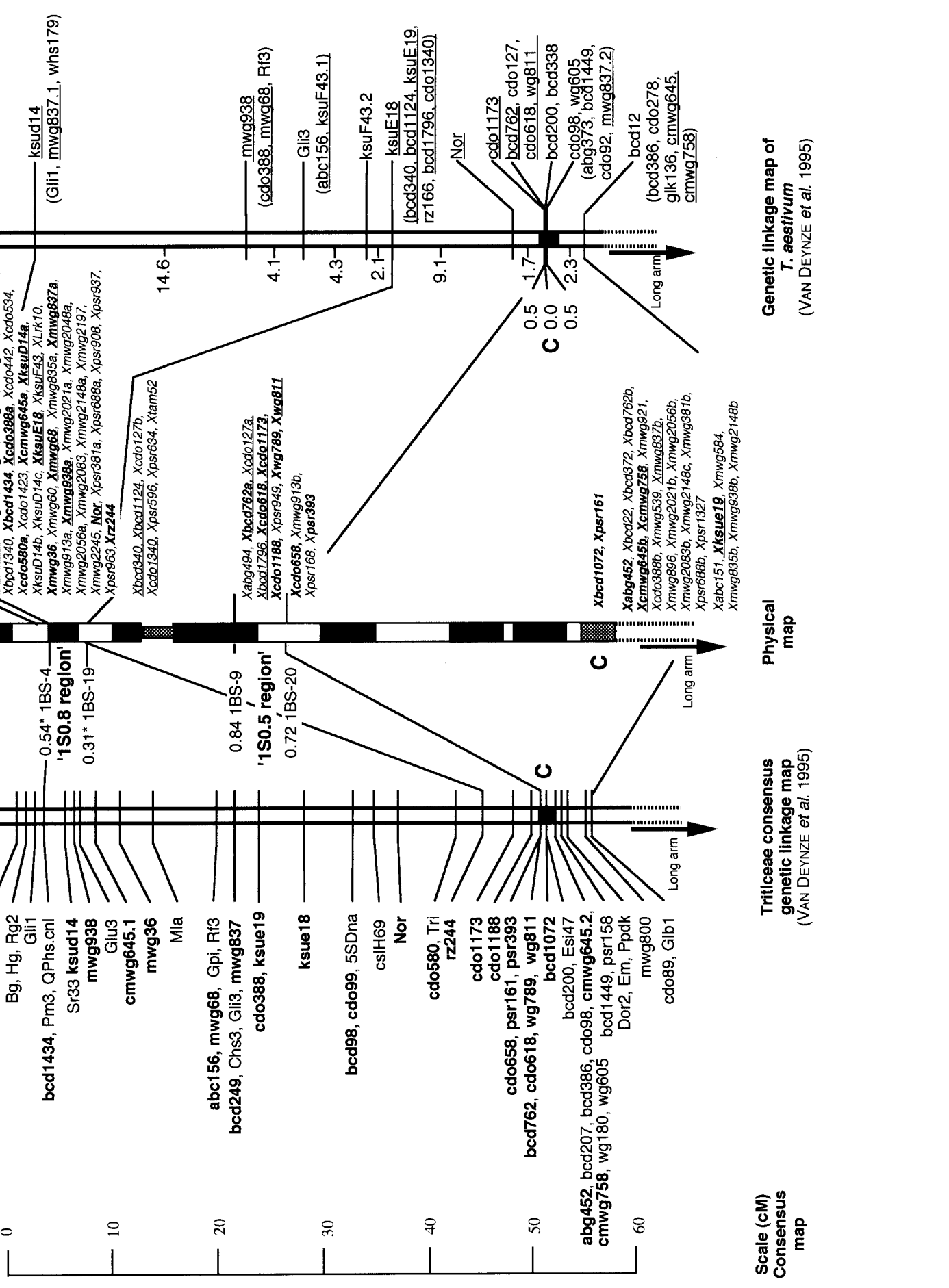
TABLE 2

(Continued)

Locus	cDNA (C)-genomic (G)	No. of fragments								Location
		*Eco*RI				*Hin*dIII				
		A	B	D	Others	A	B	D	Others	
Xmwg896	G	1	1	1	3	1	1	1	6	Long arm
Xmwg913a	G	0	0	0	8	0	1	0	5	1S0.8 region
Xmwg913b	G	0	0	0	0	0	1	0	0	Proximal to 1BS-20
Xmwg938a	G	4	2	1	1	4	2	0	1	1S0.8 region
Xmwg938b	G	0	1	0	0	0	1	0	0	Long arm
Xmwg2021a	G	2	1	1	0	2	1	1	5	1S0.8 region
Xmwg2021b	G	0	0	0	0	0	1	0	0	Long arm
Xmwg2048	G	2	1	2	2	0	2	1	3	1S0.8 region
Xmwg2056a	G	1	3	1	4	2	4	1	3	1S0.8 region
Xmwg2056b	G	0	1	0	0	0	1	0	0	Long arm
Xmwg2083a	G	1	1	1	7	2	2	1	12	1S0.8 reg
Xmwg2083b	G	0	0	0	0	0	1	0	0	Long arm
Xmwg2148a	G	2	1	2	11	2	2	1	10	1S0.8 region
Xmwg2148b	G	0	1	0	0	0	2	0	0	Long arm
Xmwg2148c	G	0	0	0	0	0	2	0	0	Long arm
Xmwg2197	G	1	1	0	0	1	1	0	0	1S0.8 region
Xmwg2245	G	2	2	1	1	2	3	1	2	1S0.8 region
Nor(*pTa71*)	C	0	1	0	8	—	—	—	—	1S0.8 region
Xpsr161	C	1	3	2	5	1	1	1	0	Centromere
Xpsr168	C	1	1	1	0	1	1	1	1	Proximal to 1BS-20
Xpsr381a	G	1	1	2	1	2	2	2	0	1S0.8 region
Xpsr381b	G	0	0	0	0	0	1	0	0	Long arm
Xpsr393	G	2	2	1	4	2	0	0	4	Proximal to 1BS-20
Xpsr596	G	1	1	1	1	1	1	1	0	Proximal to 1BS-19
Xpsr634	G	1	1	1	0	1	1	1	0	Proximal to 1BS-19
Xpsr688a	G	1	3	2	8	3	1	3	12	1S0.8 region
Xpsr688b	G	0	2	0	0	0	2	1	0	Long arm
Xpsr908	G	1	1	1	0	1	0	0	5	1S0.8 region
Xpsr937	G	2	0	1	1	2	1	1	1	1S0.8 region
Xpsr949	G	0	0	0	4	2	1	1	1	1S0.5 region
Xpsr963	G	0	1	1	0	0	1	1	2	1S0.8 region
Xpsr1327	G	2	1	0	3	2	0	0	1	Long arm
Xrz244	C	0	1	1	0	1	1	1	0	1S0.8 region
Xtam52	G	1	1	1	2	1	1	1	2	Proximal to 1BS-19
Xwg789	G	1	1	1	1	1	1	1	0	1S0.5 region
Xwg811	G	1	1	1	1	1	1	1	0	1S0.5 region

1S0.8 region is gene-rich region at fraction length 0.8. 1S0.5 region is gene-rich region at fraction length 0.5. Letters a, b, c, at the end of probe names represent probes detecting multiple loce. A dash represents missing data.

RiceGenes, http://ars-genome-cornell.edu/rice/; Oryzabase, http://shigen.nig.ac.jp/rice/oryzabase/; MaizeDB, http://nucleus.agron.missouri.edu/). Since a majority of the markers are RFLPs, many of which can be used across the family, comparative mapping can be a very powerful approach for targeted mapping and cross-referencing of any chromosomal region of interest. In this study, comparative mapping identified 195 markers for the target region (the 1S0.8 region), which is only about 1/1119th of the wheat genome. About 56% (41/73) of the putative gene-rich region probes, selected by comparative mapping, mapped in the target region. The reason for 44% of the probes mapping outside the target region is that during the comparative mapping we were inclusive rather than exclusive in the selection of markers since eliminating false positives was relatively easy. Further, a group of three markers is present just distal to the breakpoint of 1BS-4 and a group of seven markers is present just proximal to the breakpoint of the 1BS-19. As there is no reason for the deletion line to have a break exactly at the end of the gene-rich region, it is likely that these markers are part of the 1S0.8 region. Furthermore, genetic linkage maps were used for comparative mapping. On average, only ~18% of the wheat group *1* arm recombination occurs proximal to the 1S0.8 region. Therefore, many of the 1S0.5 region probes


Scale (cM) Consensus map
Triticeae consensus genetic linkage map (VAN DEYNZE et al. 1995)
Physical map
Genetic linkage map of T. aestivum (VAN DEYNZE et al. 1995)
Long arm
C
'1S0.8 region'
'1S0.5 region'
0.54* 1BS-4
0.31* 1BS-19
0.84 1BS-9
0.72 1BS-20
Lr, Lr21
Bg, Hg, Rg2
Gli1
bcd1434, Pm3, QPhs.cnl
Sr33 ksud14
mwg938
Glu3
cmwg645.1
mwg36
Mla
abc156, mwg68, Gpi, Rf3
bcd249, Chs3, Gli3, mwg837
cdo388, ksue19
ksue18
bcd98, cdo99, 5SDna
cslH69
Nor
cdo580, Tri
rz244
cdo1173
cdo1188
cdo658, psr161, psr393
bcd762, cdo618, wg789, wg811
bcd1072
bcd200, Esi47
abg452, bcd207, bcd386, cdo98, cmwg645.2, cmwg758, wg180, wg605
bcd1449, psr158
Dor2, Em, Ppdk
mwg800
cdo89, Glb1
Xbcd98, Xcdo99, Xcdo580b
Xabc156, Xabg53, Xabg59, Xabg74, Xabg500, Xbcd249,
Xbcd1340, Xbcd1434, Xcdo388a, Xcdo442, Xcdo534,
Xcdo580a, Xcdo1423, Xcmwg645a, XksuD14a,
XksuD14b, XksuD14c, XksuE18, XksuF43, XLrk10,
Xmwg36, Xmwg60, Xmwg68, Xmwg835a, Xmwg837a,
Xmwg913a, Xmwg938a, Xmwg2021a, Xmwg2048a,
Xmwg2056a, Xmwg2083, Xmwg2148a, Xmwg2197,
Xmwg2245, Nor, Xpsr381a, Xpsr688a, Xpsr908, Xpsr937,
Xpsr963, Xrz244
Xbcd340, Xbcd1124, Xcdo127b,
Xcdo1340, Xpsr596, Xpsr634, Xtam52
Xabg494, Xbcd762a, Xcdo127a,
Xbcd1796, Xcdo618, Xcdo1173,
Xcdo1188, Xpsr949, Xwg789, Xwg811
Xcdo658, Xmwg913b,
Xpsr168, Xpsr393
Xbcd1072, Xpsr161
Xabg452, Xbcd22, Xbcd372, Xbcd762b,
Xcmwg645b, Xcmwg758, Xmwg921,
Xcdo388b, Xmwg539, Xmwg837b,
Xmwg896, Xmwg2021b, Xmwg2056b,
Xmwg2083b, Xmwg2148c, Xmwg3811b,
Xpsr688b, Xpsr1327
Xabc151, Xksue19, Xmwg584,
Xmwg835b, Xmwg938b, Xmwg2148b
ksud14
(Gli1, mwg837.1, whs179)
mwg938
(cdo388, mwg68, Rf3)
Gli3
(abc156, ksuF43.1)
ksuF43.2
ksuE18
(bcd340, bcd1124, ksuE19,
rz166, bcd1796, cdo1340)
Nor
cdo1173
bcd762, cdo127,
cdo618, wg811
bcd200, bcd338
cdo98, wg605
(abg373, bcd1449,
cdo92, mwg837.2)
bcd12
(bcd386, cdo278,
glk136, cmwg645,
cmwg758)
14.6
4.1
4.3
2.1
9.1
1.7
2.3
0.5
0.0
0.5
0
10
20
30
40
50
60

and even some of the long arm probes appeared to be linked to the 1S0.8 region probes and, thus, were selected.

Recently, it has been demonstrated that genes in cereals are present in clusters encompassing physically small chromosomal regions (Gill *et al.* 1993, 1996a,b; Civardi *et al.* 1994; Descenzo *et al.* 1996; Wei *et al.* 1999). Gene-containing regions of wheat are the best defined and account for 5–10% of the genome (Gill *et al.* 1996a,b). In this study we have not only confirmed these observations but have also precisely marked the gene-containing regions and revealed the distribution of genes on the wheat homeologous chromosome group *1* short arm. The 1S0.8 region is physically ~6% of chromosome arm *1BS* but contains ~63% (41/65) of short arm markers. The 1S0.5 region is ~8% of the chromosome arm and contains 15% (10/65) of the markers. Marker density in the 1S0.8 region is more than five times as compared to the 1S0.5 region. Most of the group *1* short arm specific markers and genes are present in the two gene-rich regions, which physically encompass only ~14% of the arm. The gene-containing regions of barley, maize, and rice were estimated to be 12%, 17%, and 24% of their total genome size, respectively (Carels *et al.* 1995; Barakat *et al.* 1997). All the recent studies on gene cloning and structural genome analyses support these observations (Rogowsky *et al.* 1993; Dunford *et al.* 1995; Kilian *et al.* 1997; Wei *et al.* 1999).

Distribution of markers in this study most likely depicts distribution of wheat genes. Thirty-two of the 73 group *1* probes are cDNA and 38 of the 41 genomic clones were generated using *Pst*I, which is known to cut preferentially in the gene-containing regions (Burr *et al.* 1988). Distribution of cDNA and genomic clones was similar along the chromosome length. Highly conserved genes and multicopy gene families may not be proportionally represented in this study. Sequences representing high copy number gene families are less likely to be included in our study as they will be eliminated as "bad probes" during the initial search for probes that can be mapped. Twelve of the 73 markers, however, detected >15 bands, suggesting that these represent multigene families. The distribution of these markers was similar to that of the single/few copy probes. Most of the markers in this study were selected from genetic linkage maps and thus have been selected for their ability to detect polymorphism. Centromeric regions in most organisms are recombination poor (Gill *et al.* 1996b; Puechberty *et al.* 1999). The genes present around the centromeric region are, therefore, more likely to be conserved and are less likely to be represented in our study.

Organization of genes in clusters encompassing physically small chromosomal regions seems to be true for all wheat chromosomes and perhaps for the whole Poaceae family. High-density mapping revealed that the organization of genes for group *5* is similar to that of group *1* (Gill *et al.* 1996a; Faris *et al.* 2000). It was shown that markers on chromosome *5L* were present in five gene-rich regions, of which three were major regions and two were minor. The region between FL 0.75 and 0.79 was 4% of *5L* arm, but contained 55% (77/139) of the markers. The estimated size of the region is ~20 Mb, which makes at least one marker every 260 kb (Gill *et al.* 1996a; Faris *et al.* 2000). Distribution of genes in barley showed a striking similarity to that of wheat (Kunzel *et al.* 2000). In maize 70% of the genome consists of repetitive DNA, which is made up of transposons (Hake and Walbot 1980). Detailed studies on genome organization revealed that maize genes are present in clusters and are interspersed by long stretches of repeat units of retrotransposons (Bennetzen *et al.* 1998). The *Sh2/A1* region of maize is also conserved in sorghum and rice but lacks numerous retrotransposons present in maize (Bennetzen *et al.* 1998). By molecular clock criteria sorghum and rice have undergone ~50 million years of independent evolution, but still the colinearity of gene-rich regions has been maintained (Bennetzen *et al.* 1998). Arabidopsis lacks abundant transposons and repeat DNA in intergenic regions, but distribution of genes in Arabidopsis still is not completely uniform (Schmidt *et al.* 1995).

Uneven distribution of recombination along chromosome length appears to a be the rule in all organisms (Dvorak and Chen 1984; Ganal *et al.* 1989; Gill *et al.* 1993, 1996a,b; Choudhuri and Messing 1995; Umehara *et al.* 1995; Cai *et al.* 1997; Tranquilli *et al.* 1999; Faris *et al.* 2000; Kunzel *et al.* 2000; Spielmeyer *et al.* 2000). In this study, 99% of the recombination occurred in the distal 60% of the arm. Recombination in the distal 25% of the arm was five times higher as compared to the rest of the arm. Recombination near the centromere was negligible. Because of the nonrandom distribution of recombination along the chromosomal length, base pair per centimorgan estimates would differ among regions. The predicted estimate for 1-cM genetic distance in rice is 273 kb. However, comparisons of

Figure 1.—Physical map of short arm of chromosome *1B* of wheat in comparison with consensus genetic linkage map of Triticeae (Van Deynze *et al.* 1995) and genetic linkage map of *T. aestivum* (Van Deynze *et al.* 1995). Markers common between physical map and consensus genetic linkage map of Triticeae are represented in boldface type and markers common between physical map and genetic linkage map of wheat are underlined. Some common markers are joined by lines. Fraction breakpoints and the corresponding line numbers are indicated to the left of each chromosome. Asterisk represents fraction length for satellite. C, centromere.

actual physical distances with the genetic distances reveal that this estimate may vary from 120 kb to 1 Mb (Umehara *et al.* 1995). Cytogenetic and genetic linkage maps of *Drosophila melanogaster* revealed widespread variation in the rates of recombination among different chromosomal regions (Lindsley and Sandler 1977). In wheat it has been shown that the distal one-third of any arm shows 8–15 times the recombination compared to the proximal one-third (Hohmann *et al.* 1995; Gill *et al.* 1996a,b). Heterochromatic regions of the genome are devoid of recombination. Centromeric regions were highly suppressed in recombination in many organisms (Tanksley *et al.* 1992; McKee and Handel 1993; Laurent *et al.* 1998; Puechberty *et al.* 1999).

As reported previously, a strong correlation was also observed between the distribution of genes and recombination (Gill *et al.* 1996a,b; Kunzel *et al.* 2000). Of the total short arm recombination, 82% occurred in the 1S0.8 region and 17% in the 1S0.5 region. Almost all of the short arm recombination occurred in 14% of the short arm corresponding to the two gene-rich regions and no recombination was observed in the remaining 86%. A high correlation of recombination with genes or gene-rich regions has been observed in a wide range of organisms. The base pair per centimorgan estimate for the chromosomal region around the cyst nematode-resistant gene in sugar beet was ~30 kb/cM compared to 677 kb/cM for the whole genome (Cai *et al.* 1997). Recombination in the *A1* locus (12–25 kb/cM) and *Bronze 1* locus (14 kb/cM) was comparable and was two orders of magnitude higher than the estimation from the overall maize genome (Brown and Sundaresan 1991).

In barley, the base pair per centimorgan estimates for the chromosomal regions around the *Mlo* and *Rar1* loci (both resistant genes in different chromosomal regions) were ~50 kb/cM (Buschges *et al.* 1997; Lahaye *et al.* 1998). Hot spots of recombination correspond to ~4.9% of the total barley genome and contain 47.3% of the total number of mapped markers (Kunzel *et al.* 2000). Based on the genome size and RFLP linkage map length, the predicted size of tomato chromosomal region per centimorgan is 550 kb (Ganal *et al.* 1989). However, estimates for regions around various disease resistance genes range from 43 to 90 kb (Ganal *et al.* 1989; Segal *et al.* 1992; Zhang *et al.* 1994). All these genes showing a higher level of recombination probably are part of some gene-rich regions. The bp/cM estimates from high-density composite maps of wheat homeologous groups *1* and *5* range from 118 kb for the gene-rich regions to 22 Mb for the gene-poor regions (Gill *et al.* 1996a,b). In this study, for the 1S0.8 region, the base pair per centimorgan estimate was 365 kb in comparison to the whole *1BS* arm, where it was 5 Mb/cM. The base pair per centimorgan estimates for the regions around genes are comparable among various crop plants with the average of 100 kb/cM. However, the upper limit for such estimates depends upon the genome size. Therefore, the sizes of gene-poor compartments of the genome will determine the upper limit for base pair per centimorgan estimates. The rice genome is 35 times smaller than that of wheat and the upper limit in rice is 1 Mb/cM compared to 22 Mb/cM in wheat. The total length of linkage maps is fairly constant among eukaryotes and the average frequency of crossing over per unit of DNA decreases by several levels of magnitude from lower to higher eukaryotes (Thuriaux 1977). These relationships have profound evolutionary implications. Intragenic recombination can result in the creation of chimeric alleles (Choudhuri and Messing 1995). Thus the evolutionary advantage of creating genetic diversity in a population may have fostered a mechanism that preferentially selected genes as sites of recombination.

The haploid wheat chromosome complement is 235 μm in length (Gill *et al.* 1991a), containing 16 million kb of DNA (Bennett and Smith 1976). The 1S0.8 region is flanked by the breakpoints of deletions 1BS-4 and 1BS-19 and is present in the middle of the chromosome *1BS* satellite. The *1BS* satellite is ~1 μm in length, comprising about 68 Mb of DNA (calculated by dividing 16 million kb, the genome size, by the total chromosome size of 235 μm). The gene-rich region is ~23% of the satellite (between FL 0.31 and 0.54 of the satellite; Endo and Gill 1996). The gene-rich region should, therefore, be ~15 Mb in size. In this study, we have identified 41 markers for this region with an average distribution of a marker every 365 kb. The region syntenic to 1S0.8 region in barley is ~20 cM (GrainGenes). The 1-cM region spanning the barley *Mla* cluster centered between markers bcd249.1 and mwg036 is ~1 Mb (Wei *et al.* 1999). If the kilobase/centimorgan ratio is uniform within the region, the total size of the region will be ~20 Mb. However, physical to genetic distance within the *Mla* region may vary over 10-fold, with 176 kb/cM being the most favorable ratio. These observations indicate that the gene and recombination distributions within the gene-rich region are not uniform. Recombination seems to occur only in the gene-containing regions; however, accuracy of the observation within the gene-rich regions has not been tested yet. If the relationship between the distribution of genes and that of recombination holds within the gene-rich region also, only a part of the 1S0.8 region should contain genes. The 1S0.8 region markers most likely mark only the gene-containing parts of the region. The current marker density should therefore be sufficient to construct a contiguous map of the gene-containing regions of the gene-rich region.

In conclusion, the comparative mapping-based enrichment of a gene-rich region with markers is a powerful technique. Comparative mapping combined with targeted physical mapping strategy physically localized ~75 useful genes to the 1S0.8 region along with 41

markers, which should be adequate to construct a contiguous map for the region and eventually allow cloning of these genes. Most wheat genes are present in clusters. Many of these gene-rich regions have been bracketed by the breakpoints of single-break deletion lines, which are available for all wheat chromosomes. Therefore, the approach outlined in this study can be used to target any gene-rich region in the wheat genome.

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