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Use of recombinant substitution lines in the construction of RFLP-based genetic maps of chromosomes 6A and 6B of tetraploid wheat (*Triticum turgidum* L.)

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Abstract RFLP-based genetic maps of chromosomes 6A and 6B of *Triticum turgidum* have been constructed using data obtained by the study of *Triticum turgidum* var '*durum*' cv 'Langdon'-*T. t.* var '*dicoccoides*' recombinant substitution lines (RSLs) supplemented with data obtained from F_3 families derived from 'Langdon'-*dicoccoides* 6A and 6B disomic substitution lines. The average RFLP frequencies detected for the two chromosomes in a test of 45 DNA clones with six restriction enzymes were 56% and 53%, respectively, and a subset of 32 clones gave frequencies of 75% and 72%, respectively. Seventeen loci were mapped in 6A and 18 in 6B. With the possible exception of 5 loci in the centromeric region of 6A, all of the mapped 6A and 6B loci are located in the same arm as are homologous loci in hexaploid wheat, and the linear order of the loci is the same in the two chromosomes, except possibly close to the centromere. Major differences in genetic distances exist between homologous loci located in the proximal regions of the 6AL and 6BL linkage groups, however, the distances being much larger in the former than in the latter. The 6B maps that were constructed using data from both the RSL and the F_2 populations and using data from the RSL population alone closely resemble one another, indicating that the 6B RSL population, composed of 85 lines, can be reliably used for genetic mapping. Additional studies must be conducted before the utility of the 6A RSL population, composed of 66 lines, can be adequately assessed.

Key words RFLP · Genetic mapping · *Triticum turgidum* Recombinant substitution lines (RSLs) · Mapmaker G-Mendel

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Introduction

Due to the low level of genetic variation that exists among wheat varieties, the analysis of populations derived from wide crosses has been the method of choice for developing molecular-marker maps of wheat (Chao et al. 1989; Liu and Tsunewaki 1991, Devos et al. 1992). F_2 plants or, more commonly, F_3 families have been studied in most of the investigations reported to date. A major advantage of the study of such populations is the ease with which they can be constructed. There are advantages as well, however, to developing genetic maps in populations in which segregation of alleles is not occurring, such as populations of recombinant inbred lines (RILs) and recombinant substitution lines (RSLs). The utility of RILs for genetic mapping is well established (Bailey 1981; Burr et al. 1988; Burr and Burr 1991). The development and use of RSLs is a recent innovation.

Populations of RSLs containing recombined *Triticum turgidum* var 'durum' cv 'Langdon' and *T.t.* var '*dicoccoides*' chromosomes have been produced or are being developed in cv 'Langdon' for all of the chromosomes of *T. turgidum* ($2n$=28, genomes A and B) (Joppa 1993). The development of each RSL population was initiated by crossing the appropriate 'Langdon'-*dicoccoides* disomic chromosome substitution line with 'Langdon'. The F_1 plants that were produced by this series of crosses, each of which was heterozygous for the *dicoccoides* chromosome that had been present in the substitution line and the 'Langdon' homologue that it replaced, were then crossed with a 'Langdon'-*dicoccoides* substitution line containing the D-genome homoeologue of the recombined A- or B-genome chromosome. The progeny that resulted from this cross were monosomic for both the recombined chromosome and the D-genome homoeologue. These plants were

allowed to self-fertilize and from among their progeny plants were selected that were disomic for the recombined 'Langdon'-*dicoccoides* chromosome. Different recombination events produced the recombined chromosomes present in the different plants of this generation, so the progeny of each such plant constitutes a different RSL.

RSLs and RILs differ in that the former contain only one recombined chromosome, which is fully homozygous as of the time that the line is produced, while all of the chromosomes in the latter are recombined and homozygosity is established gradually by selfing for several generations. The two types of lines are alike, however, in that they are homozygous and therefore permanent. Consequently, with both types of lines, the map position of a newly-studied marker can be determined relative to all previously-mapped markers merely by adding the genetic data obtained for the marker to the data previously available, whether or not the data were obtained by the same or different investigators and in the same or different years. Also, being homozygous, large numbers of plants with identical genotypes can be studied in experiments replicated in time and space, thereby providing a high level of resolution in the study of quantitative trait loci (QTLs).

This paper reports the development of restriction fragment length polymorphism (RFLP) maps of chromosomes 6A and 6B of *T. turgidum* using data obtained from the study of 'Langdon'-*dicoccoides* 6A and 6B RSLs and from F_3 families derived by crossing the 'Langdon'-*dicoccoides* 6A and 6B substitution lines with 'Langdon' followed by selfing. Genetic maps of chromosomes 6A and 6B of tetraploid wheat have not been reported previously. RFLP maps of the homoeologous group 6 chromosomes of hexaploid wheat were developed by Liu and Tsunewaki (1991).

Materials and methods

Plant material

The 66 available *T. turgidum* var *'durum'* cv 'Langdon'-*T.t.* var *'dicoccoides'* chromosome 6A recombinant substitution lines and the 85 available 'Langdon'-*dicoccoides* 6B RSLs (Joppa 1993), the 'Langdon'-*dicoccoides* 6A and 6B substitution lines (Joppa and Cantrell 1990), and cv 'Langdon' were obtained from Dr. L. R. Joppa.

F_3 families segregating for 'Langdon'-*dicoccoides* 6A and 6B alleles were produced locally by crossing the substitution lines to 'Langdon' and then allowing the F_1 and F_2 progeny of the crosses to self-fertilize. Fifty 6A F_3 families and 31 6B F_3 families were studied. The available homoeologous group 6 ditelosomic (Dt) lines of *T. aestivum* cv 'Chinese Spring' (CS) were used to determine the chromosomal arm locations of some DNA fragments. These lines are maintained at Texas A&M University.

DNA clones

Clones known to hybridize to DNA fragments located in the homoeologous group 6 chromosomes of *T. aestivum* or in chromosome 6D of *T. tauschii* were used. Included among the clones were 16 TAM (=Texas A & M University) wheat genomic DNA (gDNA) clones (Devey and Hart 1993), 8 KSU (=Kansas State University) *T. tauschii* gDNA clones obtained from Dr. B. S. Gill (Gill et al. 1991), and 5 WG (=wheat gDNA), 6 BCD (=barley cDNA), and 2 CDO (=oat cDNA) clones obtained from Dr. M. E. Sorrells (Anderson et al. 1992). Also used were 8 gene clones, the names and sources of which are shown in Table 1.

Genomic DNA preparation and Southern hybridization

Genomic DNA preparation, digestion, and Southern hybridization were performed as described by Devey and Hart (1993). In brief, genomic DNA was extracted from freeze-dried tissue, digested with 1 unit of restriction enzyme per microgram of DNA, electrophoresed in 0.8% agarose gels, and transferred to Zeta-probe or Zeta-bind membrane using 0.4 *N* NaOH. Prehybridization and hybridization were performed at 65°C using 5×SSPE, 5×Denhardt's solution, 0.5% SDS, and 200 μg/ml of herring sperm DNA. DNA probes were labeled with ^{32}P-dATP using random primers, and unincorporated nucleotides were removed with Sephadex G-50 spin columns. After hybridization, membranes were washed two times at room temperature using 5×SSC and 0.5% SDS followed by one or two times at 65°C using 0.1×SSC and 0.1% SDS. The membranes were exposed to Kodak X-ray film with intensifying screens at – 80°C for 3–5 days.

Analysis of data

Segregation analyses, tests of the significance of linkage, and estimations of recombination frequencies between linked loci were performed with the data obtained from each of the four mapping populations using Mapmaker Macintosh 2.0 (Lander et al. 1987) obtained from S. V. Tingey of the Dupont Company. The Kosambi mapping function (Kosambi 1944) was used to calculate map distances in centiMorgans (cMs). Two-point analysis with LOD=3.0 and theta=0.30 was used to establish linkage groups. The best order of the loci in the linkage groups was then determined by three-point analysis, accepting only orders preferred over all others with LOD=3.0 and theta=0.40. Other loci were then mapped to the degree possible by multipoint analysis. RSL and F_2 data were combined and analyzed to construct one map for each chromosome using the computer program G-Mendel 2.0 (Liu and Knapp 1992) and a recombination frequency (R) of ≤0.25 and *P* value of ≤0.0001, except other R and *P* values were used for some loci located distally in 6AL and 6BL (see results).

Table 1 'Known-function' gene clones that were used in this study

Gene function and symbol	Clone	Reference
Wheat carboxypeptidase, *Xpsr8(Cxp3)*	2437	Baulcombe et al. 1987
Lophopyrum elongatum salt-stress-induced mRNA, *XEsi18*	ESI18	Patrick and Dvorak 1990
Wheat phosphoribulokinase, *Xpsr463(Prk)*	R6.1	Raines et al. 1989
Barley dehydrin (not used for mapping)	B17	Close et al. 1989
Barley dehydrin, *XDhn5*	HV5	Close et al. 1989
Wheat protein synthesis initiation factor, *Xuta1(Psif)1* and *2*	p26	Metz et al. 1992
Wheat DNA binding protein, *Xrsq805(Embp)*	pGC19	Guiltinan et al. 1990
Wheat gliadin multigene locus, *Xpsr10(Gli-2)*	pTag53	Bartels and Thompson 1983

Table 2 Chromosomal arm locations of DNA fragments and polymorphism detected with 45 probes (*nd* not determined)

Clone[a]	Type of clone	Arm locations of fragments in hexaploid wheat[b]	RFLP detected in Langdon-*dicoccoides* substitution lines[c]	
			6A	6B
pTaTAM3	gDNA	nd[d]	–	–
pTaTAM6	gDNA	6AS, 6BS, 6DS	+	+
pTaTAM9	gDNA	6AL, 6BL, 6DL	+	–
pTaTAM10	gDNA	6AS, 6BS, 6DS	+	+
pTaTAM17	gDNA	6AL, 6BL, 6DL	+	–
pTaTAM21	cDNA	6AL, 6BL, 6DL	–	+
pTaTAM25	gDNA	6AL, 6BL, 6DL	+	+
pTaTAM26	gDNA	6AL, 6BL, 6DL	+	+
pTaTAM27	cDNA	6AL, 6BL, 6DL	+	–
pTaTAM28	gDNA	6BL, 6DL	+	+
pTaTAM31	gDNA	6AS, 6BS, 6DS	–	+
pTaTAM36	cDNA	6AL, 6BL, 6DL	+	+
pTaTAM57	gDNA	6AS, 6BS, 6DL	+	+
pTaTAM60	gDNA	6AS, 6BS	+	+
pTaTAM68	gDNA	5A, 6DL	+	+
pTaTAM74	gDNA	6AL, 6BL, 6DL	+	+
pTtKSUB6	gDNA	nd	–	–
pTtKSUE14	gDNA	nd	–	+
pTtKSUF19	gDNA	nd	–	+
pTtKSUF37	gDNA	nd	+	+
pTtKSUG8	gDNA	6AS, 6BS, 6DS	+	+
pTtKSUG48	gDNA	6AS, 6BS, 6DS	+	+
pTtKSUH11	gDNA	nd	+	–
pTtKSUI28	gDNA	nd	–	–
BCD1	cDNA	6AL, 6BL, 6DL	–	+
BCD102	cDNA	6AL, 6BL, 6DL	–	–
BCD276	cDNA	6AL, 6BL, 6DL	–	–
BCD340	cDNA	6AL, 6BL, 6DL	–	–
BCD342	cDNA	6AS, 6BS, 6DS	–	–
BCD758	cDNA	6AL, 6BL, 6DL	–	–
CDO497	cDNA	6AL, 6BL, 6DL	–	–
CDO534	cDNA	6AS, 6BS, 6DS	–	–
WG286	gDNA	6AL, 6BL, 6DL	–	–
WG341	gDNA	6AL, 6BL, 6DL	–	–
WG405	gDNA	6AL, 6BL, 6DL	–	–
WG522	gDNA	6AL, 6BL, 6DL	–	–
WG933	gDNA	6AL, 6BL, 6DL	+	–
2437	cDNA	6AS, 6BS, 6DS	+	+
B17	cDNA	nd	+	+
ES18	cDNA	6AL, 6BL, 6DL	+	+
F-6-1	cDNA	6AL, 6BL, 6DL	+	–
HV5	gDNA	6AL, 6BL, 6DL	+	+
p26	cDNA	nd	–	+ (two loci)
pGC19	gDNA	6AL, 6BS	+	–
pTag53	cDNA	6AS, 6BS, 6DS	+	+

[a] The nomenclature for TAM DNA clones is that adopted at the 7th Int Wheat Genet Symp (Hart and Gale 1988). KSU clones were obtained from Dr. B. S. Gill (Gill et al. 1991) and BCD, CDO, andWG clones were obtained from Dr. M. E. Sorrells (Anderson et al. 1992). See Table 1 for sources of other clones

[b] Determined using ditelosomic lines; see Results and Discussion

Results

Chromosomal locations of RFLP loci in hexaploid wheat

The chromosome and chromosome arm locations in *Triticum aestivum* (2*n*=42, genomes A, B and D) of the DNA fragments to which most of the clones used in this study hybridize were determined by study of aneuploid lines of CS. The locations are shown in Table 2 and were originally reported in the references cited in Table 1 and in the Materials and methods section. The CS chromosome arm locations of the fragments to which clones HV5, ES18, KSUG8, and KSUG48 hybridize were determined in this study using Dt strains as described by Devey and Hart (1993).

RFLPs detected between 'Langdon' and 'Langdon'-*dicoccoides* substitution lines

'Langdon'-*dicoccoides* chromosome 6A and 6B RFLPs were identified by hybridizing genomic DNA from

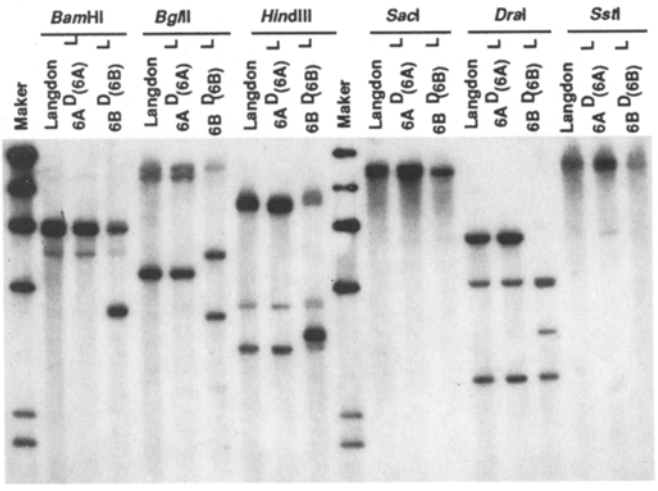

Fig. 1 Autoradiogram obtained by probing genomic DNAs of 'Langdon' and 'Langdon'-*dicoccoides* 6A and 6B substitution lines (L-6A^{dic} and L-6B^{dic}, respectively) with probe p26 using six restriction enzymes. Polymorphic DNA fragments were revealed in 6B with restriction enzymes *Bam*HI, *Bgl*II, and *Dra*I. Molecular weight markers (*Hin*dIII-digested lambda DNA) are present in the *first* and *middle* lanes

'Langdon' and its 6A and 6B substitution lines with each probe. An autoradiogram showing the polymorphic bands detected by probe p26 is shown in Fig. 1. As seen in the figure, a RFLP was detected in 6B but not 6A. Of the 32 TAM, KSU, and 'known-function' probes that were studied, 18 detected a polymorphism in both chromosomes 6A and 6B, 6 detected a polymorphism only in chromosome 6A, and 5 only in 6B (Table 2). Of the 32 probes, 3 failed to identify a 'Langdon'-*dicoccoides* RFLP using seven restriction enzymes. The probes detected single- and low-copy-number sequences, except probe B17, a barley dehydrin cDNA clone, which detected multiple-copy-number sequences.

The average RFLP frequency detected per clone with seven restriction enzymes by the 32 TAM, KSU and 'known-function' clones was 41.7% for 6A and 40.3% for 6B (Table 3). For 6A, the range for different enzymes was from 35.7% to 50.0%, and for 6B it was from 34.4% to 46.9%. The RFLP level detected by anonymous gDNA probes and 'known-function' gene probes was higher than that by anonymous cDNA probes (Table 4), but this finding must be interpreted with caution since only 3 of the 32 clones were anonymous cDNA probes.

Table 3 RFLP frequencies detected between 'Langdon' and *T. dicoccoides* chromosomes 6A and 6B with 32 TAM, KSU, and 'known-function' probes and seven restriction enzymes

Restriction enzymes	Chromosome 6A					Chromosome 6B					6A and 6B
	Number of probes tested	A[a]	B	C	D	Number of probes tested	A	B	C	D	B
*Bam*HI	32	16	50.0	16	50.0	32	11	34.4	11	34.4	42.2
*Hin*dIII	32	14	43.8	22	68.8	32	12	37.5	17	53.1	40.7
*Sst*I	32	13	40.6	24	75.0	32	15	46.9	21	65.6	43.8
*Sac*I	32	13	40.6	24	75.0	32	14	43.8	21	65.6	42.2
*Dra*I	23	10	43.4	24	75.0	23	10	43.5	23	71.9	43.5
*Bgl*II	32	12	37.5	24	75.0	32	13	40.6	23	71.9	39.1
*Eco*RV	14	5	35.7	24	75.0	14	5	35.7	23	71.9	35.7
Average			41.7					40.3			41.0

[a] A, number of probes that detected a RFLP with the restriction enzyme listed; B, percentage of probes that detected a RFLP with the restriction enzyme listed; C, cumulative number of probes that detected a RFLP; D, cumulative percentage of probes that detected a RFLP

Table 4 Comparison of RFLP frequencies detected between 'Langdon' and *T. t.* var '*dicoccoides*' chromosomes 6A and 6B with anonymous cDNA and gDNA clones and with 'known-function' gene clones with six restriction enzymes

Type of probe	Chromosome 6A			Chromosome 6B			Average RFLP frequency
	Number of probes tested	A[a]	B	Number of probes tested	A	B	
TAM and KSU gDNA	21	15	71.4	21	15	71.4	71.4
TAM and KSU cDNA	3	2	67.7	3	2	67.7	67.7
'Known-function'	8	7	87.5	8	6	75.0	81.3
Subtotal	32	24	75.0	32	23	71.9	73.5
Cornell gDNA	5	1	20.0	5	0	0.0	10.0
Cornell cDNA	8	0	0.0	8	1	12.5	6.3
Subtotal	13	1	7.7	13	1	7.7	7.7
Total	45	25	55.6	45	24	53.3	54.5

[a] A, Number of probes that detected a RFLP; B, percentage of probes that detected a RFLP

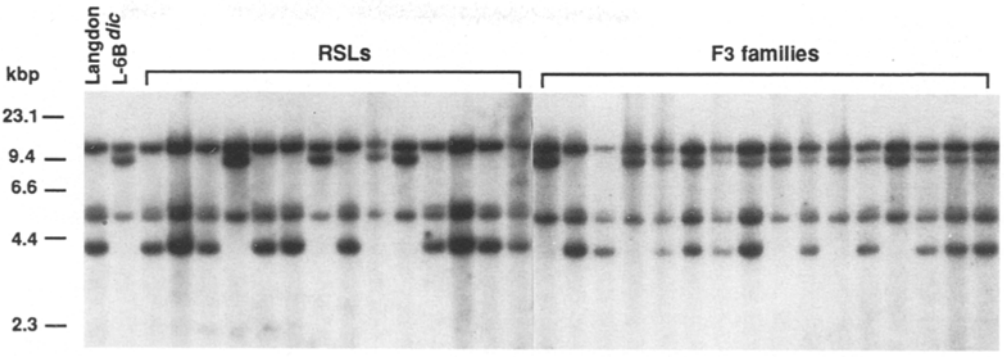

Fig. 2 Autoradiogram obtained by probing genomic DNAs of the two parental lines ('Langdon' and the 'Langdon'-*dicoccoides* 6B substitution line), the 6B RSLs and the F_3 families with probe TAM36. Data obtained from 14 RSLs and 16 F_3 families are shown. Each RSL displays one of the polymorphic bands seen in the two parents, whereas each F_3 family displays one or both of the polymorphic bands, depending upon whether there is homozygosity or heterozygous at the locus

Table 5 Polymorphic DNA fragments analyzed in RSLs and F_2 populations

Locus	Polymorphic fragments scored (in kb)			Polymorphic fragments scored (in kb)		
	RE [a]	L [b]	6A [c]	RE	L	6B [d]
Xtam6	*Hin*dIII	9.3	4.6, 2.8	*Hin*dIII	3.3, 2.4	4.8, 2.5
Xtam9	*Bam*HI	2.0	1.8			
Xtam10	*Hin*dIII	9.4	3.0	*Hin*dIII	2.5	2.7
Xtam21				*Bgl*II	7.2	6.4
Xtam25	*Sac*I	5.1	5.4	*Bgl*II	6.3	6.6
Xtam26	*Bam*HI	2.8	5.8	*Sac*I	3.5	4.2
Xtam27	*Sac*I	11.0	7.6			
Xtam28	*Hin*dIII		10.8	*Sst*I	9.1	7.5
Xtam31	*Eco*RV			*Eco*RV	7.5	8.5
Xtam36	*Dra*I	4.6, 2.5	4.3	*Hin*dIII	4.9, 3.5	9.2
Xtam57	*Sac*I	7.0		*Dra*I	16.7	15.3
Xtam60	*Hin*dIII	2.6		*Sac*I		6.9, 5.5
Xtam68	*Sac*I	4.6		*Eco*RV	6.9	
Xtam74	*Hin*dIII	19.5	6.2			
Xbcd1				*Bam*HI	11.6, 8.2	6.8
XksuE14				*Dra*I	5.7, 5.3	4.7, 4.5
XksuG8				*Sac*I	4.9, 4.7	5.2
XksuG48	*Sac*I		7.5	*Dra*I	4.5	3.0
XksuH11	*Sac*I	6.0	5.5			
Xpsr8(Cxp3)	*Hin*dIII	7.0	9.8, 3.3	*Dra*I	9.5, 6.0	11.5, 4.4
XDhn5	*Bam*HI	4.4	4.0	*Sst*I	9.6	25.0, 6.0
Xrsq805(Embp)	*Hin*dIII	8.0	6.5			
Xpsr10(Gli-2)	*Sac*I	5.0, 4.7, 2.0	6.0			
Xpsr463(Prk)	*Bam*HI	13.5				
Xuta1(Psif)1				*Bgl*II	10.7, 4.5	
Xuta1(Psif)2				*Bgl*II		4.8, 3.5
XEsi18				*Dra*I		7.5

[a] Restriction enzyme
[b] Polymorphic fragments (in kb) from 'Langdon' parent
[c] Polymorphic fragments (in kb) from 'Langdon'-*dicoccoides* 6A substitution line
[d] Polymorphic fragments (in kb) from 'Langdon'-*dicoccoides* 6B substitution line

Markedly different results were obtained with the BCD, CDO and WG clones. Genomic DNAs digested with six restriction enzymes were probed with the 13 clones and only two RFLPs were identified, one in 6A detected by WG933 and one in 6B detected by BCD1 (Table 2), giving a RFLP frequency of 7.7%. As shown in Tables 3 and 4, the average RFLP frequency detected by the 32 TAM, KSU and 'known-function' clones with five to seven restriction enzymes was 73.5%. The overall RFLP frequency with six restriction enzymes for the 45 clones studied was 54.5% (Table 4).

Segregation data

Probes that detected polymorphic fragments between 'Langdon' and 'Langdon'-*dicoccoides* substitution lines were hybridized to DNAs isolated from the RSLs and F_3 families. Data obtained with probe TAM36 in the study of 6B RSLs and F_3 families is shown in Fig. 2. As expected, each F_3 family displays either one or both of the polymorphic 6B fragments, whereas each RSL displays only one of the polymorphic fragments. This is consistent with the alleles at the locus detected, *Xtam36-6B*, being codomi-

nant, and with each F_2 plant being either heterozygous or homozygous at the locus and with all of the RSLs being homozygous.

Polymorphic DNA fragments scored in the RSL and F_2 populations are listed in Table 5. Of 39 loci studied (19 in 6A and 20 in 6B), 28 had codominant alleles [i.e., polymorphic DNA fragment(s) were present in both of the parents] and 11 had dominant alleles [i.e., polymorphic fragment(s) were present in only one parent]. 'Langdon' 6A and 6B had 4 and 2 dominant alleles, respectively, and *T. dicoccoides* 6A and 6B had 2 and 3 dominant alleles, respectively. Most of the polymorphic DNA fragments were from 4 to 9 kb in size, and the range in sizes was from 1.8 kb to 25.0 kb.

Anomalous findings were obtained with 3 probes, namely, with TAM26, TAM31, and BCD1. TAM26 detected four polymorphic fragments in the 6B RSLs. Two fragments, one located in *T. dicoccoides* 6B and the other in 'Langdon' 6B, behaved as alternative alleles at a locus designated herein as *Xtam26-6B*.The behavior of the other two fragments was anomalous in that both of the fragments were present in several 6B RSLs. This is unexpected because 6B should be fully homozygous in these lines. A similar finding was obtained with TAM31 in study of the 6A RSLs. In addition, the pair of polymorphic fragments to which TAM31 hybridized and which behaved as 6B alleles in the 6B RSLs and in the 6B F_2 populations also segregated in the 6A RSLs and the 6A F_2 populations.

The probable cause of the aforementioned anomalies is residual heterozygosity in the homoeologue of the chromosome being mapped. The 'Langdon'-*dicoccoides* 6A and 6B substitution lines that were used to develop the RSLs and the F_2 populations were backcrossed to 'Langdon' five times after the substitutions were effected (Joppa and Cantrell 1990), after which the lines were maintained by selfing. This makes it highly probable that a few *dicoccoides* chromosomal segments were fixed in the 13 unsubstituted chromosomes in the lines. If so, the crosses of the substitution lines to 'Langdon' that initiated development of the RSL and F_2 populations would have produced heterozygosity at some loci. Since the plants we studied were early-generation derivatives of these crosses, the presence of some residual heterozygosity in the homoeologues of the chromosomes being mapped is to be expected.

Two anomalies were observed with BCD1. One was the absence from a few 6B RSLs of a hybridizing fragment that was present in both parents, in the other RSLs, and in all of the 6B F_3 families. The same phenomena was observed for a few 6B RSLs when probed with TAM31. The second anomaly observed with BCD1 was the presence in 5 6B RSLs of a hybridizing fragment that was not present in the other 6B RSLs, in any F_2 plant, or in either parent. The causes of these anomalies are unknown.

Mapmaker maps of chromosomes 6A and 6B

The most probable Mapmaker Mcintosh linkage maps of chromosome 6B constructed using RSL data only and

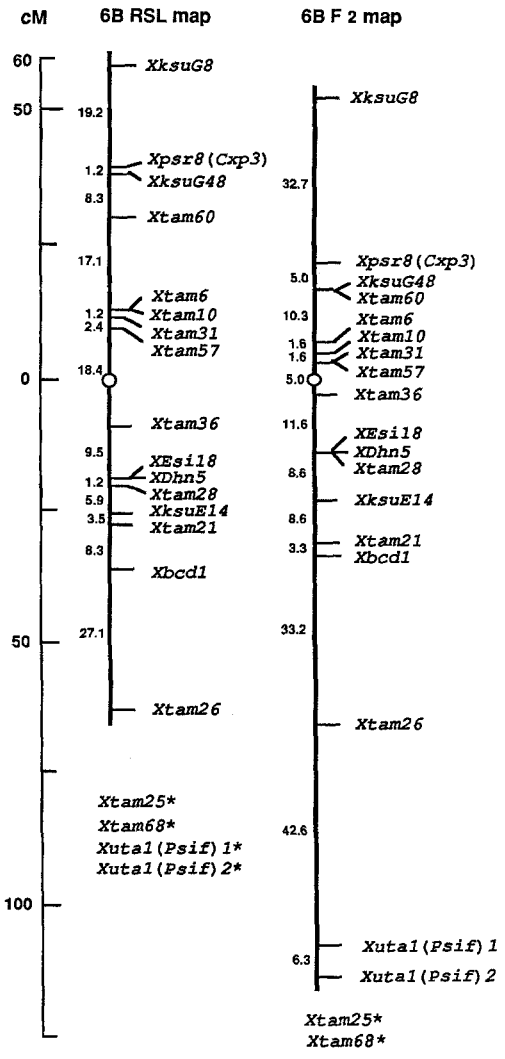

Fig. 3 Linkage maps of chromosome 6B of *T. turgidum* constructed using the Mapmaker Macintosh computer program with a LOD > 3.0. 6BL markers for which linkage data were obtained but which were not linked to the distal-mapped marker are listed *below the arm*. *Asterisks* indicates loci at which distorted segregation occurred at the 1% level. Locus designations are abbreviated by *omission* of the chromosome designation

F_2 data only are shown in Fig. 3. The position of the centromere is assumed to be midway between *Xtam57* and *Xtam36*, the most proximal of the markers known to be in 6BS and 6BL, respectively, in CS. It should be noted that with this centromere position, all of the mapped 6B markers whose arm locations have been determined in CS chromosome 6B [namely, all mapped markers except *XksuE14* and the two *Xutal(Psif)* loci; see Table 2] are in the same 6B arm in *T. turgidum* as they are in CS. Also, the linear order of the loci is the same on both maps. The linkage distances between some loci differ markedly between the two maps; however, this finding is not surprising given the small sizes of the individual populations. Two 6BL markers, namely, *Xutal(Psif)-6B.1* and *Xutal(Psif)-6B.2*, were mapped in the F_2 population but not in the RSL popula-

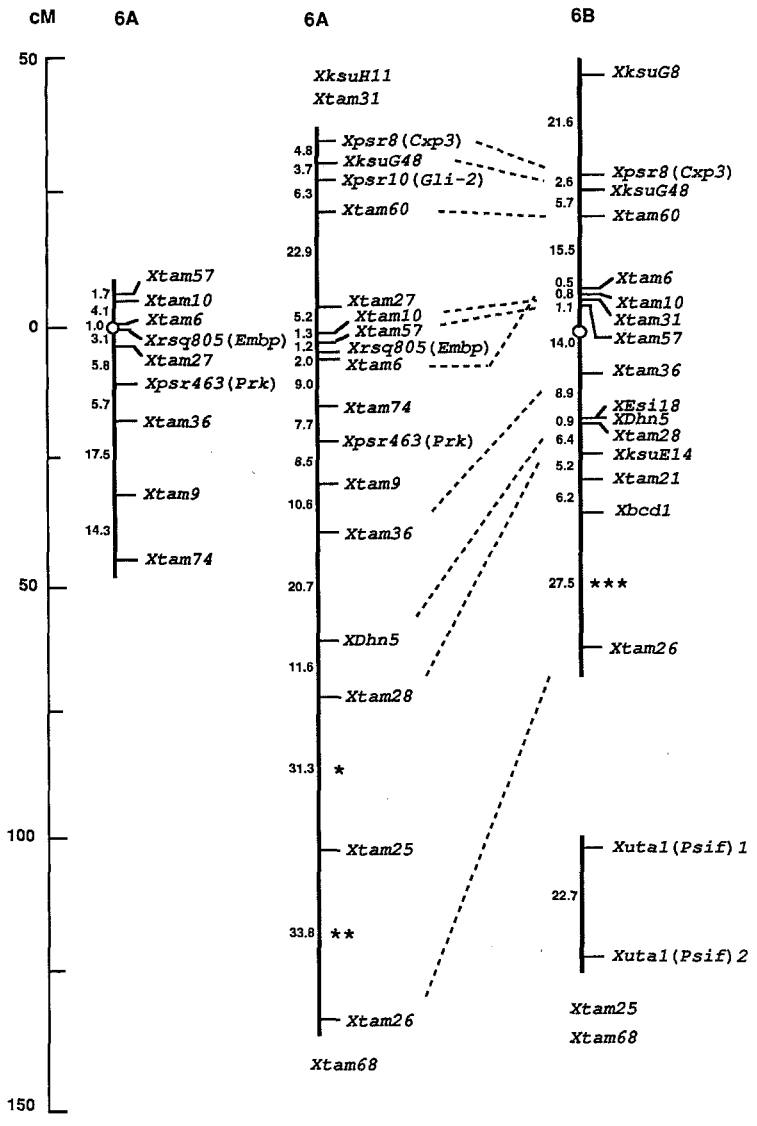

Fig. 4 Linkage maps of chromosomes 6A and 6B of *T. turgidum*. The maps of 6A and 6B on the *right side* of the figure were constructed with data obtained from both the RSL and F_2 populations using G-Mendel 2.0 with a R of ≤ 0.25 and P of ≤ 0.0001, except for regions marked with *asterisks*, where *, **, and ***, respectively, indicate $R \leq 0.35$ and $P \leq 0.0001$, $R \leq 0.40$ and $P \leq 0.0001$, and $R \leq 0.30$ and $P \leq 0.0001$. The map on the *left side* of the figure of the centromeric region of 6A and the proximal region of 6AL was constructed using F_2 data and Mapmaker Macintosh 1.0. Loci for which linkage data were obtained but which could not be mapped are listed either *above* (6AS) or *below* (6AL and 6BL) the *chromosome arm* on which they are located. Locus designations are abbreviated by *omission* of the chromosome designation

tion. All other 6B markers that were mapped in one population were also mapped in the other population.

A linkage map of *T. turgidum* 6B constructed using the computer program G-Mendel 2.0 and the data obtained from both the RSL population and the F_2 population is shown in Fig. 4. *Xuta1(Psif)1* and *Xuta1(Psif)2* are linked to each other on this map, but they are not linked to *Xtam26*, the distal 6BL marker. As is to be expected, the G-Mendel map closely resembles the two Mapmaker maps. However, being based on 147 gametes rather than either 85 gametes (the Mapmaker RSL map) or 62 gametes (the Mapmaker F_2 map), it presumably more accurately reflects the linkage distances between loci then either of the Mapmaker maps. It should be noted that the locations of *XksuG8*, *XDhn5*, and *XksuE14* in 6B are in good agreement with their locations in *T. tauschii* chromosome 6D (Gill et al. 1991).

On the most probable Mapmaker Mcintosh RSL and F_2 maps of 6A, not all of the markers whose chromosome-arm locations are known in CS are in the same arm in *T. turgidum* as they are in CS. (The CS arm locations of all mapped 6A loci, except *Xtam28*, are known; see Table 2). *Xtam57*, *Xtam10*, and *Xtam6* are in 6AS and *Xrsq805 (Embp)* and *Xtam27* are in 6AL in CS, and they are positioned across the centromere in this order on the most probable *T. turgidum* Mapmaker RSL and F_2 maps on which all markers are located in the same arm in *T. turgidum* as they are in CS (Chen 1993; data not shown). However, when the CS arm locations are disregarded, the most probable order of these markers, from short arm to long arm, is *Xtam27*, *Xtam57*, *Xtam6*, *Xrsq805(Embp)*, and *Xtam10* on the F_2 map and *Xtam10*, *Xtam6*, *Xrsq805(Embp)*, *Xtam57*, and *Xtam27* on the RSL map. Except for this, the two F_2 maps are identical to one another as regards the linear order of loci and they are almost identical as regards map distances between loci. The same is true of the RSL maps (results not shown).

Apart from the centromeric region, the linear order of 6A markers determined with Mapmaker using F_2 data only and with RSL data only is the same, except for 3 markers

located in the proximal region of 6AL, namely, *Xtam9*, *Xtam36* and *Xtam74*. The order of these loci on the F_2 map is shown in Fig. 4, while on the RSL map it is (from distal to proximal) *Xtam74*, *Xtam36, and Xtam9*.

Linkage maps of chromosomes 6A and 6B

A linkage map of 6A constructed with G-Mendel using data from both of the populations is shown in Fig. 4. On this map, *Xtam27*, a CS 6AL marker, is located to the short-arm side of 3 CS 6AS markers (*Xtam10*, *Xtam57*, and *Xtam6*), and *Xrsq805(Embp)*, another CS 6AL marker, is located to the short-arm side of one CS 6AS marker (*Xtam6*). The third map shown in Fig. 4, which is derived from F_2 data alone (the 6A F_2 data comes from 100 gametes, whereas the 6A RSL data comes from only 66 gametes) and which shows the centromeric region of the linkage group only, is the most likely of the possible Mapmaker maps in which all markers are assumed to be in the same chromosome arm as they are in CS. A prominent feature of this map is that the order of *Xtam57*, *Xtam10,* and *Xtam6* in 6AS is reversed relative to their order in 6BS. A 6A map with the same marker order is among the other possible maps that are produced by Mapmaker using RSL data only. It should also be noted the *Xrsq805(Embp)* has been reported to be in the long arm of 6A and the short arm of 6B in hexaploid wheat (Jia et al. 1993, cited in Hart et al. 1993). Tests with five restriction enzymes in addition to those listed in Table 3, namely, *Apa*I, *Eco*RI, *Kpn*I, *Xba*I, and *Xho*I, failed to reveal polymorphism for the 6A markers *Xrsq805(Embp)*, *Xpsr463(Prk)*, *Xtam9*, *Xtam27*, and *Xtam74*. The available data are suggestive of a rearrangement difference between the centromeric regions of 6A and 6B, but a definitive map of the centromeric region of 6A must await the obtaining of additional data.

Other than the possible centromeric region difference just noted, the order of mapped homologous markers in 6A and 6B is the same. The 6A and 6B maps differ markedly, however, in terms of the distances between markers in their long arms.

Distorted segregation

At most loci mapped in the two chromosomes, the observed genotypic frequencies did not differ significantly from the expected segregation ratio of 1: 2: 1 in the F_2 population and 1: 1 in the RSL population. At some loci, however, including most of the loci located in the distal regions of both 6AL and 6BL (*Xtam25*, *Xtam26*, and *Xtam68* in 6AL and *Xuta1(Psif)1*, *Xuta1(Psif)2*, *Xtam25*, and *Xtam68* in 6BL), segregation of alleles was distorted in one or both mapping populations (Chen 1993; data not shown). Distorted segregation ratios were observed in both of the 6A and 6B mapping populations (i.e., the RSL and the F_2 populations) for *Xtam68,* in both of the 6A populations for *XksuH11,* and in both of the 6B populations for *Xtam25* , and it was not possible to map these loci in these chromosomes. Loci at which distorted segregation occurred in one of the mapping populations only, such as *Xtam9*, *Xtam26,* and *XDhn5* in 6A and *Xuta1(Psif)1*, *Xuta1(Psif)2*, *XDhn5*, and *XEsi18* in 6B were mapped, however.

Discussion

RFLP frequencies

The RFLP frequencies detected in this study for 6A and 6B with 24 TAM and KSU cDNA and gDNA clones and 8 'known-function' clones were approximately 41% and 74% with one and five restriction enzymes, respectively. This is a considerably higher RFLP level than has been detected in derivatives of other wide crosses involving wheat (Chao et al. 1989; Liu and Tsunewaki 1991; Devos et al. 1992). For example, in an F_2 population derived from a cross of CS × *Triticum spelta 'duhamelianum'*, Liu and Tsunewaki (1991) found RFLP levels of 23% and 38% using one and five restriction enzymes, respectively. Markedly different 6A and 6B RFLP levels were revealed with WG, BCD, and CDO clones, however. The frequency detected by these clones with six restriction enzymes was only about 8%. The difference may be due in part to the manner in which the clones were selected; in choosing WG, BCD, and CDO clones for RFLP mapping of wheat, only clones hybridizing to six or fewer fragments were utilized and preference was given to clone/enzyme combinations that yielded three fragments displaying approximately equal signal level (Anderson et al. 1992). Nevertheless, the overall RFLP frequency detected for the 45 clones studied was still quite high, about 55% with five restriction enzymes (Table 4), a level much higher than that among wheat varieties (Chao et al. 1989).

Frequencies of polymorphic DNA fragments have been found to vary among the A-, B-, and D- genomes in hexaploid wheat. Chao et al. (1989) found 16.1% polymorphism for 7B and only 3.5% and 4.7% for 7A and 7D, respectively, while Devos et al. (1992) found 12% for the D genome and 18% and 22% for the A- and B- genomes, respectively. A similar result was observed by Liu and Tsunewaki (1991), who found that 21% of the fragments localized to the D- genome were polymorphic, while 42% and 38% of the fragments localized in the A- and B- genomes, respectively, were polymorphic. The low RFLP in D-genome chromosomes is consistent with the relatively recent origin of hexaploid wheat. In this study, the average RFLP frequency detected per restriction enzyme was quite similar for 6A and 6B (41.7% and 40.3%, respectively, using TAM, KSU, and 'known-function' probes).

Linkage maps

Given the similarity between the results obtained in mapping 6B using RSLs and F_2s, a high degree of confidence in the linear order of the mapped 6B markers seems mer-

ited. The G-Mendel map should be more accurate than the individual RSL and F_2 Mapmaker maps as regards map distances because it is based on the largest number of progeny. Linkage distances between loci on the 6B Mapmaker RSL map and the G-Mendel map are very similar, however, indicating that the 6B RSL population can be reliably used for mapping, independent of the F_2 population.

Confidence in the linear order of the 8 loci located in the distal regions of 6AS [*Xpsr8(Cxp3)*, *XksuG48*, *Xpsr10(Gli-2)*, and *Xtam60*] and 6AL (*XDhn5*, *Xtam28*, *Xtam25*, and *Xtam26*) also seems merited. The same order was determined for these markers with the RSL and F_2 populations, and the order of mapped homologous 6A and 6B loci is the same in these regions. Questions remain, however, regarding the map locations of the markers located in the proximal region of 6AS and in the centromeric region of the chromosome. If all mapped 6A markers are in the same arm in *T. turgidium* as they are in CS, then a small inversion difference between 6A and 6B in the proximal region of the short arm in the segment in which *Xtam6*, *–10,* and *–57* are located will readily explain the difference in marker order between the G-Mendel 6A and 6B maps. Another significant difference between the 6A and 6B maps would remain, however; namely, much larger genetic distances between 6AL markers than between 6BL markers (see Fig. 4). This could be due, among other things, to a proximal 6BL 'Langdon'-*T .t. var 'dicoccoides'* inversion difference which markedly reduces the frequency of recovery from hybrids of gametes that contain chromosomes that are recombined in this region.

It seems unlikely that the mapping of loci at which dominant and recessive alleles are present is a factor in either of the two aforementioned matters. Five such loci, *Xtam28*, *Xtam57*, *Xtam60*, *XksuG18,* and *Xpsr463(Prk)*, were mapped in 6A and 2 such loci, *Xtam60* and *XEsi18*, were mapped in 6B. *Xtam57* is the only one of these markers that is in the group of 5 centromeric 6A markers whose arm locations are problematic and, although 3 of the markers, *Xtam28, XEsi18,* and *Xpsr463(Prk)*, have been mapped in the proximal region of the long arm in one or both chromosomes, the major differences in genetic distances between markers located in this region in the two arms would exist even if the markers were not on the maps.

Knowledge of the correct linear order among markers in chromosomes is considerably more important than knowledge of 'accurate' linkage distances between the markers. The distances that appear on linkage maps are a function not only of the locations of the markers in chromosomes but also of the genotypes of the strains studied, the environments in which the tests were conducted, and the sizes of the populations studied. Consequently, the distances represent approximations and not absolutes. There seems to be little doubt that the results of this study have established the correct linear order, the correct arm location, and the correct telomere/centromere orientation for all of the mapped 6B markers and for the 4 distal 6AS markers and the 4 distal 6AL markers. Also established is that *Xtam6*, *–10*, *–27,* and *–57* and *Xrsq805(Embp)* are located within approximately 10 cMs of each other in the centromeric region of 6AS and that 4 other markers, *Xtam9*, *–36* and *–74* and *Xpsr463(Prk)*, are located in the proximal region of 6AL. The correct linear order among these two groups of loci is uncertain, however, and must await the results of additional studies.

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References

Anderson JA, Ogihara Y, Sorrells ME, Tanksley SD (1992) Development of a chromosomal arm map for wheat based on RFLP markers. Theor Appl Genet 83:1035–1043

Bailey DW (1981) Recombinant inbred strains and bilineal congenic strains. In: Foster HJ, Small JD, Fox JI (eds) The mouse in biomedical research, vol 1. Academic Press, New York, pp 223–239

Bartels D, Thompson RD (1983) The characterization of cDNA clones coding for wheat storage proteins. Nucleic Acids Res 11:2961–2977

Baulcombe DC, Barker RF, Jarvis MG (1987) A gibberellin responsive wheat gene has homology to yeast carboxypeptidase Y. J Biol Chem 262:13726–13735

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends Genet 7:55–60

Burr B, Burr FA, Thompson KH, Albertson MC, Stuber, CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118: 519–526

Chao S, Sharp PJ, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78:495–504

Chen Z (1993) RFLP-based genetic maps of chromosomes 6A and 6B of tetraploid wheat (*Triticum turgidum* L.). Ph.D thesis, Texas A&M University, College Station, Texas

Close S, Kortt AA, Chandler PM (1989) A cDNA-based comparison of dehydration-induced proteins (dehydrins) in barley and corn. Plant Mol Biol 13:95–108

Curtis CA, Lukaszewaski AJ (1991) Genetic linkage between C-bands and storage protein genes in chromosome 1B of tetraploid wheat. Theor Appl Genet 81:245–252

Devey ME, Hart GE (1993) Chromosomal localization of intergenomic RFLP loci in hexaploid wheat. Genome 36:913–918

Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale MD (1992) RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Theor Appl Genet 83:931–939

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TX (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Guiltinan MJ, Marcotte WR Jr, Quatrano RS (1990) A plant leucine zipper protein that recognizes an abscisic acid response element. Science 250:267–271

Hart GE, Gale MD (1988) Guidelines for nomenclature of biochemical/molecular loci in wheat and related species. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp. Bath Press, Bath, UK, pp 1215–1218

Hart GE, Gale MD, McIntosh RA (1993) Linkage maps of *Triticum aestivum* (hexaploid wheat, 2*n*=42, genomes A, B & D) and *T. tauschii* (2*n*=14, genome D). In: O'Brien SJ (ed) Genetic maps: locus maps of complex genomes, (6th edn.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. pp 6.204–6.219

Joppa LR (1993) Chromosome engineering in tetraploid wheat. Crop Sci 33:908–913

Joppa LR, Cantrell RG (1990) Chromosomal location of genes for grain protein content of *Triticum turgidum* L. var *'dicoccoides'* Crop Sci 30:1059–1064

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing linkage maps of experimental and natural populations. Genomics 1:174–181

Liu BH, Knapp SJ (1992) G-Mendel 2.0. Department of Crop and Soil Sciences, Oregon State University, Corvallis, Ore.

Liu YG, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617–633

Metz AM, Timmer RT, Browning KS (1992) Isolation and sequence of a cDNA encoding the cap binding protein of wheat eukaryotic protein synthesis initiation factor 4F. Nucleic Acids Res 20:4096

Patrick JG, Dvorak J (1990) Selective enrichment of cDNA from salt-stress-induced genes in the wheatgrass, *Lophopyrum elongatum*, by the formamide-phenol emulsion reassociation technique. Gene 95:173–177

Raines CA, Longstaff M, Lloyd JC, Dyer TA (1989) Complete coding sequence of wheat phosphoribulokinase: developmental and light-dependent expression of the mRNA. Mol Gen Genet 220:43–48