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Triticum turgidum L. 6A and 6B recombinant substitution lines: extended linkage maps and characterization of residual background alien genetic variation

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Abstract *Triticum turgidum* L. var 'durum' cv 'Langdon'-*T. t.* var 'dicoccoides' chromosome 6A and 6B recombinant substitution lines (RSLs) and a F_2 population derived from a 'Langdon'-*T. t.* var 'dicoccoides' disomic chromosome 6A substitution line × 'Langdon' cross were analyzed with the objective of markedly increasing the number of markers assigned to and the resolution of previously constructed 6A and 6B linkage maps. Fifty-seven markers were added to the 6A RSL-population map, which now consists of 73 markers that span 111 cM, and 40 markers were added to the 6B RSL-population map, which now consists of 56 markers that span 123 cM. With the exception of 2 6B loci, all of the loci on the two RSL-population maps were ordered at a LOD score ≥ 3.0. Thirty-seven orthologous markers were mapped in the two chromosomes and colinearity between them is strongly indicated. The 6A RSL-population map and the F_2-population map are highly similar, indicating that the former population, which consists of 66 lines, can be reliably used for mapping, as was previously demonstrated for the 6B RSL population. In the absence of selection and genetic drift, the lines in a RSL population, except at loci in the substituted/recombined chromosome, should be near-isogenic. An unexpected finding was that at least 26 and possibly 29 of the RFLPs detected in the RSL populations (18% of the markers analyzed) are not located in the substituted/recombined chromosomes. Linkage analysis of the markers disclosed that at least 19 of them are located in six or seven segments that span approximately 10 cM and 17 cM of the genetic lengths of 6B and 6A, respectively, in the 6A and 6B RSL populations, respectively, a finding that suggests that 40 or more alien segments spanning 8–15% of the genetic length of the 13 unsubstituted chromosomes are present in both of the RSL populations. Alien alleles are fixed in many RSLs for most of the markers, in most cases at a frequency consistent with theoretical expectations. Highly distorted segregation favoring the alien allele was detected for all of the markers in 2 of the segments, however. Nine of the markers were among those mapped in the substituted/recombined chromosomes; the linkage data obtained for the other 10 was sufficient to assign them to approximate map positions.

Key words RFLP · Wheat · *Triticum turgidum* · Genetic mapping

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Introduction

Recombinant substitution lines (RSLs; also called 'recombinant inbred chromosome lines' = RICLs), like fully-developed recombinant inbred lines (RILs), are homozygous and therefore permanent. For this reason, a population of RSLs, like a population of RILs, can be studied by different investigators at different locations and in different years, and the linkage relationships of newly studied markers relative to all previously mapped markers can be determined merely by combining and analyzing the new and the old data sets. Also, 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). For example, Joppa et al. (1997) mapped a *T. turgidum* L. chromosome 6B grain-protein-content QTL with the aid of the 6B RSL-population map that is reported in this paper.

The investigations reported in this paper were initiated with the objective of markedly increasing the number of markers assigned to and the resolution of previously constructed *T. turgidum* L. chromosome 6A and 6B maps (Chen et al. 1994), especially maps of the *T. turgidum* var 'durum' cv 'Langdon'-*T. t.* var 'dicoccoides' 6A and 6B RSL populations. The objective was obtained and the maps are reported. Also obtained, however, was evidence that a large number of alien chromosomal segments are contained in the unsubstituted chromosomes in both of the RSL populations. This was unexpected; theoretically, except for the substituted/recombined chromosomes, the lines in RSL populations should be near-isogenic. This evidence is also reported and discussed.

Materials and methods

Plant materials

Triticum turgidum L. var 'durum' cv 'Langdon'-*T. t.* var 'dicoccoides' $6A^D(6A^L)$ and $6B^D(6B^L)$ disomic substitution lines and 'Langdon' (LDN) were used to detect restriction fragment length polymorphisms (RFLPs) and 6A and 6B recombinant substitution lines (RSLs), and 6A F_3 families were used to construct linkage maps as described by Chen et al. (1994). Eighty-five 6B RSLs, 66 6A RSLs and 50 6A F_3 families were analyzed. *T. aestivum* L. em Thell. cv 'Chinese Spring' (CS) ditelosomic (Dt) lines (Sears and Sears 1979) were used to assign some markers to chromosome arms as described by Devey and Hart (1993).

The 6A F_3 families were produced locally from a $6A^D(6A^L)$ line × LDN cross. Seed stocks of LDN, the $6A^D(6A^L)$ and $6B^D(6B^L)$ lines, and the two RSL populations were obtained from Dr. L. R. Joppa and increased locally. The CS Dt lines are maintained locally. The $6A^D(6A^L)$ and $6B^D(6B^L)$ lines were produced by crossing LDN $6D^{CS}(6A^L)$ and $6D^{CS}(6B^L)$ lines to *T. t.* var 'dicoccoides' (DIC) to produce double monosomic-$6A^D$-$6D^{CS}$ nullisomic-$6A^L$ and double mono-$6B^D$-$6D^{CS}$ nulli-$6B^L$ hybrids, respectively, followed by backcrossing for five generations to the $6D^{CS}(6A^L)$ and $6D^{CS}(6B^L)$ lines, respectively, and then selecting disomic substitution lines from among the progeny obtained by selfing (Joppa and Cantrell 1990). The LDN $6D^{CS}(6A^L)$ and $6D^{CS}(6B^L)$ lines were produced by crossing nulli-6A tetra-6D and nulli-6B tetra-6D CS plants to LDN, selfing the progeny to eliminate the monosomes, backcrossing disomic progeny obtained by the selfing to LDN, making four additional backcrosses of double monosomics to LDN, and then selecting $6D^{CS}(6A^L)$ and $6D^{CS}(6B^L)$ disomic substitution lines from among progeny obtained by selfing (Joppa and Williams 1988). The RSLs were produced by crossing the $6A^D(6A^L)$ and $6B^D(6B^L)$ lines to LDN to produce $6A^D/6A^L$ and $6B^D/6B^L$ heterozygotes, respectively, crossing the F_1 progeny to the $6D^{CS}(6A^L)$ and $6D^{CS}(6B^L)$ lines, respectively, to produce progeny monosomic for $6D^{CS}$ and monosomic for a derivative of the $6A^D/6A^L$ and $6B^D/6B^L$ heterozygotes, respectively, allowing these double monosomic plants to self-fertilize, and then selecting disomic 6A and 6B plants, respectively, from among their progeny (Joppa 1993).

DNA manipulation

Genomic DNA isolation, digestion, Southern blotting, probe labeling, and hybridization were performed as described by Devey and Hart (1993).

DNA clones

DNA clones known to hybridize to Triticeae homoeologous group 6 chromosomal fragments were used. These included genomic DNA (gDNA) clones, cDNA clones and 'known-function' clones of wheat, barley, oat, *T. tauschii*, and rice. pTaTAM clones were developed by Devey and Hart (1993). Other clones were as follows: ABC (barley cDNA) and ABG (barley gDNA), obtained from A. Kleinhofs, Pullman, Washington (Kleinhofs et al. 1993); BCD (barley cDNA), CDO (oat cDNA), and WG (wheat gDNA), from M. E. Sorrells, Ithaca, N.Y. (Heun et al. 1991); ksu (*T. tauschii* gDNA), from B. S. Gill, Manhattan, Kansas (Gill et al. 1991); cMWG (barley cDNA) and MWG (barley gDNA), from A. Graner, Grunbuch, Germany (Graner et al. 1991); PSR (wheat cDNA and gDNA), from M. D. Gale, Norwich, UK (Chao et al. 1989); RZ (rice cDNA), from S. R. McCouch, Ithaca, N.Y. (McCouch et al. 1988); Tag (wheat gDNA), from R. Appels, Canberra, Australia, with the permission of K. Tsunewaki, Kyoto, Japan (Liu and Tsunewaki 1991); Hv5 (dehydrin cDNA), from T. Close, Riverside, California; R6.1 (phosphoribulokinase cDNA) from T. A. Dyer, Norwich, UK; Nar1, Nar7 [NAD(P) H nitrate reductase] and Amy1 (α-amylase), from A. Kleinhofs; p26 (wheat protein synthesis initiation factor), from K. S. Browning, Austin, Texas; 2437 (wheat carboxypeptidase), from D. Baulcomb, Norwich, UK; pTag53 (wheat gliadin multigene clone), from M. D. Gale; and ESI18 (cDNA from a salt-stress-induced gene), from J. Dvorák, Davis, California.

Linkage analysis and goodness-of-fit testing

Linkage analyses were performed with MAPMAKER Macintosh V. 2.0 (Lander et al. 1987). Loci on the RSL-population maps were ordered at a LOD score ≥ 3.0 using the 'Ripple' command, with the exception of two pairs of 6B loci (see Results and discussion). On the 6A F_2 map, markers ordered at a LOD score ≥ 3.0 were assigned to map positions, and markers ordered at a LOD score < 3.0 were assigned to intervals between the mapped markers in the order with the highest probability using the 'Near' and 'Try' commands. Recombination frequencies were converted to centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944). Chi-square tests for goodness of fit of observed to expected allelic ratios were based on an expected 1:1 DIC:LDN allelic ratio in substituted/recombined chromosomes and on expected 1:3 and 3:1 DIC:LDN and CS:LDN allelic ratios, respectively, in unsubstituted chromosomes, except an expected CS:LDN allelic ratio of 1:3 was assumed for *Xabg458-6B* and *Xglk582-6B* (see 1 of Unexpected findings below).

Results and discussion

Polymorphism

One hundred and fifteen DNA clones were used to search for RFLPs among LDN and the two LDN-DIC chromosome group 6 substitution lines in this study, and 45 clones were used in the study of Chen et al. (1994). Among these 160 clones, 119 detected 1 or more RFLPs, and 99 were used for mapping, 73 in this study and 26 in the earlier study.

Linkage maps of the 6A and the 6B RSL populations

The maps constructed with data obtained from the RSL populations, including the data of Chen et al.

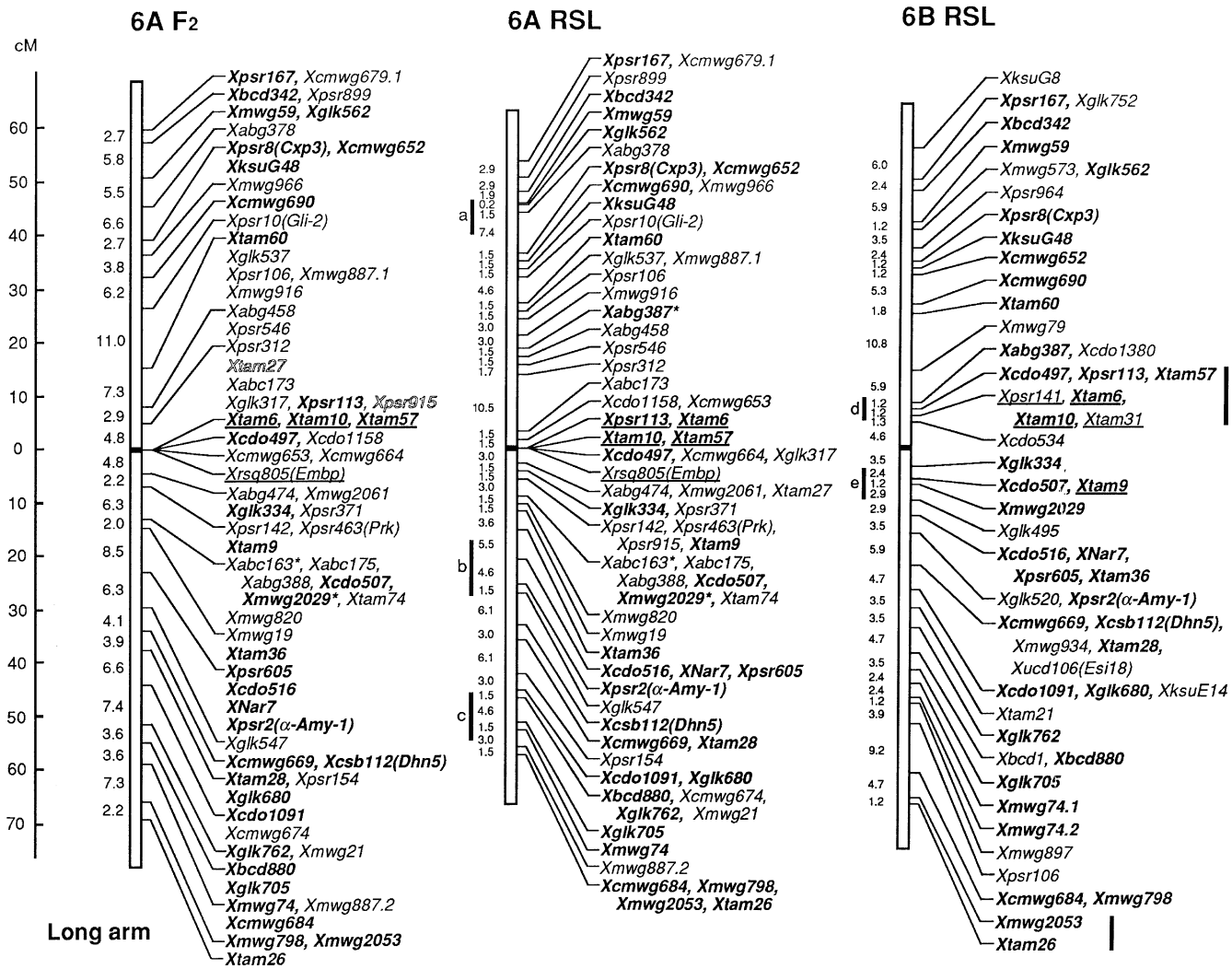

Fig. 1 RFLP linkage maps of chromosomes 6A and 6B of *T. turgidum* L. Short arms are at the *top*. *Black rectangles* designate centromeres. Orthologous 6A and 6B markers are in *boldface type*. Markers between which recombination did not occur are assigned to the same map position and their symbols are separated by *commas*. The most proximal marker(s) in each arm that were assigned to it using CS Dt stocks are *underlined*. Markers mapped in only one of the 6A populations are designated with an *asterisk* (see text). All loci on the RSL-population maps were ordered at a LOD score ≥ 3.0, with the exception of two pairs of 6B loci, designated by a *vertical line* to their *right*, which were ordered at LOD scores of 2.7 and 2.5, respectively. Loci ordered at a LOD score ≥ 3.0 on the 6A F_2-population map are indicated with *lines* connected to the maps. The arm locations of the 2 markers in *open type* are inconsistent with the 6A RSL map. The approximate locations of 10 markers, determined as described in the section entitled 'Unexpected findings', are indicated by *lines* to the *left* of the RSL-population maps, as follows: *a Xcmwg679.2*, *Xmwg573*, and *Xmwg620*; *b Xwg286*; *c XksuF37* and *Xmwg897*; *d Xabg458* and *Xglk582*; and *e Xpsr371* and *Xmwg820*

(1994), consist of 128 markers (Fig. 1), 72 of which were assigned to 40 positions on the 6A map and 56 of which were assigned to 35 positions on the 6B map [genotypes for all markers are contained in Du (1997)]. *Xabg163-6A*, which was mapped in the 6A F_2 population but not in the RSL population, is also shown on the 6A RSL-population map because it co-segregated in the F_2 population with 4 markers that also co-segregated in the RSL population. All of the 6A and 6B markers were assigned to map positions at a LOD score ≥ 3.0, except for the markers at two pairs of loci that were oriented at LOD scores of 2.7 and 2.5 (see Fig. 1). The maps are 111 cM and 123 cM in length, respectively, the average intervals between adjacent loci are 2.8 cM and 3.5 cM, respectively, and the largest gaps are 10.5 cM and 10.8 cM in length, respectively. These are the highest resolution maps that have been produced for Triticeae homoeologous group 6 chromosomes. Thirty-seven orthologous markers distributed throughout the linkage maps were located in 6A and 6B.

Highly significant distorted segregation favoring LDN alleles occurred in the centromeric regions of both linkage maps, extending from *Xcdo1158-6A* to *Xpsr371-6A* and from *Xmwg79-6B* to *Xtam21-6B*. Also,

significant distorted segregation favoring LDN alleles occurred in 6AL from *Xpsr142-6A* to *Xglk547-6A* and at the 6AS locus *Xabc173-6A* and the 6BL locus *Xglk762-6B*. $6A^L$ and $6A^D$ are homozygous in the 6A RSL population in 13 and 6 lines, respectively; $6B^L$ and $6B^D$ are homozygous in the 6B RSL population in 18 and 5 lines, respectively; and the ratio of LDN to DIC alleles in the centromeric regions of the recombined/substituted chromosomes is approximately 2 : 1. The probable cause of the distorted segregation is selection among male gametes when $6A^D/6A^L$ and $6B^D/6B^L$ heterozygotes were used as pollen parents in crosses to the $6D^{CS}(6A^L)$ and $6D^{CS}(6B^L)$ lines, respectively, during development of the RSL populations.

Linkage map of the 6A F_2 population

A linkage map of 6A was also constructed using F_2 data, including the data of Chen et al. (1994) (Fig. 1). *Xmwg2029*, 1 of 2 markers mapped in the 6A RSL population but not in the F_2 population (the other is *Xabg387*) also is shown on the F_2-population map because it co-segregated in the RSL population with 4 markers that also co-segregated in the F_2 population. The map is 129 cM in length, slightly longer than the RSL map. The orientation of all markers on the two maps that were ordered at a LOD score ≥ 3.0 is identical. Also, the same order was observed for all of the markers located in the distal one-third of the 6AS maps and the distal two-thirds of the 6AL maps. Inconsistencies in marker order between the maps occur only for *XksuG48*, *Xpsr915*, and *Xtam27*, none of which was ordered at a LOD score ≥ 3.0 on the F_2-population map. Also, Dt studies of *Xtam27* (Devey and Hart 1993) and *Xpsr195* (Jia et al. 1996) placed these markers in the 6L arms of hexaploid wheat, consistent with the locations indicated by the 6A RSL data. Seventy-two markers were assigned to 40 map positions on the 6A RSL-population map, all at a LOD score ≥ 3.0, and 48 markers were assigned to 26 map positions at a LOD score ≥ 3.0 (and an additional 23 markers were assigned to intervals at a LOD score < 3.0) on the 6A F_2-population map. It is thus clear both that the RSL population map is the more robust of the two maps and that the 6A RSL population can be reliably used for genetic mapping.

Statistically significant distorted segregation was not detected in the F_2 population. The same opportunity for selection among male gametes would seem to exist when $6A^D(6A^L)$ heterozygotes were selfed to produce the 6A F_2 population as when they were used as pollen parents during production of the 6A RSL population. It is possible that selection did occur among the male gametes but was not detected because of an absence of selection among female gametes and the small size of the F_2 population – only 50 plants.

Unexpected findings

In the absence of selection and genetic drift, LDN and CS chromatin should comprise approximately 98.5% and 1.5%, respectively, of the genetic length of the 13 unsubstituted chromosomes in the $6D^{CS}(6B^L)$ and $6D^{CS}(6B^L)$ lines, and LDN, CS, and DIC chromatin should comprise approximately 97%, 1.5%, and 1.5%, respectively, of the genetic length of these chromosomes in the $6A^D(6A^L)$ and $6B^D(6B^L)$ lines (see Materials and methods). In turn, segregating CS/LDN and DIC/LDN genetic material should comprise approximately 1.5% and 1.5%, respectively, of the genetic length of these chromosomes in each of the three mapping populations. Consequently, because DNA clones known to detect markers in homoeologous group 6 chromosomes in the Triticeae family were used, we expected the vast majority of polymorphic DNA fragments to map to the substituted chromosome in each mapping population. However, 14 RFLPs in the 6A populations and 15 RFLPs in the 6B population did not map to 6A and 6B, respectively (RSL-population genotypes for the 29 markers are shown in Fig. 2). These markers, 23 with co-dominant and 6 with dominant and recessive alleles, comprise 18% of the RFLPs analyzed. Also unexpected was the detection of numerous heterozygotes in the RSL populations for 17 of the 23 markers that have co-dominant alleles (see Fig. 2; several *Xtam31* heterozygotes probably were present but undetected among the 6A RSLs because genotypic data for *Xtam31* was not obtained for 14 lines that were heterozygous at either 3 or all 4 of the markers closely linked to it). It also should be noted that an alien allele is fixed in many RSLs for 26 of the 29 markers, all except *XksuH11* and *Xmwg984* in the 6A RSL population and *Xtam25* in the 6B RSL population.

Polymorphism at loci located in either the homoeologue of the chromosome being mapped or in other chromosomes was thought to be the probable cause of the aforementioned anomalies. As noted above, fixation of some alien genetic material in the 13 unsubstituted chromosomes in the $6A^D(6A^L)$ and $6B^D(6B^L)$ lines is expected, and the crosses of the substitution lines to LDN that produced the populations used in this study would have produced heterozygosity at these loci in the F_1 generation and segregation among the lines at these loci in later generations. Also, because the RSLs analyzed were early-generation derivatives of the substitution line $\times$ LDN crosses, heterozygosity at some of the loci in some RSLs is expected.

The results of a linkage analysis performed on the 29 aforementioned markers are shown in Fig. 3. Both the MAPMAKER 'F_2 intercross' and 'RI self' functions were used for the RSL populations, while recognizing that the former will overestimate genetic distances for these populations (because 'post-F_2' populations are being analyzed) and the latter will disregard heterozygotes and may underestimate genetic distances. The

6A RSL population

Locus	Source of alien allele	Genotypes	Goodness of fit DIC	CS	
XksuG8-6B	CS	BHHAAHBBBH HBHHBBBBBB BBBBAAHBBB HBBBABHBBH BBBHBBBBAB AHBHABABAB HAHBBH	NA	-	
Xabg458-6B	CS	HHABBBBBBH ABHHBBBABH HHHBAABAB- BBHBHHBBHB BBAAHHHAAB BBBBABBBBB BBBBHB	NA	**	
Xabg387-6B	DIC	HBHBBBBBBB ---BHBBABB HBBHBABABB BBHBHBBBBB BBABBBHHAB BBBBABBBBB B-BBHB	**	NA	
Xcdo1380-6B	DIC	HHHBBBBBBH HBHHHBBABB HBBHBABAB- BBHBHBBBBB BBABBBHHAB BBBBABBBBB BHBBHB	**	NA	
Xglk582-6B	CS	HHHB-BBBBH H-HHHBBABH HBBHBABABB BBHBHBBBBB BBABHBHHHB BBBBHBBBBB BABBHB	NA	**	
Xtam31-6B	DIC	---BBBBBBA -B---BBABB -BB-BABABB BB-B-BBBBB BBAB-B-AAB BBBBABBBBB BABB-B	**	NA	
Xpsr371-6B	DIC	BBHABBBBBB BBBB-B-BBB HHBBBBHBB- BHBBBBABHB BHABBBBHAA ABBBH-BABH AHH-HB	**	NA	
Xmwg820-6B	DIC	BBAA-BBBBB BBBBABABBB AABBBBABBB BBBBBBABAB BAAABBBABA ABBBBBBABA A-ABAA	**	NA	(LDN)
Xglk334Y	UNK	HABBBBHBBA BBHABBBBBB BBBHABBBA- BBHHBBABBH BBHABHBHAB HBAAABBBAB BHB-BB	**	-	
Xglk520.1Y	UNK	HAHB-BHBBA BHHABBBBBH BBBAAHBBHH BBHHBBABBH BBHHBHBHAB BBAAABBHAB B-BHBB	**	-	
Xglk520.2Y	UNK	BABBAABBBB B-ABBBABBB BBAABBBBBB BAABBBAABA ABAABBABAB AABBAABBBA A-BBBB	**	-	(LDN)
XksuH11Y	UNK	AAAAAAAAAA BAAAAABBBA BAAAAAAABA BAAAAAABBA AAAAABABAB AAABABAABA AAAAAA	**	**	
Xmwg984Y	UNK	HABH-BBHHH AAAHHAHHBB HAHHBHHAHH BBAHHAAHHA AHHHAABHBH AAHBH-HHHH H-BHAA	-	**	
Xtam25Y	UNK	BBAA-BBABB BBBBBBABA- BAAAABABBB BBABAAB--B BAAB--AABA BAABB-ABBB ABBAAB	-	*	

6B RSL population

Locus	Source of alien allele	Genotypes	Goodness of fit DIC	CS	
Xpsr167-6A	CS	HBAABBBBBA BHBBHBBAHH BBBHBBAHBB ABBHBA-HBB BHHBBBBBHH BHBHBBBBHB BABHBBAHBB BBABBBAABB BHHBH	NA	-	
Xbcd342-6A	CS	ABAABBBBBA BHBBABBAAH BBBHBBAABB ABBHBA-ABB BAABBBBBHA BABABBBBAB BHBABBAABB BBABBBAABB BAA--	NA	*	
Xpsr899-6A	CS	A-AABABBBA BABBHBBAHH BBBHBBAHBB AB-ABAB-BB -AABBBBBAA BABAB-BBAB BABABBAABB BBABBBAABB BAABA	NA	**	
Xmwg573-6A	CS	BBAABABBBA BABBABBABB BBBBBBBB-B ABBBBABABB BAABBBBB-B BBBBBBBBBB BABBB-AABB BBABBBAABB BBBBB	NA	-	
Xcmwg679-6A	CS	BBAABABBBA BBBBABBABB BBBBBBABBB ABBBBABBBB B--BBBBBBB BBBBBBBBBB BBBBBBABBB BBABBBAABB BBBAB	NA	-	(CS)
Xmwg620-6A	CS	BBAABBBBBA BBB--BBABB BBBBBBABBB ABBBBABBBB B--BBBBBBB BBBB-B-B-- BB--BBABBB -BABBBAABB BBBAB	NA	-	(CS)
Xwg286-6A	CS	BBBBBBBBBB BABBBAAABA AABBBBBBBB BABBBABBBB BBBBBBBBBB BBBBABBABB AABBAABBBB BBBBBBBBBB BBBBB	NA	-	(CS)
XksuF37-6A	CS	BABBBABBAB HBHABBBBBB AABBHBBBBB ABABBBBBBB A-HBBBBBHB HBBB-BBBBB BHBBBBBBAB -HBAHBBBBB BBBAH	NA	-	
Xmwg74-6A	CS	BABBBABBAB HBHABBBBBH AABBHBBBBB HBABBBBBBB ---HBBBBHB HHBBBBHBBH BHBHBBBBAB BHBAH-HHBB BBB-H	NA	-	
Xmwg897-6A	CS	BABBBABBAB ABAABBBBBA AABBABBBBB ABABBBBBBB ABAABBBBA- ABBBBBABBA BABBBBBBAB BABAABAABB BBA-A	NA	-	(LDN)
Xtam26-6A	CS	BBAABBBBAB ABAABBBBBA AABBBBBBBB ABBABBBBBA AAAABBB-AA ABBBBAAABA BABBBBBBBA BABABBBABB BBAAA	NA	*	
Xglk259	UNK	BBBABHBABH HHHABHHABA HBBABBABAH BABHBABHAB BBBBBBHBAB BBABBBBABB B-BABBH-BB BABHBBBAHB HHHHH	**	-	
Xmwg984Z	UNK	BBBHBABABH HBHBBHHABA HBBABBABAH BAHHBABAAB BBBBBBHBAB BHABBBBABB BBBABBHHBB BABHBBBAHB BBHHB	**	-	
Xtam25Z	UNK	AAAAAAAAAA AABBAAAAAA BBAABAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAA	**	**	
Xuta(Psif)Z	UNK	ABHBBBHHBB BBBBBBBHAB HABHBBBABH BHBBBHBBBH BBBBHBBHBB BBBABHHBBB BBHBABBBBA BHBHBBHBHH HHBAA	**	-	

Fig. 2 Genotypes of the RSL populations and the results of tests for distorted segregation for markers located in unsubstituted chromosomes. The chromosomal locations of markers to which a *Y* (6A RSLs) or *Z* (6B RSLs) is appended are unknown. *UNK* indicates that the source of the alien allele is unknown. *A* indicates homozygosity for the LDN allele, *B* homozygosity for the alien allele, *H* heterozygosity, and - missing data. Linked markers are grouped together. The results of Chi-square tests for goodness of fit of observed to expected allelic ratios are shown on the *right* side of the figure, with test results shown in both columns for markers for which the source of the alien allele is unknown. * and ** indicate distorted segregation at the 0.05 and 0.01 levels of significance respectively, - that segregation was not distorted, and *NA* that a test for distorted segregation is not applicable. Distorted segregation at markers with DIC and LDN alleles favored the DIC allele, except for *XksuH11Y* and *Xtam25Z*, and it favored the LDN allele at loci with CS and LDN alleles, except for *Xabg458-6B* and *Xglk582-6B*. (*LDN)* and (*CS)* on the far *right side* of the figure indicate markers with dominant LDN and CS alleles, respectively. All other markers have co-dominant alleles

'F_2 intercross' function was used to analyze the 6A F_2-population data. The markers in linkage groups A, D, F, K, and L are listed in Fig. 3 in the order that has the highest LOD score. Indeed, map D, except for the order of *Xpsr371-6B* and *Xmwg820-6B*, map K, except for the order of *Xpsr899-6A* and *Xbcd342-6A*, and map L have LOD scores > 3.0. The linkage groups assigned to 6B in the 6A populations and to 6A in the 6B populations are listed in the order that best approximates the order of the markers on the Fig. 1 maps.

The chromosomal location of the 29 markers and the probable source of the alien alleles, shown in Fig. 2, were determined to the extent possible by consideration of their genotypes and careful analysis of the parental screening blots and the mapping blots for them, as follows.

1) Clones ABG387, CDO1380, and TAM31 detected the same RFLPs in the $6A^D(6A^L)$ and $6B^D(6B^L)$ lines and the three mapping populations, and the RFLPs were placed on the 6B linkage map. This indicates both that the markers detected with these clones in the 6A populations are *Xabg387-6B*, *Xcdo1380-6B*, and

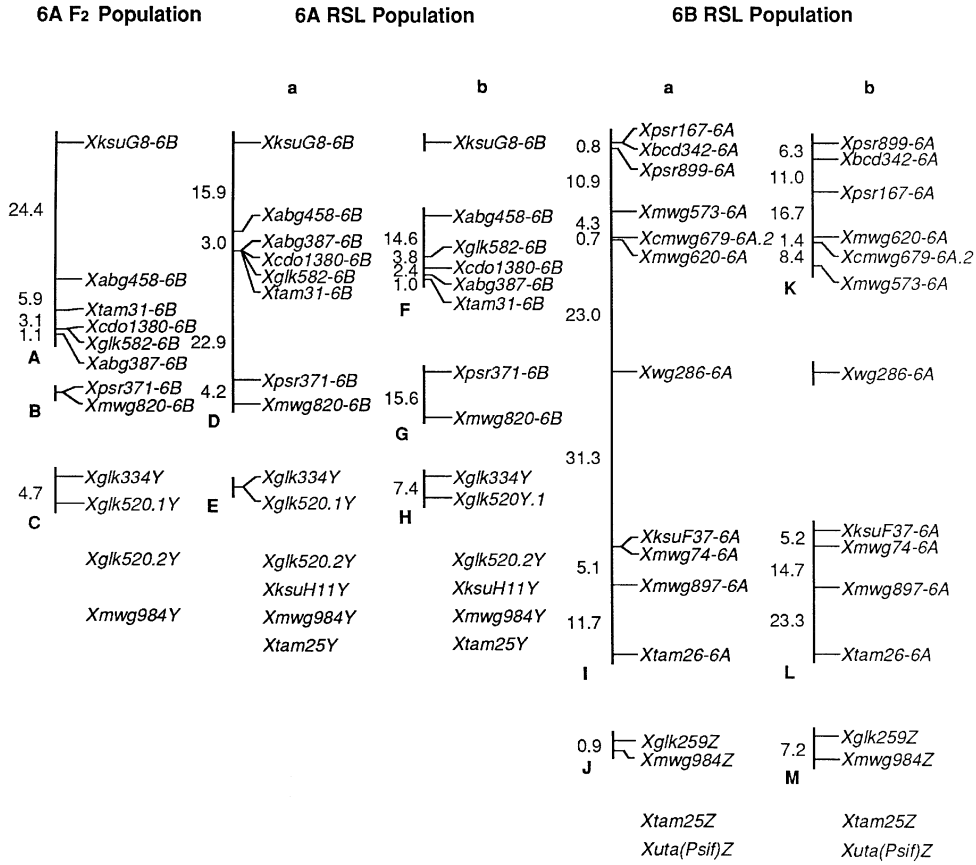

Fig. 3 Results of linkage analysis of the genotypic data for markers located in unsubstituted chromosomes. *a* maps were constructed using MAPMAKER data type 'RI self' and *b* maps and the F_2-population map using data type 'F_2 intercross'. *Boldface capital letters* designate different linkage groups. The scale used for *b* maps differs from that used for *a* maps and for the 6A F_2-population map. *Numbers* to the *left* of the linkage groups are centiMorgans. Markers listed *below* the linkage groups are not linked to any other marker. 6B markers mapped in the 6A populations and 6A markers mapped in the 6B RSL population are designated by the appropriate marker symbols, while a *Y* (6A populations) or *Z* (6B RSL population) appended to a marker symbol indicates that the chromosomal location of the marker is unknown

Xtam31-6B, respectively, and that the alien alleles at these loci were derived from DIC. Given the close linkage of *Xabg458* and *Xglk582* to these 3 markers, it is highly probable that they also are located in the proximal region of the 6BS map. The survey blots suggest that the alleles at these loci were derived from $6B^{CS}$, however (see '2' below). Contiguous CS and DIC segments that segregate opposite a LDN segment in a RSL population should arise at a very low frequency. One possible origin would be initiated by recombination, in the $6A^D/6A^L$ hybrid derived from the $6D^{CS}(6A^L) \times$ DIC cross during development of the $6A^D(6A^L)$ line, between $6B^D$ and $6B^L$ in a segment that, in the latter, contains CS chromatin. This would produce contiguous CS and DIC segments. The $6B^L$ CS segment would have been heterozygous in the progeny of the fifth backcross to LDN during production of the $6D^{CS}(6A^L)$ substitution line, and it could have been lost from the $6D^{CS}(6A^L)$ line by the time it was later used as the recurrent parent during development of the $6A^D(6A^L)$ line. This series of events would cause the expected CS : LDN allelic segregation ratio in this segment to be the same as the expected DIC : LDN ratio, namely, 1 : 3.

2) With PSR899 and several other clones, 1 or more segregating fragments in one of the RSL populations did not map to the substituted chromosome and were not present in the LDN-DIC substitution line from which the RSL population was derived, and evidence that the fragment(s) are located in the homoeologue of the substituted chromosome was obtained. Our conclusion regarding the markers detected with these clones is that the alien alleles were originally derived from CS. PSR899 will be used to illustrate the findings and the rationale for this conclusion.

Five fragments that hybridized to PSR899 were detected in LDN and the $6A^D(6A^L)$ and $6B^D(6B^L)$ lines. Numbering the fragments starting at the cathode, LDN contains fragments 2, 4, and 5, the $6A^D(6A^L)$ line fragments 2, 3, and 5, and the $6B^D(6B^L)$ line fragments 1 and 2. Fragments 2 and 5 were present but invariant, and fragments 3 and 4 segregated as alleles in the 6A mapping populations; the latter 2 fragments were placed on the 6A linkage map in both populations. Fragments 4 and 5 cosegregated opposite fragment 1, and fragment 2 was invariant in the 6B RSL population. The simplest explanation for the invariant nature of fragment 2 is that it is present in both $6B^L$ and $6B^D$. The mapping results are consistent with fragment 3 being located in $6A^D$, fragment 4 in $6A^L$, and fragment 5 in both $6A^D$ and $6A^L$. However, neither fragment 4 nor 5 is present in the $6B^D(6B^L)$ line and fragment 1 is not present in LDN.

This anomaly is resolved if the two LDN-DIC substitution lines carry different alien *Xpsr899-6A* alleles, with the allele in the $6B^D(6B^L)$ line (fragment 1) having been derived from CS. A mutation, rather than a CS allele, would also explain the results obtained, but detection of a mutant RFLP allele for even 1 or 2 markers, much less for the large number for which this phenomena was observed, is highly improbable. We conclude that CS and LDN *Xpsr899-6A* alleles are segregating in the 6B RSL population. The same types of observations led to the conclusions reported in Fig. 2 for the other markers listed as having a CS allele, including *XksuG8-6B* and *Xtam26-6A*.

3) MWG820 and PSR371 detected 2 RFLPs in the two substitution lines and in the two 6A mapping populations, 1 of which mapped to 6A and 1 of which did not (mapping data for the 6B RSL-population was not obtained with these clones). The group D *Xpsr371* and *Xmwg820* loci are linked by a distance similar to the 6 cM that separates *Xpsr371-6A* and *Xmwg820-6A* on the 6AL linkage map (groups F and G also are loosely linked), and they are linked to *Xtam31-6B*, *Xcdo1380-6B* and *Xabg387-6B*, markers that were mapped in the 6B RSL population 9.4–11.8 cM from *Xglk334-6B*, which is orthologous to *Xglk334-6A*, a marker that cosegregates with *Xpsr371-6A* (Fig. 1). Also, when group D is constructed with the data for *Xmwg820* omitted, *Xpsr371* maps only 14.8 cM from the 4 markers distal to it (although the distance between *Xpsr371* and *Xmwg820* only changes from 4.2 to 3.8 cM when these markers are mapped independently of the other group D markers). In addition, the only *Xpsr371* markers identified in hexaploid wheat are located in the 6L arms, and the 6DL marker was mapped approximately 5 cM from the centromere (Jia et al. 1996). Furthermore, *Xpsr371-6A* was mapped about 1 cM from the centromere in 6AL in *T. monococcum* L. (Dubcovsky et al. 1996a). It therefore is highly probable that the group D *Xpsr371* and *Xmwg820* loci are located in 6BL. It also is highly probable that the alien alleles at these loci were derived from DIC because the same segregating fragments were found in both RSL populations.

4) Clone MWG984 detected the same RFLP in the two substitution lines and the three mapping populations, but the RFLP did not map to either 6A or 6B and many heterozygotes were detected in both RSL populations. Graner et al. (1994) mapped *Xmwg984* markers in both 6H (at the centromere) and 1H. It is probable that *Xmwg984Y* is located in either 1A or 1B. The presence of the same segment of non-group 6 alien chromatin in both the $6A^D(6A^L)$ and the $6B^D(6B^L)$ lines is surprising, but there is no other reasonable basis for the results obtained with MWG984. A *Xglk259* marker was mapped to the distal end of the 6AS linkage group in hexaploid wheat by Liu and Tsunewaki (1991) and Tsunewaki (1991) but, given the close linkage of *Xglk259Z* to *Xmwg984Z*, it is unlikely that *Xglk259Z* is a group 6 marker. As with all markers located in non-group 6 chromosomes, whether the allele was derived from DIC or CS is unknown (see Fig. 2).

5) *Xglk334-6A*, *Xglk334-6B*, and *Xglk520-6B* were mapped in this study (see Fig. 1). It is unlikely that *Xglk334Y* and *Xglk520.1Y* (group E) or *Xglk520.2Y* are orthologous to these markers, however, because they are not linked to *Xtam31-6B* and *Xpsr371-6B* (group D) and, as noted above, *Xglk334-6A* and *Xpsr371-6A* cosegregate and *Xglk334-6B* is located in 6BL 9.4 cM from *Xtam31-6B*. As non-orthologous markers, *Xglk520.1Y*, *Xglk520.2Y*, and *Xglk334Y* could be located elsewhere in 6B or, more likely, elsewhere in the genome; *Xglk520* markers were detected in 1B, 2A, 3B, 5A and 6B by Liu and Tsunewaki (1991).

6) *Xpsr899* markers are located close to the distal end of the linkage maps of 6AS and 6DS (Dubcovsky et al. 1996a; Jia et al. 1996; Marino et al. 1996; this study), and the *Xpsr899* marker detected in the 6B RSL population is closely linked to 3 other markers detected with clones that detect distally mapped 6S markers. This makes it probable that all of the group K markers are located in 6AS. *Xwg286* loci were physically mapped in the distal parts of 6AL, 6BL and 6DL by Gill et al. (1993) and, in the case of 6AL, in a small segment that includes *Xpsr149*. The latter is located in the proximal regions of the 6BL and 6DL linkage maps of Jia et al. (1996). *Xtam26* is a distal 6L marker, and the *Xtam26* marker that did not map to 6B in the 6B RSL population is linked to 4 other markers detected with clones that identify distally mapped 6L markers. This makes it probable that the 4 markers that compose group L also are located in 6AL and thus that all of the group I markers are located in 6A.

7) *Xuta*(*Psif*) *Z* may be located in 6A with an alien allele derived from CS or it may be located elsewhere in the genome with either a CS or a DIC allele.

8) The allelic ratios for *XksuH11* in both of the 6A populations and for *Xtam25* in the 6B RSL population are highly skewed toward the LDN allele and, although the alleles for these markers are co-dominant, heterozygotes were not detected in the RSL populations. A *XksuH11* marker was mapped in 6D of *T. tauschii* (Gill et al. 1991), and three orthologous *XksuH11* loci were mapped in 4B, 4D, and 5A of wheat (Dvorák et al. 1995; Dubcovsky et al. 1996a,b). Given a lesser degree of distorted segregation, the absence of *XksuH11* heterozygotes in the 6A RSL population would be evidence that *XksuH11Y* is a 6A marker that is unmapped because of distorted segregation. On the basis of the available evidence, however, there is no basis for suggesting the chromosomal location of *XksuH11Y* in *T. turgidum*. A *Xtam 25* marker is present in each of the 6L chromosome arms of hexaploid wheat (Devey and Hart 1993), and *Xtam25Y* and *Xtam25Z*, like *XksuH11Y*, have co-dominant alleles. The *Xtam25* marker(s) segregating in the three mapping populations did not link to any other marker, however, and the chromosomal

location(s) of the marker(s) must be considered unknown.

In summary, the evidence indicates that, with the possible exceptions of *XksuH11* and *Xtam25*, the markers shown in Figs. 2 and 3 are located either in the homoeologue of the substituted/recombined chromosome or in a non-homoeologous chromosome. Both 6B in the 6A RSL population and 6A in the 6B RSL population contain a minimum of 3 alien segments. In 6B, these include a segment marked by *XksuG8-6B* located in the distal part of the 6BS linkage map, a segment located in the proximal part of the 6BS map that includes the markers in group F, and 1 or 2 segments located in the proximal part of the 6BL map that include *Xpsr371-6B* and *Xmwg820-6B*. A segment located in the distal part of the 6AS map that includes the group K markers, 2 segments located in the proximal and distal parts of the 6AL map that include *Xwg286-6A* and the markers located in group L, respectively, and possibly a segment marked by *Xuta(Psif) Z* comprise the alien segments located in 6A in the 6B RSL population. If the *XksuH11* and *Xtam25* loci are disregarded, the data indicate that a minimum of 3 non-group 6 alien segments are located in the 6A RSL population (the group H segment and the 2 segments marked by *Xglk520.2Y* and *Xmwg984Y*) and that at least 1 non-group 6 segment is present in the 6B RSL population (the group M segment).

Given the thorough coverage provided by the markers directly mapped in 6A and 6B, it is probable that few, if any, alien segments in the homoeologue of the chromosome under study were missed. As noted above, the theoretical expectation is that segregating LDN/alien genetic material comprises about 3% of the genetic length of the 13 unsubstituted chromosomes in the RSL populations. Linkage data for some of the markers shown in Fig. 3 and for other markers that co-segregated with markers shown in Fig. 3 are given in Fig. 1. These data indicate that the markers in groups F, K, and L may span as little as 3, 10, and 5 cM, respectively. The markers in group G may be located in either one or two alien segments. Assuming that they are located in 1 segment that spans 5 cM and that the alien segments that contain *XksuG8-6B* and *Xwg286-6A* span 2 cM each (and disregarding the *Xuta(Psif) Z* segment), this indicates that approximately 10 cM of alien genetic material is present in 6B in the 6A RSL population and that approximately 17 cM of alien genetic material is present in 6A in the 6B RSL population, i.e., that alien genetic material comprising approximately 8% and 15%, respectively, of the genetic lengths of the homoeologues of the substituted chromosomes is segregating in the 6A and 6B RSL populations, respectively. Also, if the number and size of the alien chromosomal segments present in non-group 6 chromosomes are approximately the same as in the homoeologues of the substituted/recombined chromosomes, then 40 or more alien segments that span 8–15% of the total genetic length of the unsubstituted chromosomes are present in both of the RSL populations.

The mapping performed in the homoeologues of the substituted chromosomes allowed 10 additional markers to be assigned to approximate positions on the 6A and 6B maps, as shown in Fig. 1.

It is well-established that a significantly larger amount of genetic recombination occurs per unit of physical length in the distal parts of wheat chromosome arms than in the proximal parts of the arms. Three of the alien segments detected in the homoeologues of the substituted/recombined chromosomes are located in the distal parts of the linkage maps (*XksuG8-6B* and the segments containing the markers in groups K and L), the segment marked by *Xwg286-6A* is located in the distal part of 6AL (see above), and the segments that contain the markers in group F and G also may be located in the distal halves of 6BS and 6BL, respectively. It seems safe to conclude, therefore, that the alien segments detected in the homoeologues of the substituted/recombined chromosomes comprise a significantly smaller proportion of the physical lengths of these chromosomes than of their genetic lengths.

Distorted segregation occurred at 5 of the 14 loci located in unsubstituted chromosomes at which a CS allele is thought to be segregating and at all of the 5 loci at which a DIC allele is thought to be segregating (Fig. 2). Highly distorted segregation favoring alien alleles was detected at all of the loci that compose groups F and G, and highly distorted segregation favoring LDN alleles occurred in the centromeric regions of the substituted chromosomes in both RSL populations (see above). These findings suggest that some small alien chromosomal segments cause the male gametes containing them to be favored under some circumstances, such as when the $6B^{D}/6B^{L}$ hybrid was used as the pollen parent in a cross to the $6D^{CS}(6B^{L})$ line during production of the 6B RSL population, and that male gametes containing chromosomes with large centromeric LDN segments are favored when major alien segments are present in recombined wheat/alien chromosomes, such as in the progeny of the $6A^{D}/6A^{L}$ and $6B^{D}/6B^{L}$ hybrids during production of the $6A^{D}(6A^{L})$ and $6B^{D}(6B^{L})$ lines. Including the segments that display distorted segregation, a minimum of 3 alien segments in 6B and 2 alien segments in other chromosomes are fixed in many 6A RSLs, and a minimum of 3 alien segments in 6A and 1 alien segment in another chromosome are fixed in many 6B RSLs. Undoubtedly, fixation also will occur in later generations in some of the 6A and 6B RSLs for some alien alleles that were heterozygous when assayed. The use of molecular markers to select backcross derivatives that lack background alien genetic material could be helpful during future development of lines of this nature, but the elimination of derivatives that contain genetically short alien segments of the type found it this study would be a labor-intensive and costly task because it would be necessary

to assay closely spaced markers distributed uniformly throughout the genome.

The results of this investigation make it clear that future users of these and other RSL populations need to be cognizant of the possible effects of residual background alien genetic material on the outcome of studies performed with the populations. It should be noted, however, that residual background alien genetic material had no discernible effect on the recent mapping of a *T. turgidum* L. 6BS grain-protein-content QTL at a high LOD score using the 6B RSL population (Joppa et al. 1997).

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