Theor Appl Genet (2001) 102:177–186

M.M. Nachit · I. Elouafi · M.A. Pagnotta · A. El Saleh
E. Iacono · M. Labhilili · A. Asbati · M. Azrak
H. Hazzam · D. Benscher · M. Khairallah
J.-M. Ribaut · O.A. Tanzarella · E. Porceddu
M.E. Sorrells

Molecular linkage map for an intraspecific recombinant inbred population of durum wheat *(Triticum turgidum* L. var. durum*)*

Received: 14 February 2000 / Accepted: 28 April 2000

Abstract Durum wheat (*Triticum turgidum* L. var. durum) is an economically and nutritionally important cereal crop in the Mediterranean region. To further our understanding of durum genome organization we constructed a durum linkage map using restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs) known as Gatersleben wheat microsatellites (GWMs), amplified fragment length polymorphisms (AFLPs), and seed storage proteins (SSPs: gliadins and glutenins). A population of 110 F_9 recombinant inbred lines (RILs) was derived from an intraspecific cross between two durum cultivars, Jennah Khetifa and Cham 1. The two parents exhibit contrasting traits for resistance to biotic and abiotic stresses and for grain quality. In total, 306 markers have been placed on the linkage map – 138 RFLPs, 26 SSRs, 134 AFLPs, five SSPs, and three known genes (one pyruvate decarboxylase and two lipoxygenases). The map is 3598 cM long, with an average distance between markers of 11.8 cM, and 12.1% of the markers deviated significantly from the expected Mendelian ratio 1:1. The molecular markers were evenly distributed between the A and B genomes. The chromosome with the most markers is 1B (41 markers), followed by 3B and 7B, with 25 markers each. The chromosomes with the fewest markers are 2A (11 markers), 5A (12 markers), and 4B (15 markers). In general, there is a good agreement between the map obtained and the *Triticeae* linkage consensus maps. This intraspecific map provides a useful tool for marker-assisted selection and map-based breeding for resistance to biotic and abiotic stresses and for improvement of grain quality.

Keywords Durum · RFLP · SSR · AFLP · Seed storage proteins · Genetic linkage mapping

Communicated by P. Langridge

M.M. Nachit (✉) · I. Elouafi · A. El Saleh · M. Labhilili
A. Asbati · M. Azrak · H. Hazzam
ICARDA, CIMMYT/ICARDA, Durum Improvement Program,
P.O. Box 5466, Aleppo, Syria
e-mail: M.Nachit@CGIAR.ORG

A. Pagnotta · E. Iacono · O.A. Tanzarella · E. Poirceddu
Dipartimento di Agrobiologia e Agrochimica,
Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy

M. Khairallah · J.M. Ribaut
CIMMYT, Applied Biotechnology Center, Lisboa 27, A.P. 6–641,
06600 Mexico, D.F. Mexico

D. Benscher · M.E. Sorrells
Department of Plant Breeding, Cornell University, Ithaca,
NY14853, USA

Introduction

Durum wheat (*Triticum turgidum* L. *var. durum*) is an allotetraploid (genome AABB, 2n = 4X = 28) with seven homoeologous groups. It is an especially popular cereal crop of the Mediterranean region, and its grain is used for food products such as pasta, couscous, and burghul. In contrast to both hexaploid wheat (AABBDD), for which several molecular linkage maps of chromosomes have been developed (Chao *et al.* 1989; Liu and Tsunewaki 1991; Devos *et al.* 1992, 1995; Nelson *et al.* 1995a,b,c; Röder *et al.* 1998; Van Deynze *et al.* 1995a; Marino *et al.* 1996; Jia *et al.* 1996) and to diploid relatives of wheat, for which linkage maps have been constructed (Gill *et al.* 1991; Lagudah *et al.* 1991; Dubcovsky *et al.* 1996), relatively little attention has been given to developing genetic linkage maps for durum. Indeed, it was only recently that the first linkage map of durum chromosomes, based on 65 recombinant inbred lines (RILs) and restriction fragment length polymorphism (RFLP) markers was reported (Blanco *et al.* 1998); microsatellite markers from hexaploid bread wheat were subsequently integrated into this genetic linkage map (Korzun *et al.* 1999). Further, because of low polymorphism among cultivated varieties of wheat (Anderson *et al.* 1993; Devos *et al.* 1995) most linkage maps involve interspecific crosses. However, a linkage map based on cultivated varieties may be more useful than an interspecific cross in detecting agriculturally important genes for grain yield and abiotic stress tolerance.

The construction of a molecular linkage map represents the first step in the genetic dissection of a target trait of interest. Once the genomic regions involved in the expression of the target trait are identified, marker-assisted experiments to trace elite alleles at these key regions can be conducted. Different types of molecular markers based on RFLP, microsatellites, and amplified fragment length polymorphism (AFLP) have different characteristics that make them more useful for certain purposes.

RFLPs are most useful for constructing comparative maps across species (Kurata *et al.* 1994; Devos *et al.* 1993; van Deynze *et al.* 1995a) because such probes can detect similar loci with some variation in DNA sequences. AFLPs are highly efficient for saturating a linkage map because up to 20 or 30 segregating loci can be revealed with each primer combination. However, RFLPs are more costly and time-consuming; and as for AFLPs, they may not be transferable across different populations. Microsatellite markers generally exhibit a higher level of polymorphism that is critical for detecting differences among related crop cultivars, and they can even discriminate among closely related wheat breeding lines (Plaschke *et al.* 1995). In addition to their high polymorphism, microsatellites are stable and usually inherited in a co-dominant Mendelian manner. The abundance of information derived from such markers, together with the ease by which they can be identified, make them ideal markers for the construction of genetic linkage maps and useful in marker-assisted selection experiments.

The objective of the study presented here was to construct a saturated linkage map in a recombinant inbred population from a cross between two cultivated durum wheat varieties. This population is ideally suited for studies designed to map genes controlling traits of agronomic importance to durum grown in the Mediterranean region.

Materials and methods

Plant materials

The durum population used in this study consisted of 110 F_9 lines derived by single-seed descent from the cross ICD-MN91–0012 between Tamgurt (Jennah Khetifa) and Cham 1 made in 1991 at the Tel Hadya research station (Aleppo province, Syria) by the CIMMYT/ICARDA durum breeding program for Mediterranean dryland. Tamgurt is a local durum variety collected in 1990 from the southeast high plateau of the Atlas Mountains of Morocco. It is also known as Jennah Khetifa, a landrace grown in the dry areas of Algeria and Tunisia that shows specific adaptation to the North African continental dryland: tall, moderately resistant to drought, cold, leaf rust (*Puccinia recondita* Rob.), powdery mildew (*Erysiphe graminis* DC.), common bunt (*Tilletia caries* D.C.), and septoria leaf blotch *(Mycosphaerella graminicola* {Fuckel} Schroeder), but susceptible to yellow rust (*Puccinia striiformis* Westend.). With respect to quality traits Jennah Khetifa has red grain color, high gluten strength, but low yellow pigment content. Cham 1 was released for commercial production in several countries of the Mediterranean basin under different names (Celta in Portugal; Waha in Algeria and Iraq; Cham 1 in Syria, Turkey, Saudi Arabia, and Sudan; Maru in Jordan; Karpasia in Cyprus). It combines wide adaptation with high yield potential and yield stability. Cham 1 is early, resistant to yellow rust and Russian wheat aphid, but is susceptible to leaf rust, septoria leaf blotch, and powdery mildew. Additionally, it shows high osmotic adjustment, and with respect to quality traits, has white grain color, high yellow pigment content, but low gluten strength.

Probes

The probes used were from wheat (UTV, KSU, WG), barley (BCD, MWG), oat (CDO), rice (RZ), and the Gatersleben wheat microsatellites (GWM). Additional markers included AFLPs, genes, and gliadin and glutenin subunits. Clones were selected based on previously published aneuploid and linkage data from *Gramineae* species. The probes have been described by the following authors: BCD, CDO, WG (Heun *et al.* 1991), RZ (Causse *et al.* 1994), KSU (Gill *et al.* 1991), MWG (Graner *et al.* 1991), GWM (Röder *et al.* 1998), and UTV (Blanco *et al.* 1998). For AFLP, we adopted the nomenclature as follows: *X* for unknown, and P+nucleotides= *Pst*I+nucleotides, M+nucleotides= *Mse*I+ nucleotides.

DNA extraction, Southern blotting, and hybridization

DNA extraction and RFLP analyses were conducted according to Heun *et al.* (1991) and Sharp *et al.* (1988). The parents were surveyed for polymorphism using the restriction enzymes *Eco*RI, *Eco*RV, *Dra*I, *Hind*III, and *Xba*I. The AFLP and simple-sequence repeat (SSR) assays were carried out at ICARDA and CIMMYT using total genomic DNA isolated from young fresh leaves as described by Saghai-Maroof *et al.* (1984) with minor modifications.

AFLP assay

The AFLP method used was as described by Vos *et al*. (1995). The *Pst*I selective primers were labeled by digoxigenin. Nineteen (19) *Mse*I+3/*Pst*I+3 combinations were used: PaagMctg; PaccMcat; PacgMcag; PaggMctg; PaagMcga; PaccMctc; PaagMcgc; PaagMcac; PacgMcgc; PacgMcgg; PaggMcgt; PaccMcga; PaccMcag; PaggMcag; PaggMcat; PacgMctg; PaggMcgg; PaccMcgt; PaagMcag.

Microsatellite assay

Forty-five SSRs (*gwm*) were used to screen the parents. Twenty six were polymorphic and used in mapping. The polymerase chain reaction (PCR) amplifications were performed in 20-µl aliquots of 10 m*M* Tris-HCl (pH 9.0), 50 m*M* KCl, 2.5 m*M* $MgCl_2$, 0.2 m*M* dNTPs, 5% glycerol, 0.25 µ*M* primer, 1 U *Taq* DNA polymerase (Boehringer), and 50 ng of durum genomic DNA. The PCR cycling program used had a touchdown from 63°C to 56°C, and it was carried out in a Perkin-Elmer 9600 thermal cycler. The PCR-amplified fragments were separated on 12% acrylamide gels (39:1), and the fragments were visualized by silver staining. Polymorphisms were scored according to the two parents, either as a pattern when all loci were co-segregating or scored as locus-by-locus when the co-segregation was shown to be broken.

Seed storage protein assay

Gliadin subunits were extracted and fractionated according to the modified procedure of Tkachuk and Metlish (1980). For the glutenin subunit assay, the extraction was with alkylation (Singh *et al.* 1991) on gradient gels. The low-molecular-weight (LMW) glutenins were scored as electrophoretic patterns instead of as individual fragments.

Table 1 Distribution of molecular markers, assignment, and centiMorgan (cM) coverage across the 14 durum A and B genomes

Chromosome	RFLPs	SSRs	AFLPs	Genes	SSPs	Markers		cM	cM/marker
						Number	%		
1A	10	2	10	0	0	22	7.2	201.2	9.1
1B	23	1	14	0	3	41	13.4	459.4	11.2
2A	4	4	3	0	0	11	3.6	138.8	12.6
2B	11	1	11	1	0	24	7.8	320.8	13.4
3A	11	2	9	0	0	22	7.2	300.2	13.6
3B	11	4	10	0	0	25	8.2	345.7	13.8
4A	11	2	17	0	0	30	9.8	302.6	10.1
4B	8	2	4	1	0	15	4.9	133.9	8.9
5A	8	3	1	0	0	12	3.9	172.3	14.4
5B	7	1	7	1	0	16	5.2	239.5	15.0
6A	10	1	10	0	1	22	7.2	325.3	14.8
6B	10	1	8	0	1	20	6.5	251.3	12.6
7A	7	2	12	0	0	21	6.9	174.6	8.3
7B	7	0	18	0	0	25	8.2	232.2	9.3
Total	138	26	134	3	5	306	100.0	3597.8	11.8

Data analyses and linkage mapping

The observed segregation ratios were tested by Chi-square analyses (1:1). Unstable or weak markers were not considered. The data were analyzed using MAKMAKER version 2.0 (Lander *et al.* 1987), and the Haldane function (Haldane 1919) was used to convert the recombination frequencies to centiMorgans (cM). The first linkage groups were constructed via the ''Two-Point/Group'' command with a LOD threshold of 3.0 and maximum recombination of 40%. Whenever possible the ''FirstOrder'' command combined with the LOD table were used to determine the most likely order, and the three-point analysis ''Ripple'' command was used to check the final order. All the groups were constructed at an LOD score of 3.0 except for some chromosomal subgroups (see Fig. 1). The relative order of RFLPs and SSRs markers were compared with the consensus *Triticeae* maps and recently published individual maps (Van Deynze *et al.* 1995a,b; Nelson *et al.* 1995a,b,c; Röder *et al.* 1998; Blanco *et al.* 1998; Korzun *et al.* 1999).

Results and discussion

The distribution of the molecular markers (RFLPs, SSRs, and AFLPs), genes, and seed storage proteins (SSPs) for all chromosome groups are shown in Table 1. The loci are fairly evenly distributed between the A and B genomes except for SSPs. However, in all homologous groups with the exception of group 4 and 6, the B genome shows a slightly higher percentage of molecular markers (Table 1). Out of 444 markers, 306 (138 RFLPs, 26 SSRs, 134 AFLPs, 3 genes, and 5 SSPs) were assigned to the different durum chromosomes. As for the mapping frequency of different kind of markers in relation to chromosomes, the highest number of RFLPs was mapped on 1B, of SSRs on 2A and 3B, of AFLPs on 7B, and of SSPs on 1B.

The chromosome with the most markers is chromosome 1B (41 markers), followed by 4A (30 markers) and 3B and 7B (each 25 markers). The chromosomes with the fewest markers are 5A (12 markers), 2A (13 markers), and 4B (15 markers). The homoeologous group with the most markers is group 1 (63 markers); – 20.6% of the total markers mapped; – followed by group 3 (47 markers). Group 5 has the fewest, with 28 markers; i.e., 9.1%. The 306 markers cover a total of 3598 cM, almost 2.5 times longer than the linkage map reported by Blanco *et al.* (1998) for the cross durum × *Triticum dicoccoides*. This longer map is due mainly to the intraspecific cross durum × durum and to the greater number of mapped markers. The average distance between marker pairs is 11.8 cM, ranging from 8.3 cM for chromosome 7A to 15 cM for 5B (Table 1), with some gaps wider than 30 cM on all linkage groups, except for 1A and 4B (Fig. 1). Chromosomes 1B and 5A showed marker clustering around the centromeric region. Marker clusters are usually associated with reduced recombination in the proximal region of chromosome arms. However, in this mapping population the marker distribution was relatively adequate and few clusters of tightly linked loci were revealed, as all RFLP and SSR marker locations were known and the markers were selected to avoid closely linked multiple loci.

Analysis of the segregation data for the percentage of Jennah Khetifa genotypes for each of the 306 markers mapped showed a mean value of 49.9%, whereas for Cham 1 this was 48.4%, with a residual heterozygosity of 1.7%. Most of the molecular markers, seed storage proteins, and genes (87.9%) showed Mendelian segregation (1:1 ratio; $\alpha = 0.01$) in the 110 RILs, however 37 (12.1%) had significant deviations from the expected ratio (Tables 2 and 3). These markers with skewed segregation occur in all chromosomes, except in 2A and 4B. The chromosomes with the most mapped skewed markers are 3 A, 5 A, 6 A, and 6B. Distorted markers favoring Jennah Khetifa were found on 5A, 5B, 3B, and 7B; those favoring Cham 1 on 1A, 1B, 2B, and 7A (Table 3). Additionally, distortions favoring both Jennah Khetifa or Cham 1 in the same chromosome were also found (Table 3).

There was no major difference between RFLPs and AFLPs in the frequency of disturbed segregation, but the percentage of disturbed segregation for microsatellites was higher (30%) than that of RFLPs (11.6%) or AFLPs

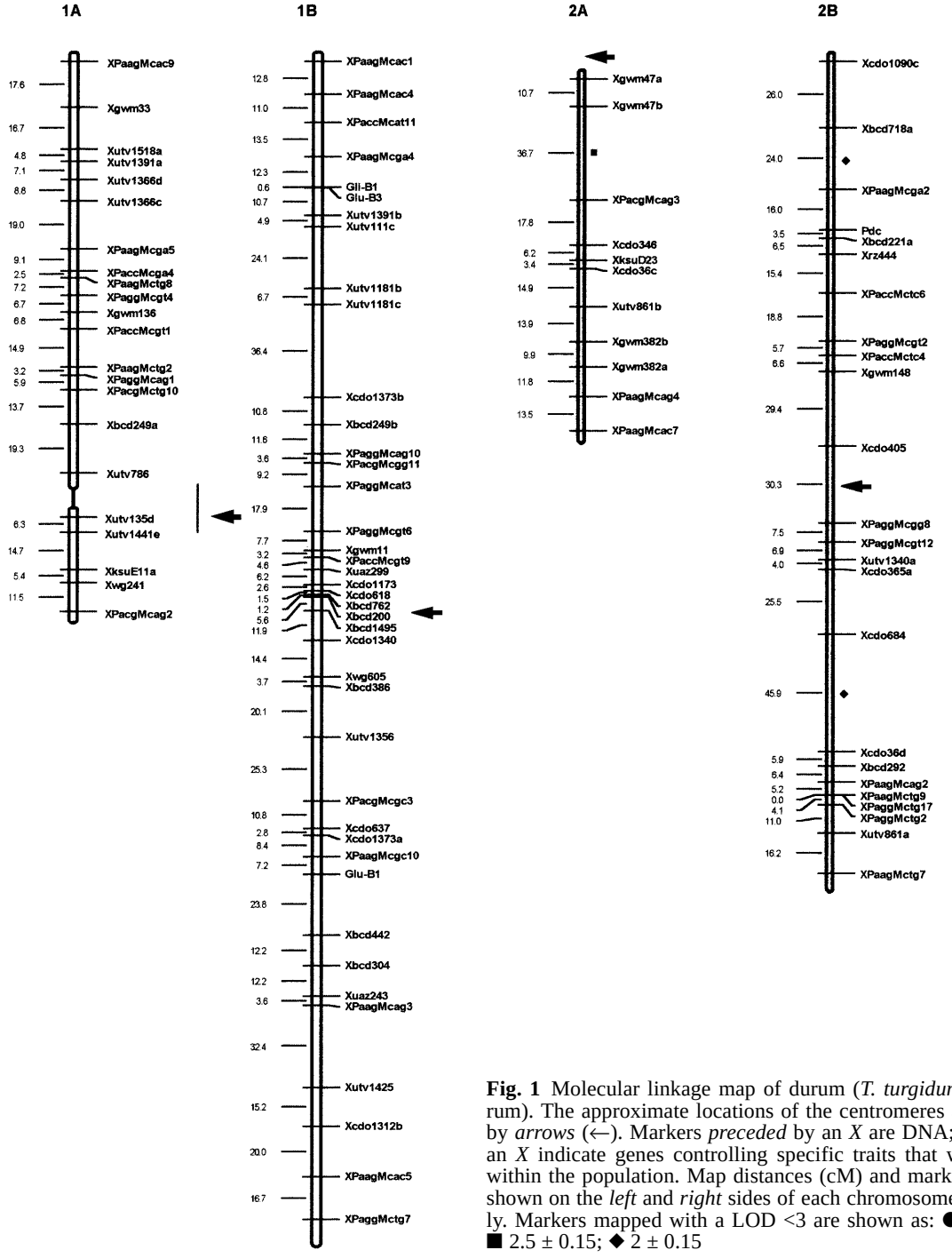

Fig. 1 Molecular linkage map of durum (*T. turgidum* L. var. durum). The approximate locations of the centromeres are indicated by *arrows* (←). Markers *preceded* by an *X* are DNA; and *without* an *X* indicate genes controlling specific traits that were assayed within the population. Map distances (cM) and marker names are shown on the *left* and *right* sides of each chromosome, respectively. Markers mapped with a LOD <3 are shown as: ● 1.5 ± 0.15; ■ 2.5 ± 0.15; ◆ 2 ± 0.15

(9.7%). However, fewer microsatellites (26) were mapped than RFLPs (138) or AFLPs (134). Molecular markers representing skewed segregation have already been reported in other *Triticeae* species (Heun *et al.* 1991; Blanco *et al.* 1998). Chromosomal rearrangements (Tanksley 1984), alleles inducing gametic or zygotic selection (Nakagarha 1986), parental reproductive differences (Foolad *et al.* 1995), and the presence of lethal genes (Blanco *et al.* 1998) have been suggested as potential causes of distortion. Furthermore, the wide genetic background of the parents could also be a cause of distortion, as Jennah Khetifa and Cham 1 are genetically distant from each other. In addition, the use of the single seed descend method to generate the RILs could also be another cause of segregation distortion.

In general, there was a good agreement for marker order between this map and the consensus maps published by Van Deynze *et al.* (1995a,b), the consensus physical map (Gill *et al.* 1991) and other linkage maps (Gale *et al.* 1995; Blanco *et al.* 1998; Korzun *et al.* 1999). How-

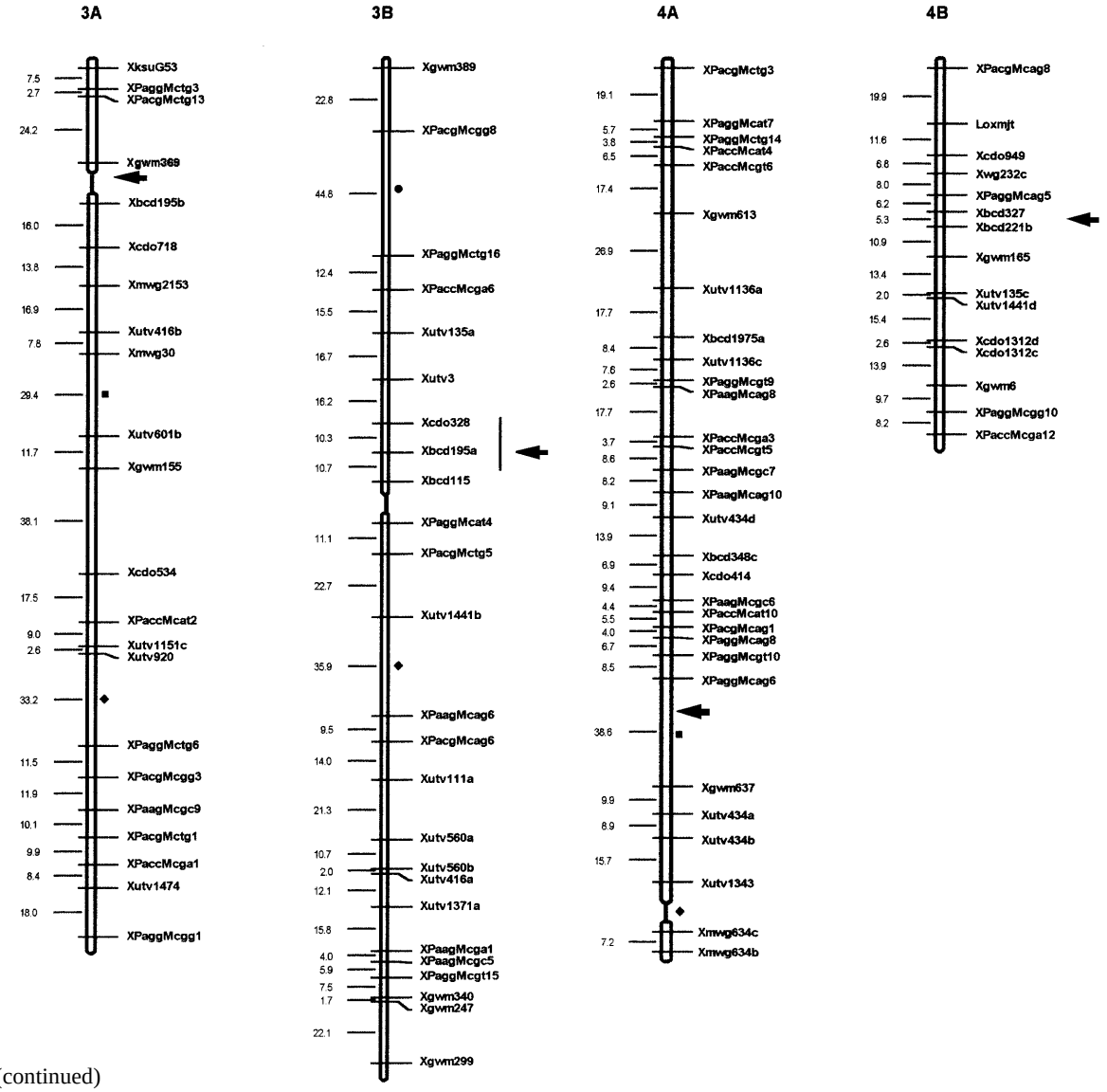

Fig. 1 (continued)

Table 2 Percentage of skewed segregation for different markers in the durum chromosomes of the population Jennah Khetifa × Cham 1

Chromosome	RFLPs	SSRs	AFLPs	Genes	SSPs	Skewed markers	
						Number	%
1A	0	1	0	0	0	1	2.7
1B	2	0	1	0	0	3	8.1
2A	0	0	0	0	0	0	0.0
2B	1	0	2	0	0	3	8.1
3A	1	1	2	0	0	4	10.8
3B	0	0	2	0	0	2	5.4
4A	1	1	1	0	0	3	8.1
4B	0	0	0	0	0	0	0.0
5A	3	3	1	0	0	7	18.9
5B	1	0	0	0	0	1	2.7
6A	2	0	2	0	0	4	10.8
6B	3	1	0	0	0	4	10.8
7A	2	1	0	0	0	3	8.1
7B	0	0	2	0	0	2	5.4
Total	16	8	13	0	0	37	100.0

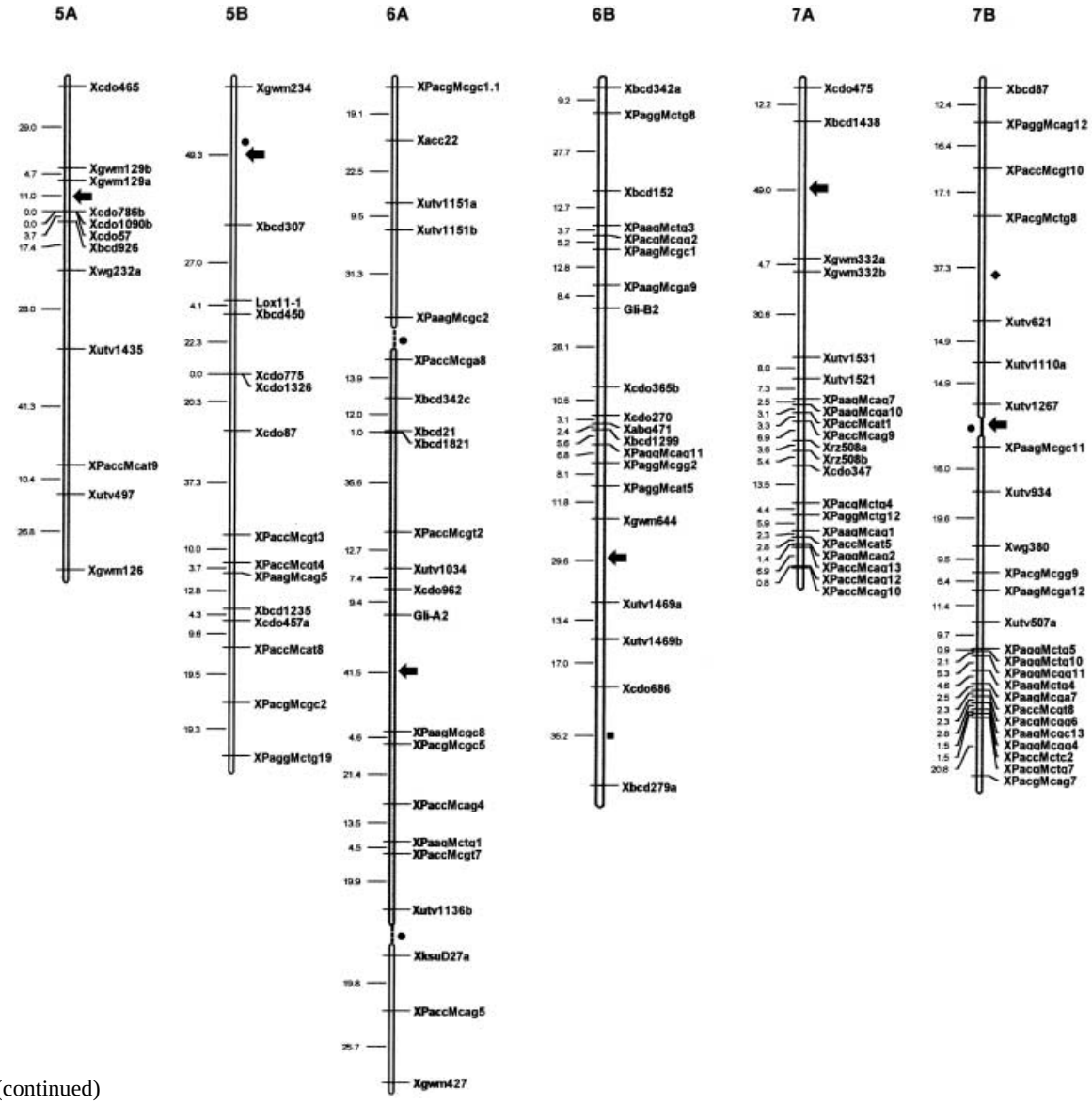

Fig. 1 (continued)

ever, discrepancies were also detected, and details are presented here for each specific chromosome. For unpublished marker assignments, the mapping libraries of Cornell and Tuscia Universities were consulted (M.E. Sorrells and M.A. Pagnotta, personal communication). The centromeres were positioned at the midpoints between the most proximal markers on the short and long arm according to previously published maps (Nelson *et al.* 1995a,b,c; Röder *et al.* 1998; Korzun *et al.* 1999; McGuire and Qualset 1997).

Chromosomes 1A and 1B

The highest level of polymorphism was found in chromosome 1B, which had 13.4% of the total markers mapped (Table 1). These results confirm earlier reports (Van Deynze *et al.* 1995a). No clustering of markers was shown for 1A; however, for 1B some clustering of markers was revealed near the centromere. This is in agreement with earlier findings in wheat (Snape *et al.* 1985; Dvorak and Appels 1986; Curtis and Lukaszewski 1991; Devos *et al.* 1992; Werner *et al.* 1992). The linkage map for group-1 chromosomes (Fig. 1) comprises 63 markers. Chromosomes 1A and 1B consist of 22 and 41 markers, respectively. The centromere was placed on 1A between *Xutv786* and *XksuE11*; the location of X*utv135d* is unknown, and of *Xutv1441e* was mapped on 2AS (Blanco *et al.* 1998; Korzun *et al.* 1999). As for 1B, the centromere was positioned between *Xbcd762* and *Xbcd200*.

The relative order of the markers on 1AS is similar to that reported for the Messapia × MG4343 population (Korzun *et al.* 1999). Compared to the consensus map of 1A (Van Deynze *et al.* 1995a), the common markers, *Xbcd249* on the short arm and *XksuE11* and *Xwg241* on the long arm, are in the same order. In contrast, there are

Table 3 Molecular markers with significant skewed segregation ratios

Chromosome	Marker[a]	%Cham 1[b]	Chromosome	Marker[a]	%Cham 1[b]
1A	*Xgwm33*	65	1B	*Xbcd386*	62
				Xutv1356	68
3A	*Xgwm155*	64		*XPaggMcgc10*	63
	XPacgMctg1	37			
	XPacgMctg13	36	2B	*Xcdo405*	62
	Xutv920	65		*XPaccMctc6*	67
				XPaggMcgg8	64
4A	*Xwg232a*	26			
	Xutv1136a	80	3B	*XPaagMcag6*	36
	Xgwm613	71		*XPaccMcga6*	37
5A	*Xcdo786b*	24	5B	*Xcdo457a*	36
	Xgwm126	23			
	Xgwm129a	22	6B	*Xbcd279a*	62
	Xgwm129b	30		*Xgwm644*	34
	XPaccMcat9	34		*Xutv1469a*	16
	Xutv1435	37		*Xutv1469b*	35
	Xutv497	27	7B	*XPaggMcgc11*	32
				XPaggMcgt10	38
6A	*Xbcd342c*	68			
	XPaccMcga8	63			
	XPacgMcgc1.1	33			
	Xacc22	35			
7A	*Xgwm332b*	66			
	Xutv1521	62			
	Xutv1531	62			

[a] Markers that did not fit the expected 1: 1 ratio for α =0.01
[b] Percentage of segregates that the carried Cham 1 allele

two discrepancies between this map and the map published by Röder *et al.* (1998). On the short arm, the chromosomal segment between the two microsatellites *Xgwm136* and *Xgwm33* was switched; however, we could not detect an inversion of *XksuE11* and *Xwg241*, and our results are similar to those of the consensus map.

In 1B chromosome, the marker positions agreed with the consensus map: *Gli-B1, Glu-B3, Xbcd249, Xcdo1173, Xcdo618, Xbcd762, Xbcd200, Xbcd1495, Xwg605, Xbcd386, Xcdo637, Glu-B1, Xbcd442*, and *Xbcd304*. With the durum map by Korzun *et al.* (1999), there was also consistency for the markers: *Gli-B1*, *Glu-B3*, *Xutv111*, *Xutv1181*, *Xgwm11*, *Glu-B1*, and *Xutv1425*. However, there are two discrepancies in the relative order of markers: The segment of 1B genome *Gli-1B* and *Xutv111* is inverted between the two maps. In our map the distance between *Gli-B1* and *Glu-B3* is 0.5 cM versus 3.3 cM in the map reported by Korzun *et al.* (1999). Secondly, the microsatellite *Xgwm11* was mapped in our study in the short arm of 1B chromosome as reported for the M6 x Opata hexaploid wheat map (Röder *et al.* 1998).

As is the case for *T. aestivum*, genes that control grain quality are located in this group. Homoeologous group-1 chromosomes are important in marker-assisted selection as they contain chromosomal regions of major interest to the breeder for improvements in grain quality and biotic stress resistance. The largest chromosomal effects on gluten strength (gliadin and glutenin subunits) are found in chromosome group 1. Further, there are several genes located in this group for resistance against the three rusts and powdery mildew (McGuire and Qualset 1997). The parental varieties Jennah Khetifa and Cham 1 show contrasting gluten strength and reactions to the rusts and powdery mildew diseases.

Chromosomes 2A and 2B

The linkage map of the group 2 chromosomes (Fig. 1) comprises 35 markers. With respect to 2A, four RFLPs, 4 SSRs, and 3 AFLPs were mapped on 2AL. For 2AL, the mapped RFLP and SSRs were consistent with previous reports (Korzun *et al.* 1999; Röder *et al.* 1998; Nelson *et al.* 1995a). The short arm of 2A was not mapped with any molecular marker used in this study, the RFLP markers *Xcdo1090c* and *Xbcd718a* are different fragments than those mapped in the M6 × Opata (Nelson et al. 1995a). The RFLP and microsatellite markers found on the long arm are consistent with previous reports (Korzun et al. 1999; Röder et al. 1998; Nelson et al. 1995a). The centromere was drawn above the marker *Xgwm47*, as reported by Röder *et al.* (1998). The SSRs *Xgwm47* and *Xgwm382* each amplified two nearby loci. The AFLP markers were from different primer combinations. Two of them were located near the telomere of 2AL, while the *XPacgMcag3* locus was located between a SSR and a RFLP.

The markers mapped in 2B include 11 RFLPs, 11 AFLPs, one SSR, and one gene, *Pdc* (pyruvate decarboxylase). These markers are consistent with the consensus map (Blanco *et al.* 1998; Röder *et al.* 1998; Korzun *et al.* 1999). For chromosome 2B, the centro-

mere was positioned between the markers *Xcdo405* and *Xutv1340a*, and between these 2 markers, 2 AFLP loci (*XPaggMcgg8 and XPaggMcgt12*) were mapped nearer to *Xutv1340a* (Fig. 1). Chromosome 2B is relatively well covered with markers; however, for 2A, additional markers are needed to map 2AS.

The homoeologous group 2 chromosomes are important determinants of *Triticum* adaptation, with loci controlling photoperiod and vernalization responses, plant height (Nelson *et al.* 1995a), and early heading (Scarth and Law 1984). This group is also important for resistance to diseases as numerous resistance genes are located on chromosomes 2A and 2B against rust, powdery mildew, *septoria nodorum* blotch, and bunt. Two semi-dwarfing genes also lie on these chromosomes as well as a region associated with resistance to preharvest sprouting (Anderson *et al.* 1993). The parents Jennah Khetifa and Cham 1 show contrasting values for photoperiod and vernalization requirements, earliness, height, and resistance to bunt. Finer mapping in this population, in conjunction with accurate phenotypic data, may yield the locations of QTLs for several traits in this chromosome group.

Chromosomes 3A and 3B

Twenty-two and twenty-five markers were mapped on chromosomes 3A and 3B, respectively; most were mapped on the long arms. Chromosome 3A markers, *XksuG53, Xgwm369, Xcdo718, Xutv416,* and *Xgwm155* correspond to previously published work (Röder *et al.* 1998; Korzun *et al.* 1999). The centromere was positioned between the microsatellites *Xgwm369* and *Xbcd195b*. In the case of chromosome 3B, map locations of *Xgwm389, Xutv3, Xcdo328, Xbcd115, Xutv111, Xgwm340, Xgwm247,* and *Xgwm299* were also similar to earlier reports (Röder *et al.* 1998; Blanco *et al.* 1998; Korzun *et al.* 1999). The centromere of chromosome 3B was located between *Xcdo328* and *Xbcd115* (the arm location of *Xbcd195a* is unknown).

A dense linkage map enables the selection of evenly distributed markers over the entire genome for loci controlling traits of interest (Nelson *et al.* 1995b). The markers mapped on this homoeologous group provide a good coverage of 3A and 3B. Chromosome group 3 also contains several genes for resistance to rusts, *septoria nodorum* blotch, powdery mildew, and root knot nematode. The genes for red grain color are located on the short arms of this group. The parents of this population have contrasting grain colors red – for Jennah Khetifa red and white for Cham 1.

Chromosomes 4A and 4B

Homoeologous group 4 had a total 45 markers mapped: chromosome 4A has 30 markers, including 11 RFLPs, 2 SSRs, and 17 AFLPs (Table 1), while chromosome 4B 15 markers were mapped, 6 on the short arm, and 9 on the long arm; these include 8 RFLPs, 2 SSRs, *Loxmjt*, and 4 AFLPs. The lipoxygenase gene (*Loxmjt*) was mapped on the short arm of 4B as expected. The suggested centromere position of chromosome 4B is between *Xbcd327* and *Xbcd221b*. *Loxmjt* was cloned and sequenced at the Plant Breeding Department (Cornell University; M.E. Sorrells, personal communication). It mapped in same position as *Lpx-B1*.

Chromosome group 4 carries several genes on the short arms for resistance to leaf and stem rust and for reduced plant height. Chromosome 4D, colinear with 4B, also carries resistance to several abiotic stresses, such as salinity and aluminium toxicity. Several of the markers mapped on 4A or 4B have been mapped previously to 4D in the synthetic M6 × Opata population (Nelson *et al.* 1995c; Röder *et al.* 1998); these include *Xmwg634c and Xmwg634b* on 4A, and *Xcdo949, Xbcd327, Xcdo1312d,* and *Xcdo1312c on* 4B.

Chromosomes 5A and 5B

On chromosome 5A, 12 markers were mapped, 9 of which were located on the long arm. The centromere of 5A was positioned between *Xgwm129a* and *Xcdo786b* based on previously published maps (Nelson *et al.* 1995c; Röder *et al.* 1998). For chromosome 5B, only 1 marker, *Xgwm234,* was located on the short arm with LOD 2, whereas 15 markers are on the long arm. The AFLP fragments *XPaccMcat7* and *-8* were mapped to the same position. The centromere is located between *Xgwm234* and *Xbcd307*. The RFLP loci *Xcdo1326* and *Xcdo775* co-segregated in this population, but *Xcdo775* was mapped on 7L using aneuploid stocks and *Xcdo1326* mapped to the long arm of 5A in the synthetic M6 × Opata population (Nelson *et al.* 1995c). The gene *Lox11–1* for α–lipoxygenase was also cloned and sequenced at the Plant Breeding Department (Cornell University; M.E. Sorrells, personal communication). It mapped in the same position as *Lpx-B2*.

Several other useful genes present on group-5 chromosomes have already been tagged with molecular markers. These include copper efficiency, grain hardness, lipoxygenase, accumulation of abscissic acid, reduced height, response to vernalization, insensitivity to tan spot and resistance to *Cochliobolis* root rot, leaf rust, and yellow rust.

Chromosomes 6A and 6B

The homoeologous group 6 consists of 42 markers 20 RFLPs (10 in 6A and 10 in 6B), 2 microsatellites (1 in 6A and 1 in 6B), 18 AFLPs (10 in 6A and 8 in 6B), and 2 SSPs (1 in 6A and 1 in 6B). The centromere for 6A was positioned between *Gli-A2* and *Xutv1136*; several AFLPs were between the 2 markers (Fig. 1). The centromere of chromosome 6B was located between *Xgwm644* and *Xutv1469a*. Chromosome group 6 is the location for sever-

al resistance genes to biotic stresses, including resistance genes for leaf, yellow, and stem rust, and powdery mildew.

Chromosomes 7A and 7B

Chromosome group 7 contains 46 markers, 15 RFLPs (7 in 7A and 8 in 7B), 2 microsatellites (both in 7 A), 30 AFLPs (12 in 7A and 18 in 7B). In chromosome 7 A, 2 markers were located on the short arm and 19 on the long arm. The centromere was positioned between *Xbcd1438* and *Xgwm332*. As for chromosome 7B, 25 markers were mapped. The centromere of chromosome 7B was located between *Xutv1267* and *Xutv934*; *Xpaag-Mcgc11* was mapped closer to *Xutv934*.

Chromosomes 7A and 7B also carry loci controlling reduced height and response to vernalization; in addition to carrying genes for rust resistance, they carry resistance genes to powdery mildew, barley yellow dwarf virus, and Russian wheat aphid. As reported earlier, the parents also show contrasting resistance levels to Russian wheat aphid.

Conclusion

This population is derived from a cross between genetically diverse parents and is therefore ideal for identifying loci controlling both qualitative and quantitative traits. The traits associated with abiotic stresses (drought, cold, and heat) will be given high priority for quantitative trait loci (QTL) analysis, as will the grain quality traits that are linked to the endproducts (pasta, couscous, and burghul). The Jennah Khetifa × Cham 1 population is also of interest for localization of QTLs for biotic stresses such as the three rusts, powdery mildew, tan spot, common bunt, gall nematode (*Anquina tritici*), and Russian wheat aphid.

Acknowledgements We are thankful for the helpful discussions and review by O. Abdalla, A. Amri, M. El Bouhssini, A. Yahyaoui, J. Ryan, S. Grando, R.S. Malhotra, A. Sarker; and for the valuable support during this study by N. Fadda, A. El Beltagy, S. Rajaram, J. Dubin, D. Hoisington, R. Coffmann, and W. Erskine. The authors also thank Obeid Jassem, Reem Shab-Kalyeh, and Douha Terrisy for excellent technical support. The authors gratefully acknowledge the Ministry of Foreign Affairs of Italy, ICARDA, CIMMYT, USAID/Egypt (ATUT durum project), GRDC / Australia, INIA-Ministry of Agriculture of Spain, Cornell University, and Tuscia University for financial support of this project. The experiments comply with the laws of the countries where they were performed.

References

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1993) Optimizing parental selection for genetic linkage maps. Genome 36:181–186

Blanco A, Bellomo MP, Cenci A, De Giovanni C, D'Ovidio R, Iacono E, Laddomada B, Pagnotta MA, Porceddu E, Sciancalepore A, Simeone R, Tanzarella OA (1998) A genetic linkage map of durum wheat. Theor Appl Genet 97:721–728

Causse M, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SB, Second GA, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

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

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

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

Devos KM, Dubcovsky J, Dvorak, J, Chinoy CN, Gale MD (1995) Structural evolution of wheat chromosomes 4A, 5A, and 7B and its impact on recombination. Theor Appl Genet 91:282–288

Devos KM, Millan T, Gale MD (1993) Comparative RFLP maps of homoeologous group 2 chromosomes of wheat, rye and barley. Theor Appl Genet 85:784–792

Dubcovsky J, Luo MC, Zhong GY, Bransteitter R, Desai A, Kilian A, Kleinhofs A, Dvorak J (1996) Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics 143:983–999

Dvorak J, Appels R (1986) Investigation of homologous crossing over and sister chromatid exchange in the wheat Nor-B2 locus coding for rRNA and Gliadins. Genetics 113:1037–1056

Foolad MR, Arulsekar S, Becerra V, Bliss FA (1995) A genetic map of *Prunus* based on an interspecific cross between peach and almond. Theor Appl Genet 91:262–269

Gale MD, Aktinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. In: Li ZS, Xin ZY (eds) Proc 8th Int Wheat Genet Symp. China Agric Scientech Press, Beijjing, pp 29–40

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

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Haldane JBS (1919) The combination of linkage values, and the calculation of distance between linked factors. J Genet 8:299–309

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Jia J, Devos KM, Chao S, Miller TE, Reader SM, Gale MD (1996) RFLP-based maps of the homoeologous group 6 chromosomes of wheat and their application in the tagging of *pm12*, a powdery mildew resistance gene transferred from *Aegilops speltoides* to wheat. Theor Appl Genet 9:559–565

Korzun V, Röder MS, Wendekake K, Pasqualone A, Lotti C, Ganal MW, and Blanco A (1999) Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat. Theor Appl Genet 98:1202–1207

Kurata N, Moore G, Nagumara Y, Foote T, Yano M, Minobe Y, Gale M (1994) Conservation of genome structure between rice and wheat. Biotechnology 12:276–278

Lagudah ES, Appels R, Brown AHD, McNeil D (1991) The molecular-genetic analysis of *Triticum tauschii*, the D genome donor to hexaploid wheat. Genome 34:375–386

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

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

Marino CL, Nelson JC, Lu HY, Sorrells ME, Leroy P (1996) Molecular genetic maps of the group 6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). Genome 39:359–366

McGuire PE, Qualset CO (1997) Progress in genome mapping of wheat and related species. (Joint proceedings 5th and 6th public workshops of the International Triticeae Mapping Initiative). Genetic Resources Conservation Program, Division of Agriculture and Natural Resources, University of California, USA

Nakagarha M (1986) Geographic distribution of gametophyte genes in wide crosses of rice cultivars. In: IRRI (ed) Rice genetics. (Proc Int Rice Genet Symp). IRRI, Manila, The Philippines, pp 73–82

Nelson JC, van Deynze AE, Autrique E, Sorrells ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995a) Molecular mapping of wheat Homoeologous group 2. Genome 38:516–524

Nelson JC, Van Deynze AE, Lu YH, Autrique E, Sorrells ME, Negre S, Bernard M, Leroy P (1995b) Molecular mapping of wheat Homoeologous group 3. Genome 38:525–533

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris J, Anderson J (1995c) Molecular mapping of wheat. Major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141:721–731

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 92:1078–1084

Röder MS, Korzun V, Wandehake K, Planschke J, Tixier MH, Leroy P, Ganal M W (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Scarth R, Law CN (1984) The control of the daylength response in wheat by the group 2 chromosomes. J Plant Breed 92:140–150

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of α-amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–290

Singh NK, Shepherd KW, Cornish GB (1991) A simplified SDS-page procedure for separating LMW subunits of glutenin. J Cereal Sci 14:203–208

Snape JW, Flavell RB, O'Dell M, Hughes WG, Payne PI (1985) Intrachromosomal mapping of the nuclear organiser region relative to three marker loci on chromosome 1B of wheat (*Triticum aestivum*). Theor Appl Genet 69:263–270

Tanksley SD (1984) Linkage relationships and chromosomal locations of enzyme-coding genes in pepper, *Capsicum annum.* Chromosoma 89:352–360

Tkachuk R, Metlish VJ (1980) Wheat cultivar identification by high-voltage gel electrophoresis. Ann Technol Agric 29:207–212

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995a) Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genome 38:45–59

Van Deynze AE, Nelson JC, Harrington SE, Yglesias ES, Braga D, McCouch SR, Sorrells ME (1995b) Comparative mapping in grasses: wheat relationships. Mol Gen Genet 248:744–754

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Werner JE, Endo TR, Gill BS (1992) Toward a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci USA 89:11307–11311