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ORIGINAL PAPER

High-density genetic map of durum wheat × wild emmer wheat based on SSR and DArT markers

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Abstract A genetic linkage map of tetraploid wheat was constructed based on a cross between durum wheat [*Triticum turgidum* ssp. *durum* (Desf.) MacKey] cultivar Langdon and wild emmer wheat [*T. turgidum* ssp. *dicoccoides* (Körn.) Thell.] accession G18-16. One hundred and fifty-two single-seed descent derived F_6 recombinant inbred lines (RILs) were analyzed with a total of 690 loci, including 197 microsatellite and 493 DArT markers. Linkage analysis defined 14 linkage groups. Most markers were mapped to the B-genome (60%), with an average of 57 markers per chromosome and the remaining 40% mapped to the A-genome, with an average of 39 markers per chromosome. To construct a stabilized (skeleton) map, markers interfering with map stability were removed. The skeleton map consisted of 307 markers with a total length of 2,317 cM and average distance of 7.5 cM between adjacent markers. The length of individual chromosomes ranged between 112 cM for chromosome 4B to 217 cM for chromosome 3B. A fraction (30.1%) of the markers deviated significantly from the expected Mendelian ratios; clusters of loci showing distorted segregation were found on chromosomes 1A, 1BL, 2BS, 3B, and 4B. DArT markers showed high proportion of clustering, which may be indicative of gene-rich regions. Three hundred and fifty-two new DArT markers were mapped for the first time on the current map. This map provides a useful groundwork for further genetic analyses of important quantitative traits, positional cloning, and marker-assisted selection, as well as for genome comparative genomics and genome organization studies in wheat and other cereals.

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Introduction

Wheat (*Triticum* spp.) is one of the most widely grown food grain crops in the world, providing about one-fifth of the calories consumed by humans (FAOstat 2007). Construction of a genetic map with molecular markers is a key step in convenient linkage analysis of biologically or agronomically important traits. Genetic linkage maps are a fundamental tool for several purposes, such as evolutionary genomics, understanding the biological basis of complex traits, dissection of genetic determinants underlying the expression of agronomically important traits and, finally, facilitating marker-assisted selection (MAS) and map-based cloning.

Wheat genetic maps were first comprised of restriction fragment length polymorphisms (RFLPs) and later on PCR-based markers were adopted, including random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) (Gale et al. 1995; Messmer et al. 1999; Peng et al. 2000; Paillard et al. 2003; Blanco et al.

1998). Recently, simple sequence repeats (SSRs, also known as microsatellites) have become the markers of choice for cereal genetic analysis and mapping (Varshney et al. 2007). To date, over 2,000 SSR markers on 21 hexaploid wheat chromosomes have been published (see Ganal and Röder 2007). Molecular marker technologies, however, are currently undergoing a transition from largely serial technologies based on separating DNA fragments according to their size (e.g., SSR, AFLP) to highly parallel, hybridization-based technologies that can simultaneously assay hundreds to tens of thousands of markers.

Diversity arrays technology (DArT) was developed as a hybridization-based alternative which captures the values of the parallel nature of the microarray platform (Jaccoud et al. 2001). DArT simultaneously types several thousands of loci in a single assay. DArT generates whole-genome fingerprints by scoring the presence versus absence of DNA fragments in samples of genomic DNA. DArT has recently been used in genetic mapping and fingerprinting studies in cereals such as rice (Jaccoud et al. 2001), barley (Wenzl et al. 2004), and wheat (Akbari et al. 2006; Semagn et al. 2006).

Wild emmer wheat [*Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell.] is an allo-tetraploid species comprised of two sub-genomes (A and B) with $2n = 4\times = 28$ chromosomes. It is the progenitor of tetraploid (genome BBAA) durum wheat [*T. turgidum* ssp. *durum* (Desf.) MacKey] and hexaploid ($2n = 6\times = 42$; genome BBAADD) bread wheat (*T. aestivum* L.) (Feldman 2001). Wild emmer thrives across wide ecological conditions throughout the Near East Fertile Crescent (Harlan and Zohary 1966). Hence, it may offer valuable source of allelic repertoire for improvement of agronomically important traits in cultivated wheat (Nevo et al. 2002; Peleg et al. 2005). Furthermore, it is estimated that only 10–20% of the wild alleles have been used in modern wheat varieties (Langridge et al. 2006).

The objectives of the present study were to (1) construct a high-density genetic linkage map in a population of recombinant inbred lines (RILs) obtained from a cross between durum wheat cultivar and its wild progenitor, *T. dicoccoides*, and (2) analyze the distribution of DArT versus SSR markers throughout the A and B genomes.

Materials and methods

Mapping population and genomic DNA extraction

The mapping population used in this study consisted of 152 F_6 recombinant inbreed lines (RILs) derived from a cross between durum wheat (female) cultivar Langdon (LDN hereafter) and wild emmer wheat (male) accession G18-16. The wild emmer wheat accession was sampled from Gitit (35°24′N, 32°06′E; 288.4 m above sea level) (see Table 1 in Peleg et al. 2005). F_1 progeny from the initial cross were taken through five generations of selfing via single seed decent (SSD) procedure to produce homozygous RILs.

DNA was extracted from fresh leaf tissue (~200 mg) of 2-week-old wheat seedlings of the two parent lines and the RIL population using DNAzol ES kit (Molecular Research Center Inc., Cincinnati).

Microsatellite assay

A total of 914 wheat microsatellite (SSR) markers were used to screen the parental lines for polymorphism. These markers consisted primarily of Gatersleben Wheat Microsatellites (GWM; Röder et al. 1995, 1998; Ganal and Röder 2007) and a few additional markers from Wheat Microsatellite Consortium (WMC; Gupta et al. 2002), Dupont Company (DuPw; Eujayl et al. 2002), INRA Clermont-Ferrand (CFA and CFD; Sourdille et al. 2004; Guyomarc'h et al. 2002), Beltsville Agriculture Research Center (BARC; Song et al. 2005), and Cornell University and Kansas State University (cnl and ksum; Yu et al. 2004). One hundred and ninety-six markers showing codominant alternative alleles between the two parental lines and covering all 14 chromosomes of tetraploid wheat (based on previously published maps) were used to genotype the 152 RILs.

PCR reactions were carried out in a 25 µl reaction volume under the following conditions: one denaturation cycle at 94°C for 5 min followed by 35 cycles of 94°C for 60 s, 50–65°C (depending on the primer) for 60 s, and 72°C for 90 s, followed by an elongation step of 72°C for 7 min. Fragment analysis was carried out in an automated laser fluorescence (A.L.F.) sequencer and analyzed using the computer program Fragment Analyzer ver. 1.02 (Amersham Biosciences) by comparing with internal size standards (Röder et al. 1995). The bread wheat cultivar Chinese Spring and the two parental lines were used as a reference in each run to ensure size accuracy and to avoid run-to-run and gel-to-gel variations.

DArT assay

Diversity array technology (DArT) marker assays were performed by Triticarte Pty. Ltd (Canberra, Australia; http://www.triticarte.com.au) as previously described (Wenzl et al. 2004; Akbari et al. 2006; Semagn et al. 2006) and were used to provide additional genomic converge. Briefly, a genomic representation of a mixture of 13 cultivars was produced after *Pst*I–*Taq*I digestion, spotted on microarray slides, and the individual genotypes were screened for polymorphism based on fluorescence signals. DNA from the parents (G18-16 and LDN) was first screened for polymorphism and then the individual RILs were genotyped. A total

of 493 loci were scored as present (1) or absent (0). Names of loci that were previously mapped by Triticarte Pty. Ltd include the prefix "wPt" (followed by numbers corresponding to a particular clone); loci that were mapped for the first time on the current map are presented by clone ID number.

Data analysis and linkage map construction

For each segregating marker, a $\chi 2$ analysis was performed to test for deviation from the 1:1 expected segregation ratio. The genetic map was constructed as described in Mester et al. (2003a, b, 2004) and Ronin et al. (2008) using the MultiPoint package (http://www.MultiQTL.com). First, the pairwise recombination fractions (rf) were calculated for all pairs of markers using maximum likelihood estimation procedure. Then, the number of clusters (linkage groups, LG) was evaluated as a function of the threshold (maximal) value rf_0, allowing for preliminary assignment of a marker to a certain LG. For example, marker m_i may be assigned to an LG_j if recombination between m_i and at least one marker from LG_j is lower than the threshold rf_0 and is the lowest compared to its distances to any other LG. The number of scored markers may considerably exceed the number of practically resolvable markers by recombination for the given population size. Thus, only a small portion of markers (here referred to as *delegate markers*) can be included in the skeleton map, with the remainder of markers being attached to the delegates. Besides non-resolvable linkage caused by small sample size, the necessity for selection of representative markers for the skeleton map derives from non-random (clustered) recombination distribution in the genome (Korol et al. 1994), varying information content of markers (missing data, distorted segregation, and scoring errors), and negative interference (Peng et al. 2000; Esch and Weber 2002).

The reliability of the obtained multilocus map order was tested using the jackknife resampling procedure (Mester et al. 2003b) implemented in MultiPoint software. After revealing the regions of map instability, marker(s) responsible for such local instability were removed from the dataset with the objective of stabilizing the order. At the next step, we have rebuilt the map and tested again its stability based on the jackknife resampling in order to arrive at a stabilized (skeleton) map.

Results

Overview of the genetic linkage map

A total of 690 polymorphic loci were used to assemble the genetic linkage map, including 197 SSR and 493 DArT markers. After elimination of 21 unlinked loci, the mapping of the remaining 669 loci resulted in 14 linkage groups comprising 24 (5A) to 81 (3B) loci (Table 1). Markers violating map stability were removed and linkage groups reanalyzed to construct a stabilized map (Mester et al. 2003a, b; Ronin et al. 2008). The resultant skeleton map consisting of 307 markers provided a practical basic map for calculating recombination frequencies and centiMorgan (cM) distances. The skeleton map accounted for a total length of 2,317 cM, with an average density of one marker per 7.5 cM (Fig. 1, Table 1). The length of individual chromosomes ranged between 112 cM for chromosome 4B and 217 cM for chromosome 3B (Fig. 1; Table 1).

Most of the 493 DArT markers that were polymorphic between the two parental lines were scored as "present" for the durum allele (73.4%) with the rest (26.6%) as "present" for the wild G18-16 allele. Three hundred and fifty-two DArT markers were mapped in the current map for the first time.

Distribution of markers among chromosomes and genomes

The seven homologous groups of the tetraploid wheat genome varied in the number of markers, map length, and marker density. Total marker number and density was highest in homoeologous group 6 (total 104 loci, 53 skeleton loci with 6.3 cM per marker), whereas total map length was the highest (371.7 cM) in group 2. Homoeologous group 7 had the lowest marker number and density (total 78 loci, 36 skeleton loci with 8.9 cM per marker) and group 4 had the shortest map length (233.5 cM). Differences were also found between the two sub-genomes, with 339 (60%) markers mapped to the B genome (average 57 markers per chromosome) and 270 (40%) to the A genome (average of 39 markers per chromosome). The B-genome skeleton map was denser, with 185 markers that accounted for 1,261 cM of genetic distance (6.8 cM per marker). The A genome skeleton map spanned 1,056 cM with 122 markers (8.7 cM per marker).

Clustering of markers

The genetic markers were distributed non-randomly ($P\ \chi^2$ $(df\ 231) \leq 0.0001$) along the chromosomes. Clusters of markers were observed on most of the chromosomes of the A and B genomes (Figs. 1, 2). The SSR markers used in the current study were selected, according to previously published maps, to cover all 14 chromosomes, whereas DArT markers scored in the mapping population were not targeted to specific genomic regions. Therefore, clustering of markers was tested for the two types of markers separately, revealing significant clusters of DArT markers on most chromosomes. The clustering phenomenon for the A and B sub-genomes and for the entire genome was found highly

Table 1 Chromosome assignment, distribution of markers, length of linkage groups, and marker density in genetic map constructed with the G18-16 × LDN recombinant inbreed line population

Linkage group	SSR	DArT	Total markers	Skeleton markers	Added markers	Length (cM)[a]	cM/marker[a]
1A	13	27	40	18	22	183.8	10.2
1B	18	34	52	26	26	181.5	7.0
2A	16	28	44	19	25	188.8	9.9
2B	13	59	72	26	46	182.9	7.0
3A	13	22	35	17	18	118.8	7.0
3B	16	65	81	35	46	217.2	6.2
4A	11	40	51	20	31	121.5	6.1
4B	13	22	35	16	19	112.0	7.0
5A	11	13	24	12	12	144.0	12.0
5B	16	37	53	29	24	209.8	7.2
6A	15	23	38	19	19	161.2	8.5
6B	13	53	66	34	32	175.3	5.2
7A	13	25	38	17	21	138.0	8.1
7B	10	30	40	19	21	182.3	9.6
Group 1	31	61	92	44	48	365.3	8.3
Group 2	29	87	116	45	71	371.7	8.3
Group 3	29	87	116	52	64	336	6.5
Group 4	24	62	86	36	50	233.5	6.5
Group 5	27	50	77	41	36	353.8	8.6
Group 6	28	76	104	53	51	336.5	6.3
Group 7	23	55	78	36	42	320.3	8.9
A genome	92	178	270	122	148	1,056.1	8.7
B genome	99	300	399	185	214	1,261	6.8
Total	191	478	669	307	362	2,317.1	7.5

[a] Chromosome length and marker density refer to the skeleton map

significant for the DArT markers. For example, the non-random distribution pattern of both types of marker intervals on chromosomes 1A [$P\ \chi^2$ (df 18) ≤ 0.1 and $P\ \chi^2$ (df 18) ≤ 0.0001 for SSRs and DArTs, respectively], 2A [$P\ \chi^2$ (df 18) ≤ 0.0001 and $P\ \chi^2$ (df 18) ≤ 0.0001 for SSRs and DArTs, respectively], and 6B [$P\ \chi^2$ (df 16) ≤ 0.001 and $P\ \chi^2$ (df 16) ≤ 0.0001 for SSRs and DArTs, respectively] is shown in Fig. 2.

Segregation distortion of markers

A significant segregation distortion was found in 208 (30%) out of 669 markers analyzed on 152 RILs. One hundred and twenty-one markers (17.5%) showed distortion in favor of the wild emmer wheat allele whereas 87 (12.6%) showed distortion in favor of the durum wheat allele (at $P \leq 0.05$; data not shown). Out of the 307 skeleton markers that were used to construct the skeleton map, 91 (29.6%) showed significant distorted segregation with 38 markers (12.4%) showing distortion in favor of the LDN allele and 53 markers (17.3%) in favor of the wild allele (Fig. 1). It is worth mentioning that neighboring DArT and SSR markers showed the same pattern of segregation. The 121 markers that showed distortion in favor of the wild allele were distributed among seven chromosomes as follows: 1A (6), 1B (18), 2B (9), 4B (16), 6A (1), 6B (2), and 7B (1). The markers that showed distortion in favor of the cultivated allele were distributed as follows: 1A (5), 2A (2), 3B (16), 4A (1), 5B (1), 6B (3), and 7B (1) (Fig. 1). Chromosomes 4B and 1BL showed distortion of all markers in favor of the wild emmer allele (Fig. 3). Chromosome 1A showed segregation distortion in favor of the wild allele in the long arm and in favor of the wild allele in the short arm (Fig. 3). Out of the 14 chromosomes of genome A and B of tetraploid wheat, only three chromosomes (3A, 5A, and 7A) showed no segregation distortion (Fig. 1).

Frequency of RILs with parental (non-recombinant) chromosomes

Out of the 2,128 RIL × chromosome combinations, 148 chromosomes (6.9%) remained non-recombinant (parental) even after five cycles of recombination (Table 2). The highest frequency of parental chromosomes was observed in group 4 chromosomes (25 and 26 for 4A and 4B, respectively). The recovery rate of non-recombinant

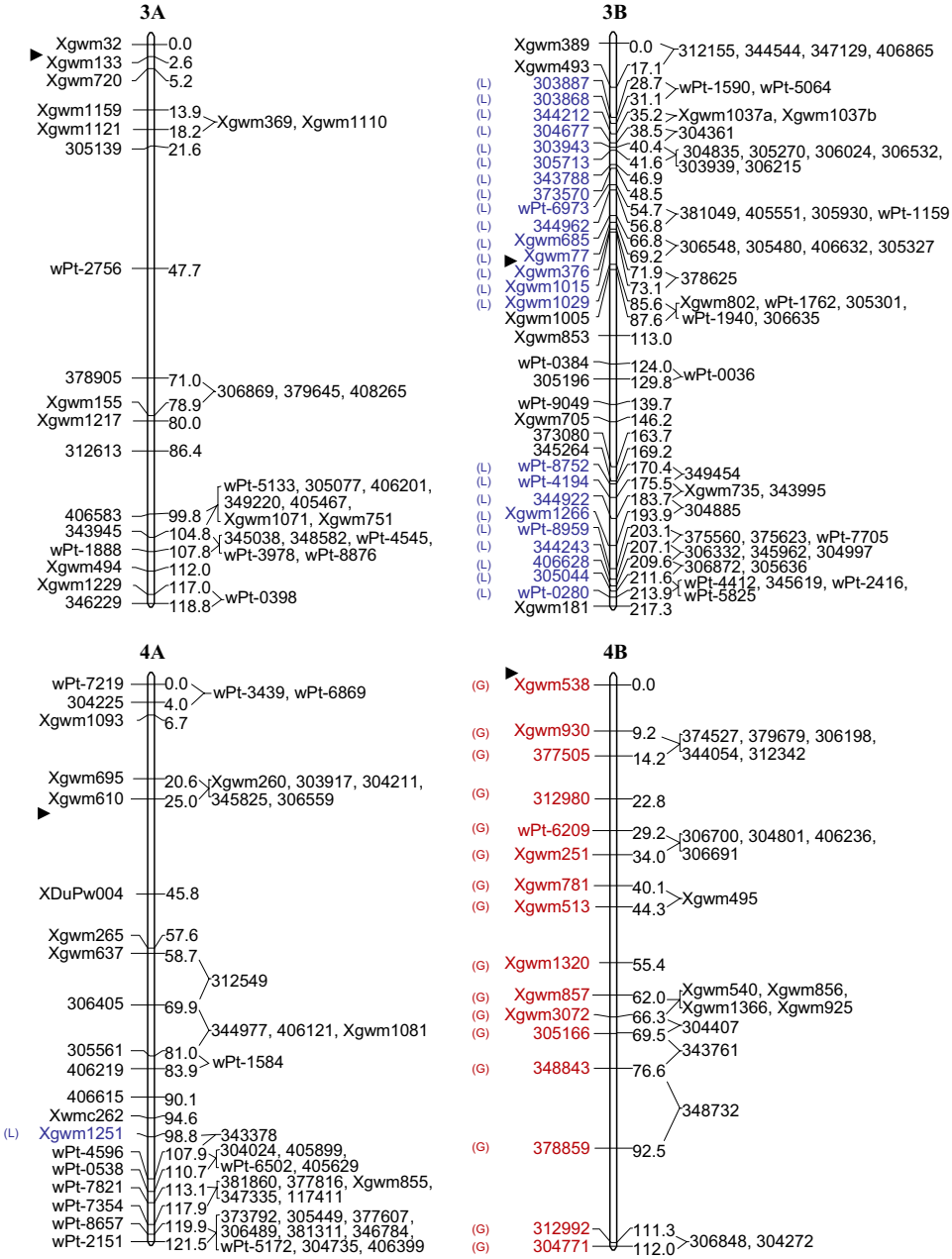
Fig. 1 Genetic linkage map of tetraploid wheat constructed from RIL population derived from a cross between durum wheat cultivar Langdon and wild emmer wheat acc. G18-16. Skeleton markers are shown on the *left* with map distances (cM; Kosambi 1944) on the *right*. The added markers assigned to chromosome intervals are shown on the *right side of each chromosome*. The approximate centromere position is indicated by *arrowhead*. Short arms are at the *top*. Markers that showed distorted segregation in favor of wild alleles and cultivated alleles were marked on the left by G or L, respectively

chromosomes correlated ($R^2 = 0.72$, $P = 0.001$; Fig. 4) with chromosome length (cM) but was unrelated ($R^2 = 0.43$, $P = 0.31$) to the number of markers per chromosome. The probability of recovering non-recombinant chromosomes was similar for both parental lines LDN (81) and G18-16 (67).

Discussion

The rapid advance in molecular marker and linkage mapping technologies exponentially increases the number of marker loci assigned to genetic maps. Dense genetic maps are a very useful tool in the identification of molecular markers closely linked to genes or QTLs of interest, isolation of genes via map based cloning, comparative mapping, and genome organization studies (Varshney et al. 2007). The genetic linkage map presented in the current study is the first published map of durum wheat × wild emmer wheat population making use of DArT markers. This genetic map spans over 2,317 cM, with each chromosome represented by one linkage group. Wild emmer wheat accession G18-16 originated from drought-prone habitat in eastern Israel, while Langdon is a spring durum wheat variety from North Dakota, USA. The two parental lines were found polymorphic in various morphophysiological traits, such as productivity, heading date, plant height, drought resistance and others (data not shown).

Fig. 1 continued



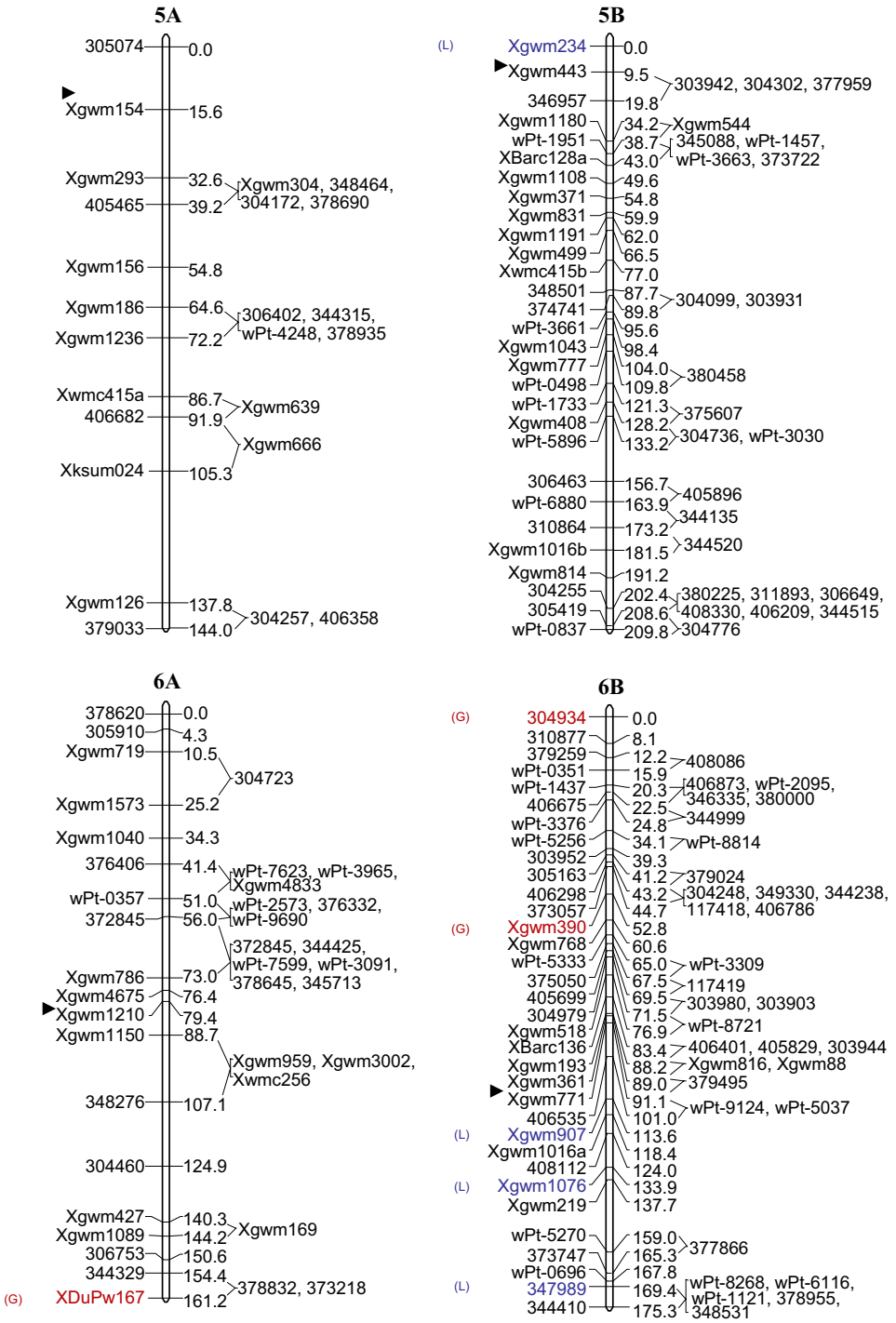
Map construction and genome coverage

The constructed map for durum wheat × wild emmer covers 2,317.1 cM, corresponding to an average of 7.5 cM per marker. In general, the current map provides a good coverage of the tetraploid wheat genome. However, a few chromosome arms (3AS, 4AS, 5AS, and 5BS) were partly covered and one arm (4BS) was not covered. Lack of complete genome coverage of homoeologous group 4 was observed in a few other wheat mapping populations (Röder et al. 1998; Paillard et al. 2003; Elouafi and Nachit 2004).

Several genetic maps were constructed for tetraploid wheat, either for *T. durum* × *T. dicoccoides* (Peng et al. 2000; Blanco et al. 1998, 2004; Elouafi and Nachit 2004) or *T. durum* × *T. durum* (Nachit et al. 2001), revealing map sizes of 2,237 to 3,598 cM (Table 3). Other maps constructed for hexaploid wheat populations reported (for genomes A and B) map sizes of 1,791.0 to 2,356 cM (Röder et al. 1998; Quarrie et al. 2005; Akbari et al. 2006). Therefore, our map is in accord with most published maps and presents a fine coverage of genomes A and B of tetraploid wheat.

The relatively large population size used for construction of the genetic linkage map presented here (152 RILs) as compared with other studies (62–120; Blanco et al. 1998, 2004; Röder et al. 1998; Nachit et al. 2001;

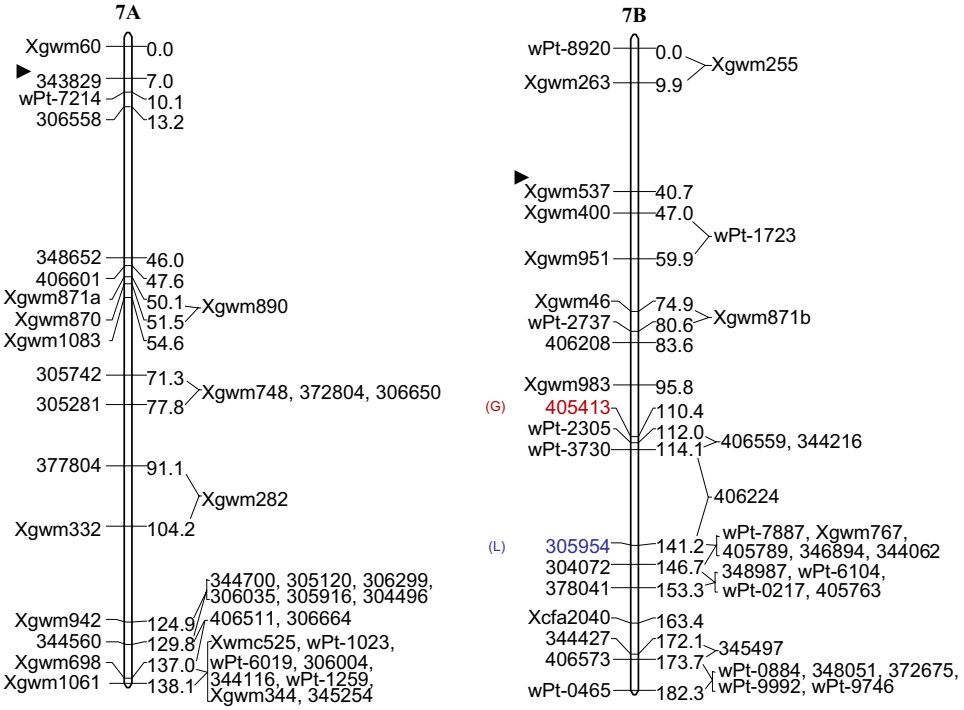
Fig. 1 continued



Elouafi and Nachit 2004; Quarrie et al. 2005; Akbari et al. 2006) is highly advantageous for further exploitation of this map. This large population size will improve the resolution of QTL mapping of agronomic traits (e.g., drought resistance, Peleg et al., in preparation). It also enables a greater resolution in the positioning of QTLs on the genetic map, while uniform genome coverage of markers is required to detect all contributing loci (Chalmers et al. 2001).

The SSR markers used in the current study, mostly derived from hexaploid bread wheat (Ganal and Röder 2007), showed high level of transferability to both tetraploid *T. turgidum* L. subspecies. In contrast, DArT markers showed a greater level of transferability to the domesticated durum wheat with 73% of the markers being scored as "present", as compared to the wild emmer wheat where only 27% of the markers scored as "present". Similar results were reported for barley mapping population

Fig. 1 continued



derived from a cross between cultivated barley (*Hordeum vulgare*) and wild barely (*H. spontaneum*), revealing 70% versus 30% DArT alleles detected as present/absent, respectively (Hearnden et al. 2007). The low level of wild alleles detected in the reported cross seems to be due to a bias in the current DArT array probes that are derived from cultivated wheat accessions. On the other hand, the allele-specificity of DArT offers an efficient path towards discovery of species-specific markers (Wenzl et al. 2004).

Clustering of markers

In wheat and many other organisms, recombination is unevenly distributed with "hot-spots" and "cold-spots" across chromosomes (Dvořák and Chen 1984; Gill et al. 1996a, b; Faris et al. 2000). Clustering around centromeres is a well known phenomenon with all types of markers, resulting from centromeric suppression of recombination (Tanksley et al. 1992; Korol et al. 1994). Contrary to other wheat mapping populations (Röder et al. 1998; Peng et al. 2000), in the current study, SSRs showed only a moderate tendency to cluster around centromeres, presumably due to the selection of markers based on their known location. However, a remarkable clustering of DArT markers was found in telomeric regions (Figs. 1, 2). Akbari et al. (2006) reported for bread wheat that DArT markers showed a stronger tendency than SSR markers in particular to map to gene-rich telomeric regions. A similar pattern was observed in barley maps using DArT markers (Semagn et al. 2006). Clusters of *Pst*I-based DArT markers may reflect the abundance of *Pst*I restriction sites in hypomethylated telomeric chromosome regions (Moore 2000). Similar clustering in telomeric regions was also found in tetraploid wheat using *Pst*I-based AFLP markers (Peng et al. 2000).

High-density physical maps in wheat revealed that more than 85% of wheat genes are present in gene-rich regions, physically spanning only 5–10% of the genome (Gill et al. 1996a, b; Faris et al. 2000). These regions are strongly associated with recombination rate in wheat (Gill et al. 1996a, b; Weng and Lazar 2002) and are predominantly located in telomeres (Qi et al. 2004). For example, the clusters of DArT markers in homoeologous group 1 (Fig. 2a) are associated with a reported gene-rich region near chromosome telomeric ends (Gill et al. 1996a). The high proportion of clustering of DArT markers may, therefore, be indicative of gene-rich regions. If this is indeed the case, DArT markers may be unique for fine mapping of genes/QTLs residing in gene-rich regions, thereby facilitating positional cloning.

Segregation distortion

Segregation distortion is defined as a deviation of observed genetic ratios from the expected Mendelian ratios in a given phenotypic or genotypic class within a segregating population. Distorted segregation may be caused by competition between gametes for preferential fertilization or from abortion of gamete or zygote (Lyttle 1991). Meiotic drive is another phenomenon affecting segregation ratios through a

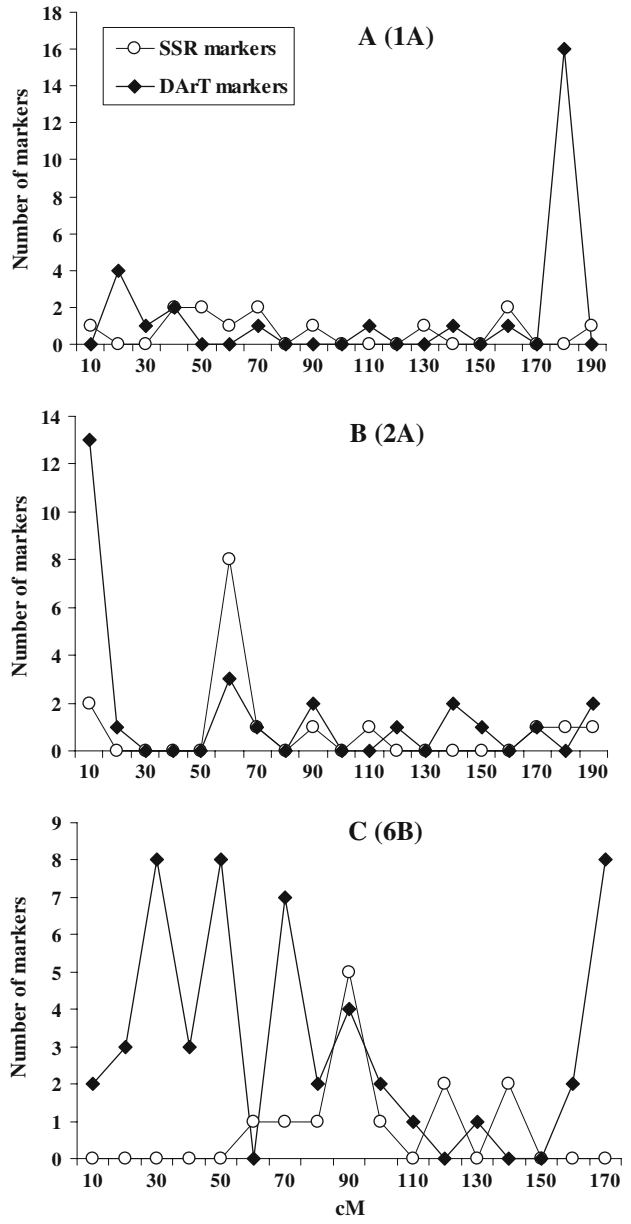


Fig. 2 Distribution of SSR and DArT loci along chromosomes 1A, 2A, and 6B of the G18-16 × LDN genetic map

variety of molecular and cytogenetic mechanisms resulting in non-equal representation of homologous alleles (or chromosomal segments) among the functional gametes (Lyttle 1991). In general, normal Mendelian segregation can be viewed as a product of evolutionary co-adaptation, and of adjustment of genomic components within a species rather than an automatic outcome of the eukaryote meiotic mechanics (Korol et al. 1994). Indirect evidence for this is provided by the fact that segregation distortions frequently occur in the progeny of interspecific hybrids and are similar in manifestation to meiotic drive systems.

Numerous examples of segregation distortion have been reported in many crop species including barley (*H. vulgare*; Graner et al. 1991; Devaux et al. 1995), rice (*Oryza sativa*; Causse et al. 1994; Xu et al. 1997), and maize (*Zea mays*; Wendel et al. 1987; Lu et al. 2002). In wheat, this phenomenon has been reported repeatedly (Blanco et al. 2004; Peng et al. 2000; Quarrie et al. 2005). While segregation distortion is a common phenomenon in different types of mapping populations, be it F_2, RILs or double haploid (DH), RIL populations have the highest probability for distortions due to repeated 5–6 generations of selection forces (Singh et al. 2007).

The F_6 RIL population used in the present study showed segregation distortion for 91 (29.6%) of the 307 skeleton loci. In most previous studies, segregation distortion in favor of the female parent was observed (Singh et al. 2007). In contrast, our data showed some tendency (58%) in favor of the wild alleles of the male parent, suggesting high level of compatibility between the parental lines. The vast majority of loci distorted in favor of the wild allele mapped to contiguous regions on chromosomes 1A, 1B, 2B, and 4B, while those distorted in favor of the cultivated allele were mapped on chromosomes 1A and 3B. Segregation distortion on group 5 chromosomes (5A, 5B, and 5D) of wheat have been reported previously in several studies (Peng et al. 2000; Faris et al. 2000), including reciprocal populations derived from a cross between LDN and wild emmer wheat (Kumar et al. 2007). In the present study, however, no distortion was noted on chromosomes 5A and 5B (Fig. 1). Segregation distortion on the short arm chromosome 2B, observed in the current study, is in agreement with previous reports (Cadalen et al. 1997; Campbell et al. 1999; Paillard et al. 2003).

Notably, all markers mapped on chromosome 4B showed skewed segregation in favor of the wild allele. Distorted segregation in chromosome 1A showed opposite patterns in the two chromosome arms: markers on 1AS were in favor of the cultivated wheat alleles while markers on 1AL were in favor of the wild alleles (Fig. 3). Likewise, all markers on the homoeologous chromosome arm 1BL showed skewed segregation in favor of the wild alleles. Blanco et al. (2004) also reported distortion of markers on chromosome 1B in favor of the wild alleles in durum wheat × wild emmer population. The alternative directions of segregation distortion found in our and in other studies should not be considered as a surprise if we take into account the variety of mechanisms that could contribute to the observed distortions. These may include meiotic drive and preferential abortion of gametes, effect of usual gametophyte factors, non-random fertilization, and viability selection at post-syngamic stages. Clearly, these factors may work simultaneously and in opposite directions, favoring the alleles of the wild or cultivated parent in different genomic regions. It is also worth recalling that our RIL mapping population was obtained via several selfing gener-

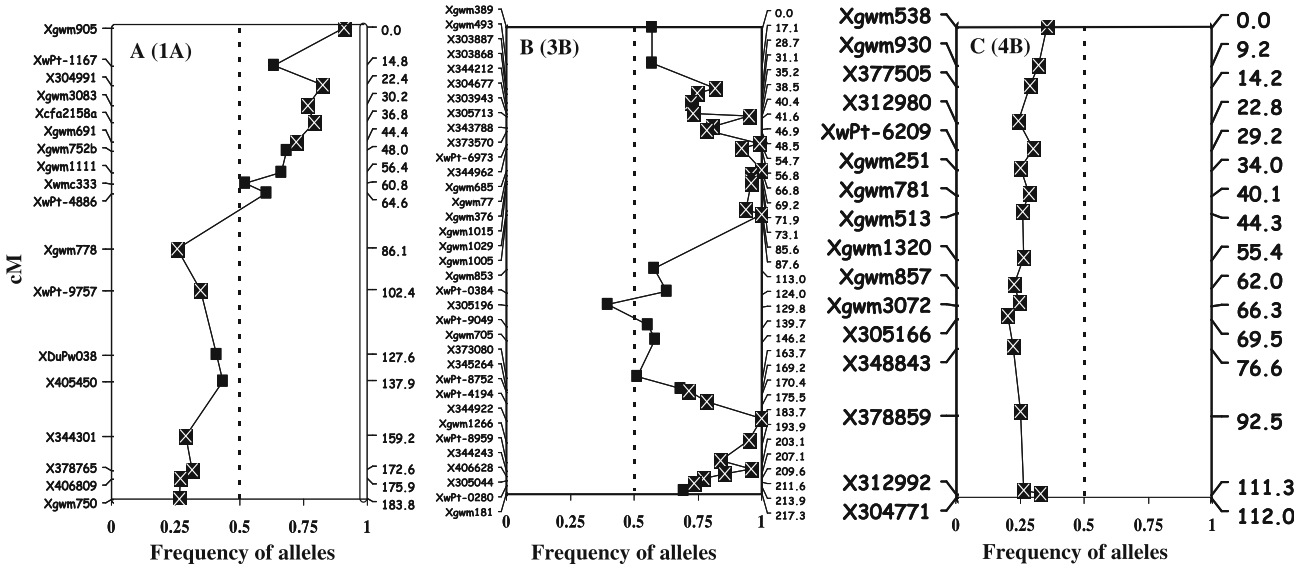


Fig. 3 Allele frequencies as a function of the genetic linkage map along chromosomes 1A, 3B, and 4B. The *x*-axis indicates the segregation distortion from the 1:1 ratio observed for each marker, and the *y*-axis corresponds to the genetic linkage map. Markers marked with *crossed square* indicate the significance threshold of $P < 0.05$

Table 2 Number of durum wheat (LDN) × wild emmer wheat (G18-16) RILs with parental (non-recombinant) chromosomes

Chromosome	Number of RILs with non-recombinant chromosomes		
	Wild emmer	Durum wheat	Total
1A	2	2	4
1B	2	8	10
2A	5	3	8
2B	1	3	4
3A	6	7	13
3B	4	1	5
4A	12	13	25
4B	6	20	26
5A	8	6	14
5B	4	0	4
6A	4	8	12
6B	2	3	5
7A	9	6	15
7B	2	1	3
Total	67	81	148

ations, i.e., the foregoing mechanisms could work in different combinations during these generations.

Non-recombinant chromosomes

The chromosomes of two *T. turgidum* subspecies, wild emmer wheat and durum wheat, are assumed to be fully compatible (congruent). Therefore, in the F_6 RIL population used in this study, normal recombination rates were

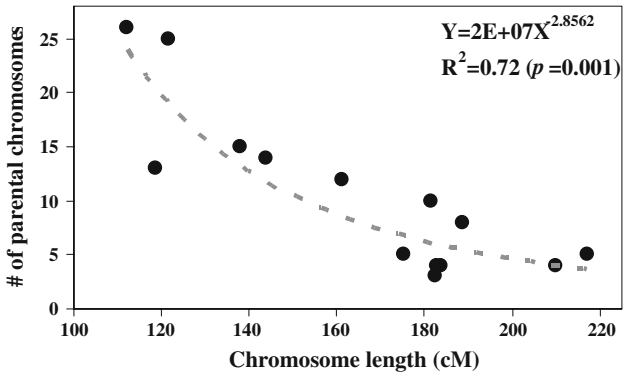


Fig. 4 Occurrence of parental chromosomes (non-recombinant chromosome) in the mapping data set as associated with chromosome length (cM)

expected for all chromosomes. Nevertheless, a relatively high frequency (6.9%) of non-recombinant chromosomes was observed in this population. The highest frequency of non-recombinant chromosomes was found in group 4 chromosomes (34%), which is close to the theoretical proportion expected for the estimated length of chromosome 4 (~ 120 cM). In accordance, Singh et al. (2007) reported the highest frequency of RILs with non-recombinant chromosome for chromosome 4A in *T. boeoticum* × *T. monococcum* RIL population. Chromosome 4A is known to be involved in cyclical interchange with chromosome 5A (Devos et al. 1995) but in our case, only 9% of the non-recombinant chromosomes were found in chromosome 5A. The parental genotypes of our cross cannot be considered as highly divergent in evolutionary terms. Still, one could explain the slightly reduced observed proportion of

Table 3 Comparison of map length (cM) of tetraploid wheat genome in various linkage maps generated in different mapping populations

Chromosome	Current map	Röder et al. (1998)	Peng et al. (2000)	Nachit et al. (2001)	Paillard et al. (2003)	Blanco et al. (2004)	Elouafi and Nachit (2004)	Quarrie et al. (2005)	Liu et al. (2005)	Akbari et al. (2006)
Cross[a]	1	3	1	2	3	1	1	3	3	3
Type	RIL	RIL	F_2	RIL	RIL	RIL	BC_1F_8	DH	RIL	DH
Number	152	70	150	110	240	65	114	95	118	62
1A	183.8	155.8	152.0/138.6	201.2	65.0	180.9	147.8	131	166.9	149.3
1B	181.5	151.8	200.4/200.2	459.4	180.0	162	208.6	167	124.1	119.4
2A	188.8	138.3	207.8/250.3	138.8	204.0	318	153.9	170.2	123	73.9
2B	182.9	180	256.2/257.9	320.8	178.0	260.7	167.3	184.0	197.6	143.4
3A	118.8	160.3	267.4/261.7	300.2	103.0	170.2	137.4	158.3	217.3	111.4
3B	217.2	265.5	250.5/266.1	345.7	182.0	181	239.4	190.1	272.7	199.8
4A	121.5	101.6	193.3/192.5	302.6	105.0	236.6	118.6	179.2	167.2	170.6
4B	112.0	66.0	82.9/104.6	133.9	80.0	200.8	180.1	145.2	83.1	91.6
5A	144.0	175.1	271.6/274.9	172.3	198.0	266.4	90.0	190.7	138.3	190.7
5B	209.8	165.6	310.0/276.7	239.5	45.0	253.4	209.4	222.2	213	147.7
6A	161.2	134.1	180.2/179.4	325.3	192.0	178.6	111.7	151.7	196.2	148.5
6B	175.3	98.7	237.1/239.2	251.3	50.0	148.8	180.2	125.1	141.9	57.8
7A	138.0	178.2	317.0/309.8	174.6	242.0	281.3	136.5	167.1	131.9	156.4
7B	182.3	191.4	243.0/227.8	232.2	141.0	199.7	156.5	173.8	117.3	179.4
A genome	1,056.1	1,043.4	1,589.3/1,607.2	1,615	1,109.0	1,632	895.9	1,148.2	1,140.8	1,000.8
B genome	1,261	1,119	1,580.1/1,572.5	1,982.8	856.0	1,406.4	1,341.5	1,207.4	1,149.7	939.1
Total	2,317.1	2,162.4	3,169.4/3,179.7	3,597.8	1,965.0	3,038.4	2,237.4	2,355.6	2,290.5	1,939.9

[a] Mapping populations: *1* durum wheat × wild emmer wheat, *2* durum wheat × durum wheat, *3* bread wheat × bread wheat

recombinant chromosomes compared to the theoretical expectation as a result of the known general trend of decrease in recombination in hybrids with genetic distance between the parental lines.

Concluding remarks

Genetic map with full genome coverage and confidence in locus order is necessary for detection, mapping, and estimation of gene effects on phenotypic traits. The present map, based on a cross between durum wheat and wild emmer wheat, most likely covers the majority of the tetraploid wheat genome. Furthermore, this map is probably the most intensive published for tetraploid wheat, both in terms of population size (152 RILs) and in number of markers mapped (669). This is the first genetic map of a cross involving wild relatives of wheat using DArT markers and, therefore, it could serve as new source to identify new markers (clones) that were not mapped before. This map provides a valuable resource for wheat genetic research, including 352 DArT markers whose chromosomal location was previously unknown, which will expand the pool of markers available for wheat research. The constructed map will be used for further QTL detection (e.g., drought response, grain minerals concentration, and powdery mildew resistance) and as a tool for marker-assisted selection and map-based breeding for resistance to biotic and abiotic stresses.

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