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ORIGINAL PAPER

An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* × *T. monococcum* RIL population

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Abstract Diploid A genome species of wheat harbour immense variability for biotic stresses and productivity traits, and these could be transferred efficiently to hexaploid wheat through marker assisted selection, provided the target genes are tagged at diploid level first. Here we report an integrated molecular linkage map of A genome diploid wheat based on 93 recombinant inbred lines (RILs) derived from *Triticum boeoticum* × *Triticum monococcum* inter sub-specific cross. The parental lines were analysed with 306 simple sequence repeat (SSR) and 194 RFLP markers, including 66 bin mapped ESTs. Out of 306 SSRs tested for polymorphism, 74 (24.2%) did not show amplification (null) in both the parents. Overall, 171 (73.7%) of the 232 remaining SSR and 98 (50.5%) of the 194 RFLP markers were polymorphic. Both A and D genome specific SSR markers showed similar transferability to A genome of diploid wheat species. The 176 polymorphic markers, that were assayed on a set of 93 RILs, yielded 188 polymorphic loci and 177 of these as well as two additional morphological traits mapped on seven linkage groups with a total map length of 1,262 cM, which is longer than most of the available A genome linkage maps in diploid and hexaploid wheat. About 58 loci showed distorted segregation with majority of these mapping on chromosome $2A^m$. With a few exceptions, the position and order of the markers was similar to the ones in other maps of the wheat A genome. Chromosome $1A^m$ of *T. monococcum* and *T. boeoticum* showed a small paracentric inversion relative to the A genome of hexaploid wheat. The described linkage map could be useful for gene tagging, marker assisted gene introgression from diploid into hexaploid wheat as well as for map based cloning of genes from diploid A genome species and orthologous genes from hexaploid wheat.

Keywords Diploid wheat · *Triticum monococcum* · *T. boeoticum* · Linkage map · Microsatellites

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Introduction

The chromosome complement of hexaploid wheat, *Triticum aestivum* L. ($2n = 6x = 42$), consists of three genomes A, B and D. Genetic analysis and gene discovery in hexaploid wheat has been arduous because of large genome size (~17,300 Mb—Bennette and Leitch 1995), abundance of repetitive DNA sequences (SanMiguel et al. 2002; Wicker et al. 2003) and limited polymorphism (Gale et al. 1990). As a result only a few successes of map based gene cloning have been reported in hexaploid wheat. Of the three homoeologous genomes of hexaploid wheat, donors of the A and

D genome are well defined. *T. urartu* Tum. contributed the A genome (Dvorak et al. 1988) and *Aegilops tauschii* Coss. contributed the D genome (McFadden and Sears 1946). *Triticum monococcum* ssp. *monococcum* L. ($2n = 2x = 14$), a diploid A genome species, is domesticated and *T. monococcum* ssp. *aegilopoides* (Link) Thell. (*T. boeoticum* Boiss.) ($2n = 2x = 14$) is a wild form of *T. monococcum* ssp. *monococcum*. Both, *T. monococcum* ssp *monococcum* (A^m) and *T. monococcum* ssp. *aegilopoides* (A^m) are very closely related to *T. urartu* (A^u) and to the A genome of hexaploid wheat, *T. aestivum* but are reproductively isolated from *T. urartu* (Johnson and Dhaliwal 1976). *T. monococcum* and *T. boeoticum* on the other hand do not show any evidence of differentiation (Dvorak et al. 1988) as hybrids between the two species form seven bivalents (Kihara et al. 1929; Dubcovsky et al. 1996) and viable seeds (Johnson and Dhaliwal 1976). *T. monococcum* was domesticated around 10,000 years ago (Heun et al. 1997) and is the only diploid cultivated species of wheat. The A^m and A^u genomes of *T. monococcum* and *T. urartu* diverged some 1.0 MYA (Huang et al. 2002) and the A and D genomes diverged some 2.7 MYA (Dvorak and Akhunov 2005). Molecular linkage map of diploid wheat using RFLP markers showed that gene content and order were well conserved between A-genome of wheat and *T. monococcum* except for the chromosomes 4A and 5A that were involved in chromosome interchanges (Dubcovsky et al. 1996). Chromosomes 4A, 5A and 7B of wheat were involved in cyclical translocations where chromosome 4A further differed from $4A^m$ of *T. monococcum* by having one pericentric and one paracentric inversion (Devos et al. 1995; Mickelson-Young et al. 1995).

Because of the presence of high-levels of polymorphism and ease of working with single genome, diploid progenitor species of wheat have been used for developing genetic linkage maps (Dubcovsky et al. 1996; Boyko et al. 1999; Luo et al. 2005) that complemented the genome analysis in wheat. Recently, progenitor diploid species were used for gene discovery in wheat by utilizing genomic resources such as BAC libraries of *T. monococcum* (Feuillet et al. 2003; Yan et al. 2003, 2004) and *Ae. tauschii* (Huang et al. 2003). The diploid nature of *T. monococcum* with smaller genome size of 5,700 Mb compared to 17,300 Mb of bread wheat (Bennett and Leitch 1995), the existence of a very high level of polymorphism for DNA based markers (Castagna et al. 1994), sequence conservation at orthologous loci (Wicker et al. 2003) and availability of a large BAC library (Lijavetzky et al. 1999) makes this species an attractive diploid model for gene discovery in wheat. The vernalization genes *VRN1* and *VRN2* were cloned in wheat by using the naturally existing variation in *T. monococcum* (Yan et al. 2003, 2004). BAC libraries of *T. monococcum* were used for cloning the disease resistance gene *Lr10* (Feuillet et al. 2003) and a domestication locus *Q* in wheat (Faris et al. 2003; Simons et al. 2006). *T. monococcum* was also used to map the genetic factors responsible for earliness per se in wheat (Bullrich et al. 2002; Valarik et al. 2006). *T. monococcum* was used for developing loss of function mutations for a large number of agronomic traits using ethyl methanesulphonate based mutagenesis (Dhaliwal et al. 1987; our unpublished results). One of the tillering mutant *tin3* from the above EMS mutant collections of *T. monococcum* was mapped on chromosome arm $3A^mL$ (Vasu et al. 2007) to isolate the tillering genes in *Triticeae*, further indicating that the *T. monococcum* could be a reference diploid genome for gene discovery in wheat. In addition, A genome linkage map based on diploid wheat will be helpful for selective introgression of desired traits from diploid A genome species to hexaploid wheat.

Triticum boeoticum, the progenitor of cultivated *T. monococcum*, is sympatric to *T. urartu* over its entire distribution (Johnson and Dhaliwal 1976) and shows immense variability for a number of biotic and abiotic stresses and for productivity traits. All the three species are a rich source of variability for resistance to several diseases like leaf rust (The 1976; Hussien et al. 1997; Bai et al. 1998; Anker and Niks 2001), stripe rust (Dhaliwal et al. 1993b; Harjit-Singh et al. 1998), Karnal bunt (Vasu et al. 2000), powdery mildew (Shi et al. 1998; Lebedeva and Peusha 2006), partial resistance to cereal aphid (Migui and Lamb 2004), grain protein and carotene content, spikelets per spike, grain weight (Castagna et al. 1995), as well as for cereal cyst nematode and many productivity related traits (our unpublished results).

Molecular linkage map of A genome diploid wheat have been developed using RFLP (Dubcovsky et al. 1996) and AFLP markers (Taenzler et al. 2002). The available series of simple sequence repeat (SSR) markers have been developed from hexaploid wheat and from *T. urartu* and *Ae. tauschii*, the A and D genome donors of wheat, respectively (http://www.wheat.pw.usda.gov/GG2/quickquery.shtml#microsats). Integration of polymerase chain reaction (PCR) based markers and RFLPs into a single linkage map will not only complement the existing hexaploid wheat map but will also serve as the base map for mapping both qualitative and quantitative traits in diploid A genome wheat, their map-based cloning and for monitoring their introgression into wheat. Here we report an integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* × *T. monococcum* RIL population. The RIL population will also allow us the molecular mapping of agronomically important traits such as resistance to leaf rust, stripe rust, Karnal bunt, cereal cyst nematode, powdery mildew, spikelets per spike, grain weight, tillering and flowering time and for domestication trait like single vs two seeds per spikelet.

Materials and methods

Plant material

Plant material consisted of a set of 121 F_6 recombinant inbred lines (RILs) derived through single seed descent, from a cross *T. boeoticum* acc. pau5088 X *T. monococcum* acc. pau14087 (hereon referred to as *T. boeoticum* and *T. monococcum*, respectively, for brevity). Seeds of both accessions were originally supplied by Dr. BL Johnson, University of California, Riverside, USA. The *T. monococcum* was a spontaneous mutant with spring growth habit, identified from an accession with otherwise winter growth habit, and shows resistance to a number of diseases like leaf and stripe rusts, cereal cyst nematode, Karnal bunt and powdery mildew. The *T. boeoticum* (G2610; PI 427839) has facultative winter growth habit and also shows resistance to a number of diseases. *T. boeoticum* acc. 5088 was chosen from a set of *T. boeoticum* and *T. urartu* accessions, based on high level of polymorphism it showed with *T. monococcum* (Dhaliwal et al. 1993a). Although *T. urartu* accessions were more diverse from *T. monococcum* than were *T. boeoticum* accessions, the RIL population was generated by crossing the diverse *T. boeoticum* accession 5088 with *T. monococcum* as F_1 hybrids between *T. monococcum* and *T. urartu* or *T. boeoticum* and *T. urartu* are male sterile and do not set seed upon selfing (Johnson and Dhaliwal 1976). From a total of 121 RILs available from this cross, a set of 93 RILs were used for generating the linkage map so as to fit the 96 well microtitre plate format for in vitro DNA amplification through PCR. The RILs, which were too late in flowering were not included for molecular analysis.

Microsatellite assay

DNA from parents and the RIL population was extracted following the CTAB method as modified by Saghai-Maroof et al. (1984) by pooling leaf tissue from five plants of each RIL. Three hundred and six SSR markers were used for screening the parental lines for polymorphism. The SSRs included the following markers: 91 GWM (Röder et al. 1998; M. Röder, unpublished data); 13 GDM (Pestsova et al. 2000); 51 WMC (Gupta et al. 2002); 56 CFD (Guyomarc'h et al. 2002a); 25 CFA (Sourdille et al. 2003), 71 BARC (Song et al. 2005) and 5 SMW (Eligio et al. 2006). The PCR were performed in 10-µl aliquots in a PTC-200 (MJ Research, Waltham, MA, USA) or in a Bio-Rad 9600 thermal cycler. The reaction mixture contained 20 ng of template DNA, 0.125 mM of each dNTPs, 1X buffer (10 mM Tris-HCl - pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 0.05 µM labeled primer (IRD700 or 800), 0.2 µM unlabelled primer and 0.5 U of Taq DNA polymerase. Thermal cycling conditions included: 94°C for 4 min followed by 29 cycles of 94°C for 1 min, 50-63°C (depending on the primer combination) for 1 min, 72°C for 2 min, followed by an elongation step of 7 min at 72°C. Fragment analysis was carried out in a LI-COR 4200 DNA analyzer. Five microliters of formamide tracking-dye (Amersham, Piscataway, NJ, USA) was added per PCR reaction and 1–1.5 µl of the reaction mix was run on 8% acrylamide gels after diluting the original PCR product to 5:1 with formamide.

RFLP assay

The RFLP analysis for parental polymorphism survey was performed with 194 probes and five enzymes (*Bam*H I, *Dra* I, *Eco*R I, *Hind* III and *Xba* I) as described by Messmer et al. (1999). The 194 RFLP probes included 128 ABC, CDO, PSR, KSU, MWG, FBA, FBB and 66 bin mapped ESTs (http://www.wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi).

Morphological markers

In addition to SSR and RFLP markers, the parents differed for two morphological traits viz. aleurone colour (*Ba*) (blue aleurone in *T. boeoticum* versus non-blue in *T. monococcum*), and coleoptile colour (*RC*) (purple coleoptile in *T. boeoticum* versus green in *T. monococcum*). The aleurone colour in parents and the RILs was recorded in mature grains. For coleoptile colour, 10–15 seeds each of parents and the RIL population were grown in 6.5 cm diameter pots at 20°C under constant light and coleoptile colour was recorded a week after germination.

Data analysis and linkage mapping

Goodness of fit for all the loci to an expected 1:1 segregation ratio was tested using chi-square analysis. Linkage analysis of polymorphic marker loci was performed with MAPMAKER (Lander et al. 1987; Lincoln et al. 1993) for selfed RILs. Recombination frequencies were converted to centi-Morgans (cM) using Haldane's mapping function (Haldane 1919) because of the independent cross-over events in different meiotic phases during the development of the population. Multipoint analysis was used on individual linkage groups, using an initial LOD threshold of 3.0 and maximum recombination fraction of 0.45. Non-distorted markers that could be mapped with a LOD threshold >3.0 were integrated first, followed by markers that showed distorted segregation. Markers that did not map at LOD 3.0 were then placed at preferred locations using LOD score 2.5. The polymorphic markers were ordered using the "compare" command. Additional markers were added afterwards on this frame using the "try" command. The final order was verified with the "ripple" command with a window-size of five and LOD threshold of

3.0. The linkage map was finally drawn using the software Mapchart, Version 2.1 (Voorrips 2002).

Results

The RIL population showed wide variation for growth habit, tillering capacity, plant height and days to flowering (data not shown). In addition to these quantitative traits, the population also showed segregation for two morphological traits viz. coleoptile and aleurone colour (Table 1). A total of 306 SSR markers were used to screen parents for polymorphism. While surveying for polymorphism, a hexaploid wheat cultivar WL711was used as a positive control. Although the majority of the markers showed amplification in both the parents, some showed amplification only in one parent while some did not show amplification in both the parents. An allele was considered as null if it showed amplification in hexaploid wheat cultivar WL 711 but no amplification either in *T. monococcum* or *T. boeoticum* or both. Out of 306 SSRs tested for polymorphism, 98 (32.0%) had null alleles in *T. monococcum*, 105 (34.3%) were null in *T. boeoticum* and 74 (24.2%) did not show amplification (null) in both the parents (Table 2). The GDM and GWM markers had the lowest frequency of null alleles in both the parents, being 15.4 and 17.6%, respectively. Excluding the SSR markers that had null alleles in both the parents, the overall polymorphism exhibited was 73.7%, with GDM markers showing lowest (45.4%) and WMC markers showing highest (91.2%) level of polymorphism (Table 2). In addition to SSR markers, 194 RFLP probes were also tested for parental polymorphism and 98 (50.5%) of these showed polymorphism (Table 2) with at least one of the five restriction enzymes used.

Inheritance of morphological traits

Data for two morphological traits viz. coleoptile colour (*RC*) and aleurone colour (*Ba*) was recorded on parents and 121 RILs. The *T. monococcum* had green coleoptile and amber colour grains whereas *T. boeoticum* had purple coleoptile and blue aleurone. However, penetrance and expressivity for blue aleurone varied in *T. boeoticum* because only about half the grains had a blue aleurone. The F_1 of the cross had green coleoptile and amber grains, thereby indicating dominance of green over purple coleoptile and amber over blue aleurone. The RILs showed variation for aleurone colour, ranging from RILs with 100% amber grains, RILs with 100% purple grains and RILs with apparently more than 50% purple grains but with varying levels of expression. Phenotype of a RIL was considered as blue aleurone whether it had half grains (like *T. boeoticum*) or 100% grains with blue aleurone. Segregation in RIL population for both the traits fitted to 1:1 ratio, expected of a single gene control (Table 1). Both *Rc* and *Ba* are known to be controlled by single dominant genes (Khlestkina et al. 2002; Dubcovsky et al. 1996).

Map construction

Of the 269 polymorphic SSR and RFLP markers (Table 2), 176 were assayed on 93 RILs (Fig. 1) and these yielded 188 polymorphic loci. The 176 markers comprised of 129 SSR and 47 RFLP markers. The SSR markers that gave unambiguous amplification were used for mapping and polymorphic RFLP markers were mapped only for some target regions. These regions were the ones where some genes of our interest are mapping. Most of the SSR markers, except five, amplified a single locus in *T. monococcum* and *T. boeoticum*. SSR markers BARC124, WMC96, WMC150, WMC470 and CFD2 amplified multiple loci and the RFLP probes MWG710, MWG2021, PSR549, BF474204 and BE443103 detected multiple loci. Markers Xwmc96 and Xmwg710 had three loci each and remaining had only two loci each. Fifty-eight loci showed segregation distortion from the expected 1:1 ratio, with most of these favouring the *T. boeoticum* allele. Chi-square values for distorted loci ranged from 3.86 for Xbarc104 to 15.7 for Xbarc140.

Of the 188 polymorphic SSR and RFLP loci, 177 could be mapped on the seven linkage groups with a total map length of 1,262 cM and four gaps in chromosomes 2, 4 and 7 (Fig. 2). In addition to 177 SSR and RFLP loci, two phenotypic loci, *Rc* and *Ba* were also mapped on the linkage map thus making a total of 179 loci. As many as 28, 42, 27, 12, 32, 14 and 24 loci including *Rc* and *Ba* were mapped on linkage groups 1A, 2A, 3A, 4A, 5A, 6A and 7A, respec-

Table 1 Segregation for morphological traits in the *T. boeoticum* × *T. monococcum* RIL population

Trait	*T. boeoticum*	*T. monococcum*	RIL population	Chi-square
Coleoptile colour (Rc)	Purple	Green	Green = 59 Purple = 59 Total = 118	χ^2 (1:1) = 0.00
Aleurone colour (Ba)	Blue	Non-blue	Non-blue = 60 Blue = 61 Total = 121	χ^2 (1:1) = 0.00

Table 2 PCR amplification of SSR markers and the level of polymorphism in *T. boeoticum* and *T. monococcum* using SSR and RFLP markers

Marker type	Total markers tested	Markers giving amplification in *T. monococcum*		Markers giving amplification in *T. boeoticum*		No amplification in both the parents	Polymorphic markers	% polymorphism[b]
		Number	%	Number	%			
BARC	70	44	62.8	47	67.1	19 (27.1)[a]	34	66.6
CFA	25	17	68.0	19	76.0	6 (24.0)	16	84.2
CFD	55	36	65.4	31	56.4	16 (29.1)	29	74.3
GDM	13	11	84.6	9	69.2	2 (15.4)	5	45.4
GWM	91	67	73.6	62	68.1	16 (17.6)	54	72.0
WMC	47	30	63.8	30	63.8	13 (27.6)	31	91.2
SWM	5	3	60.0	3	60.0	2 (20.0)	2	66.6
Sub-total	306	208	68.0	201	65.7	74 (24.2)	171	73.7
RFLP	194	–	–	–	–	–	98	50.5

[a] Numbers in parentheses are the percentages

[b] Excluding SSR markers that did not show amplification in both the parents

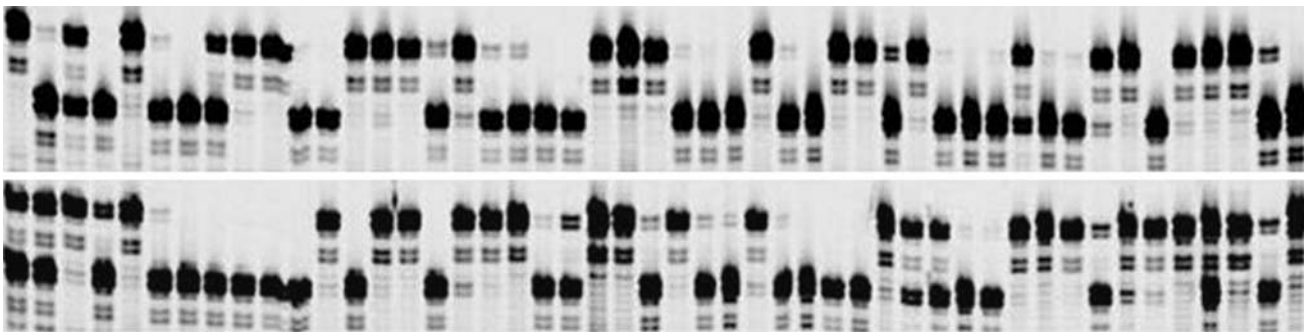

Fig. 1 PCR amplification of *T. monococcum* (lane 1), *T. boeoticum* (lane 2), F_1 (lane 3) and 93 RILs (lanes 4–48 upper panel and 1–48 lower panel) for SSR marker BARC67, resolved in 8.0% polyacrylamide gel and visualized on LICOR4200 DNA sequencer

tively (Table 3, Fig. 2). A small number of loci (11) remained unlinked to any of the seven linkage groups. These include five bin-mapped ESTs, one genomic DNA RFLP and five SSRs and three of these were detected as multiple loci. In the published maps, five of these are shown to be mapped on distal region of 1AS and four in the region proximal to the telomere of 2AL. The mapped loci include 18 bin mapped ESTs, 27 RFLPs and 132 SSRs. Largest linkage group was obtained for chromosome 3 (253 cM), and smallest for chromosome 6 (89.0 cM) (Table 3). Total map length of chromosome 4 was 80 cM with one gap spanning more than 50 cM. Similarly chromosome 7 had a total map length of 192 cM with two gaps, each spanning more than 50 cM, thus if the gaps are bridged, the linkage group 7 may turn out to be the longest. The 179 loci were distributed through out the genome and do not show any large rearrangements. Overall this map is longer than all other published maps (Table 3), except the one from Roder et al. (personal communication).

With a few exceptions, chromosomal location and marker order was similar to the one reported for *T. aestivum* (Gale et al. 1995; Dubcovsky et al. 1996; Roder et al. 1998; Sourdille et al. 2003; Somers et al. 2004; Quarrie et al. 2005; Song et al. 2005; Torada et al. 2006, http://www.shigen.nig.ac.jp/wheat/komugi/maps/markerMap.jsp). A total of 27 loci (15.3%), which include 20 SSR and seven RFLPs loci showed different chromosomal location (Table 4). Some SSR and RFLP markers like WMC96, WMC470, BARC124 and MWG2021 that amplified or detected multiple loci also showed different chromosomal locations. SSR marker WMC96 for example, amplified four loci and three of these mapped on linkage groups 3, 6 and 7 (Fig. 2) but in earlier studies, in hexaploid wheat, it has been mapped on linkage groups 4 and 5 as Xwmc96-4A and Xwmc96-5A. The two morphological traits viz. aleurone colour (*Ba*) and coleoptile colour (*RC*) map on linkage groups 4L and 7S, respectively. The traits *Ba* and *Rc* have been reported to map on 4L (Dubcovsky et al. 1996) and 7S (Khlestkina et al. 2002), respectively.

Segregation distortion

From the 189 loci analysed on 93 RILs, 58 (30.6%) showed segregation distortion. Only nine loci (4.8%)

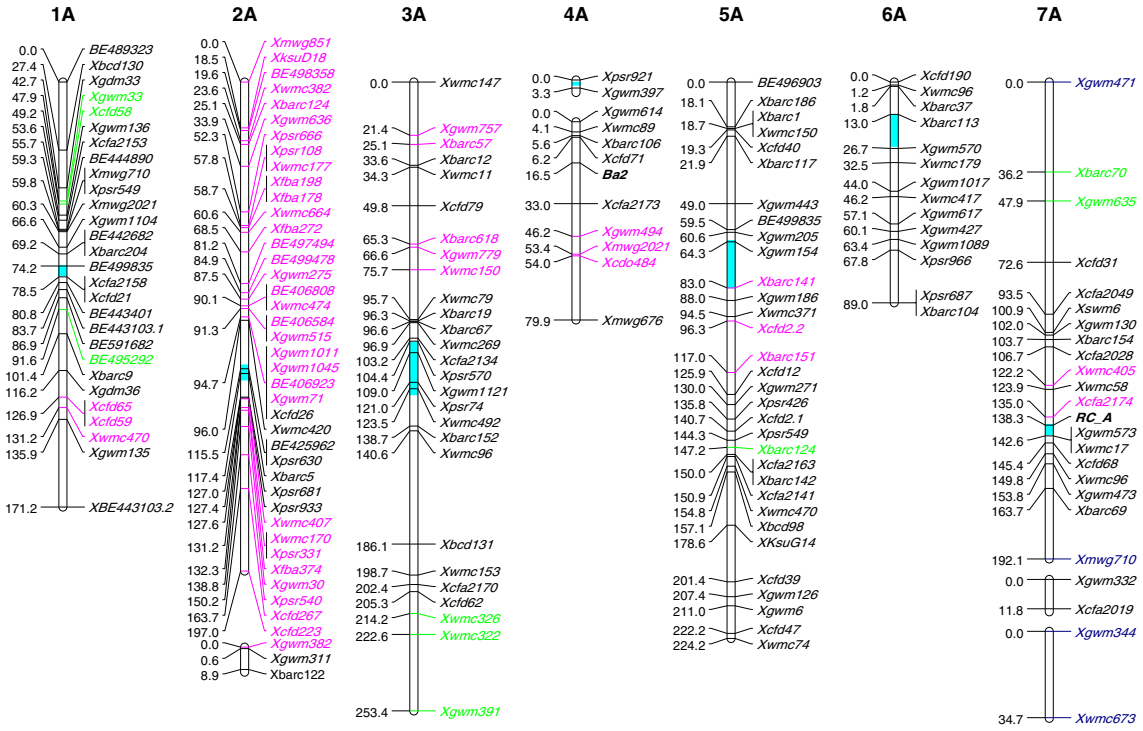

Fig. 2 Molecular linkage map of diploid A genome wheat developed using RIL population of the cross *T. boeoticum* × *T. monococcum*. Recombination fractions are in Haldane cM. Markers in blue are ones mapped at LOD score 2.5. Markers in magenta and green colours are distorted in favour of *T. boeoticum* and *T. monococcum* alleles, respectively. Shaded portions in the bar are most probable centromere positions as inferred from published records

Table 3 Comparison of map length of A genome chromosomes in various linkage maps generated in different populations

Linkage group	Present map	Torada et al. (2006)	Quarrie et al. (2005)	Song et al. (2005)	Somers et al. (2004)	Röder et al. (unpublished)[a]	Roder et al. (1998)	Dubcovsky et al. (1996)	Gale et al. (1995)
1A	171.2 (28)[b]	133.7	131.0	129.3	126.0	224.0	155.8	157.1	146.0
2A	205.9 (42)	113.0	170.2	120.1	143.0	265.0	138.3	160.0	167.8
3A	253.4 (27)	251.0	158.3	211.7	116.0	216.0	160.3	145.2	145.2
4A	79.9 (12)	156.5	179.2	122.5	88.0	196.0	101.4	126.9	122.8
5A	224.2 (32)	241.8	190.7	132.4	184.0	237.0	175.1	192.2	192.2
6A	89.0 (14)	148.7	151.7	113.4	156.0	191.0	134.1	143.6	143.6
7A	238.6 (24)	186.3	167.1	146.7	131.0	286.0	178.2	146.6	146.6
Total	1,262.2 (179)	1,231.0	1,148.7	976.1	944.0	1,615.0	1,043.2	1,071.6	1,064.2

[a] M. Roder, personal communication

[b] Figures in parentheses are the number of loci mapped on these linkage groups

showed distortion in favour of *T. monococcum* allele whereas 49 (25.9%) showed distortion in favour of *T. boeoticum* allele (Fig. 2). Among the 58 distorted loci, 28 (15.1%), 22 (11.6%) and 8 (4.2%) showed distortion at $P = 0.05$, 0.01 and 0.001, respectively, and all but one (BF474204.2) could be mapped on various linkage groups. Of the loci that showed distortions in favour of *T. boeoticum* allele, three mapped on 1A, 32 on 2A, five on 3A, three each on 4A and 5A, two on 7A, and one remained unlinked. Similarly, of the nine loci that showed distortion in favour of *T. monococcum* allele, three mapped on 1A, three on 3A, one on 5A and two on 7A. The complete chromosome 2A, except for 30 cM region around centromere, showed distortion in favour of *T. boeoticum* allele (Fig. 2) while other markers were present in short stretches on several chromosomes.

Table 4 Loci that mapped on different linkage groups in present *T. boeoticum* × *T. monococcum* RILs compared to published studies

Chromosome	Loci
1	Xbcd130 (4)[a], Xbarc204 (6), Xgdm36 (6)
2	Xmwg851 (4), Xwmc264 (3), Xcfd26 (5), Xmwc420 (4), Xcfd223 (3), Xbarc122 (5)
3	Xwmc147 (1), Xwmc79 (6), Xwmc492 (7), Xbarc152(1), Xbcd131 (5), Xcfa2170 (1), Xcfd62 (2, 7)
4	Xgwm614 (2), Xgwm494 (5, 6), Xcdo484 (1)
5	Xbcd98 (1, 7), XksuG14 (3), Xcfd39(4), Xcfd47 (6), Xwmc74 (7)
6	Xcfa2173 (4), Xpsr687 (2, 7)
7	Xgwm473 (2)

[a] Numbers in parentheses are the linkage groups where these loci have been mapped in earlier studies

Transferability of A and D genome specific SSR markers

Since *T. monococcum* and *T. boeoticum* are not the A genome donors of the hexaploid wheat, 100 per cent transferability (ability to amplify) of SSRs developed in hexaploid wheat was not expected in *T. monococcum* and *T. boeoticum*. Out of 306 SSR markers tested, 74 (24.2%) markers did not show amplification in either parent (Table 2). Overall 75.8% markers did show amplification in one or both the parents. The D genome specific primers developed from *T. tauschii* (Pestsova et al. 2000; Guyomarc'h et al. 2002a) were also tested for amplification (Table 2). Of 68 D genome specific markers, only 18 (26.5%) did not show amplification in both the parental lines, thus 73.5% of the D genome specific markers were transferable in *T. monococcum* and *T. boeoticum* compared to 76.5% transferability of A genome specific primers including CFA markers developed from *T. urartu* (http://www.wheat.pw.usda.gov/GG2/quickquery.shtml#microsats). *T. monococcum* and *T. boeoticum* showed 68.0 and 65.7% transferability, respectively, for all SSR marker groups (Table 2) and 69.1 and 58.8% transferability, respectively, for the D genome specific markers. In addition to 306 SSR markers mentioned above, *T. monococcum* DNA was amplified with additional 139 A genome specific GWM primers (600 onward series provided by Dr. M Röder, IPK, Gatersleben) and only 86 showed amplification (data not shown). Thus, in a total of 230 GWM markers tested, 153 (66.5%) markers showed amplification in *T. monococcum*. There was no significant difference in the transferability of A genome specific markers developed from hexaploid wheat *T. aestivum*, and the diploid A genome donor *T. urartu* and D genome specific markers developed from D genome progenitor *Ae. tauschii*. Polymorphism levels for the three marker groups were also comparable.

Frequency of RILs with parental (non-recombinant) chromosomes

Since F_6 RILs were used in this study, the population had undergone 5 cycles of recombination. All the chromosomes were expected to undergo recombination because the F_1 between *T. monococcum* and *T. boeoticum* show complete bivalent formation, normal fertility and seed germination (Johnson and Dhaliwal 1976). However, among the 93 RILs analysed with molecular markers, 68 (73%) had one or more parental (non-recombinant) chromosomes (Table 5). Out of 68 RILs, one RIL had three parental chromosomes, 10 RILs had two and 57 RILs had one non-recombinant parental chromosome. Thus, of the 651 RIL X chromosome combinations, 68 chromosomes (10.4%) remained non-recombinant even after 5 cycles of recombination, highest frequency of parental chromosomes being observed was for chromosome 4.

Discussion

Map construction

The A genome linkage map, based on an inter sub-specific cross, generated in the present study has 179 loci including two morphological markers and a size of 1,262 cM with four gaps in linkage group 2, 4 and 7. This map is very similar in length to an earlier map of *T. monococcum* × *T. boeoticum* and A genome maps of hexaploid wheat (Table 3). The present map is about 17.7% longer than the one reported by Dubcovsky et al. (1996) but about 21.8% shorter than the A genome map generated by Marion Röder at IPK, Gatersleben (Marion Röder, personal communication). Gaps have been reported in most of the hexaploid wheat maps generated so far (Torada et al. 2006; Quarrie et al. 2005; Pailliard et al. 2003; Sourdille et al. 2003).

Table 5 Frequency of *T. monococcum* × *T. boeoticum* RILs with parental (non-recombinant) chromosomes

Chromosome	Number of RILs with non-recombinant		Total
	T. monococcum chromosome	*T. boeoticum* chromosome	
1A	5	8	13
2A	0	8	8
3A	0	0	0
4A	11	17	28
5A	0	1	1
6A	4	7	11
7A	3	4	7
Total	23	45	68

Most of the markers maintained their position and order along a linkage group as presented in series of linkage maps, but some of the markers did map at different positions. This could be due to amplification of SSRs at multiple loci or due to small changes in A genome of *T. monococcum* and *T. boeoticum* relative to the A genome of hexaploid wheat which has its origin from *T. urartu*. Locations of centromeric positions also correspond well to other published results except for linkage group 1A. For example, loci Xcfa2158, Xcfd65, Xgwm135 have been shown to map on 1AL (Röder et al. 1998; Sourdille et al. 2003, 2004; Song et al. 2005) and ESTs BE443401, BE443103, BE591682, BE495292 and BE443103, have been reported to map in the deletion bin 1AS3. 0.86-1.00 and BE489323 maps in the deletion bin 1AS1.0.47–0.86 (http://www.wheat.pw.usda.gov/cgi-bin /westsql/ map_locus.cgi). Thus, in the linkage map, the ESTs BE443401, BE443103, BE591682, BE495292 and BE443103 should map proximal to BE489323, i.e. towards telomere of 1AS but actually these map in between Xcfa2158 and Xbarc9 (Fig. 2) which otherwise are shown to map on 1AL (Röder et al. 1998; Sourdille et al. 2003, 2004; Song et al. 2005). *Xgwm33* that maps on 1AS in this linkage map (Fig. 2) has been physically mapped in deletion bin 1AS3 0.86-1.0 (Goyal et al. 2005). Therefore, chromosome $1A^m$ of *T. monococcum* and *T. boeoticum* may have a paracentric inversion relative to 1A of *T. aestivum*. Dubcovsky et al. (1995, 1996) reported complete colinearity between $1A^m$ of *T. monococcum* and 1A of *T. aestivum* despite the fact that reduced pairing (0.76%) was observed between the 1AS and $1A^mS$ compared to 91.8% pairing observed between 1AL and $1A^mL$ (Dubcovsky et al. 1995) and reduced recombination was also observed in the terminal regions of 1AS and $1A^mS$ even in the absence of *Ph1* locus. Thus, a "sub-structural" differentiation between 1A and $1A^m$ as postulated by Dubcovsky et al. (1995) could be a paracentric inversion in less than 14% terminal region of the short arm of $1A^m$ relative to 1A, because some of the loci such as Xgwm33, that map in the terminal region in the present map also map in bin 1AS3.0.86-1.0 (Goyal et al. 2005). This paracentric inversion may be the cause of sterility in the F_1 between *T. monococcum* and *T. urartu* (Johnson and Dhaliwal 1976). Also in the F_1 between *T. durum* and *T. monococcum* the frequency of bivalents in the F_1 was always six and not seven (data not shown). Variation in relative order of markers in cultivated species and its progenitor species does occur as has been shown for D genome of hexaploid wheat and its diploid progenitor *Ae. tauschii* for chromosomes 3D, 4D and 5D (Boyko et al. 1999). In chromosome 4D for example, markers that map physically in an interstitial region of 4DL, map on terminal region of 4DS in the genetic map. On linkage group 5D, 18 of the 47 loci common to the *Ae. tauschii* genetic linkage map and the *T. aestivum* consensus physical map are in different locations. Such changes are expected to occur during genome evolution.

Segregation distortion and presence of non-recombinant chromosomes

Segregation distortion seems a common phenomenon in all populations, be it F_2, DH or RILs, with RILs having highest probability of distortions due to continued selfing for 5–6 generations. The *T. boeoticum* × *T. monococcum* population used in the present study showed segregation distortion for 58 of 188 loci. More than 80% of the distorted loci were in favour of *T. boeoticum* alleles and more than 50% of these were mapping in two contiguous regions of chromosome 2A, comprising 95 and 70 cM, respectively. One may argue that this distortion in 2A could be due to selection of RILs for earliness, as the *Ppd* genes for response to photoperiod are located on homoeologous group 2 (McIntosh et al. 2003). If that was the case then the distortion should have in been in favour of *T. monococcum* alleles and not in favour of *T. boeoticum* alleles, because *T. monococcum* accession used was spring type and flowers about 15–20 days early than the *T. boeoticum* accession used. Gametocidal activity genes, *Gc1-B1a, Gc1-B1b* and *Gc1-Sl1* from *Aegilops speltoides*. sub-sp. *aucheri, Ae. speltoides* sub-sp. *ligustica*, and *Ae. Sharonensis*, respectively, have been mapped on homoeologous group 2 (Tsujimoto and Tsunewaki 1988). In monosomic additions of chromosomes with gametocidal effects, chromosome deletions and translocations are produced in gametes not having the gametocidal genes. Likewise the *T. boeoticum* accession 5088 used in the present study might contain a gametocidal gene, resulting in preferential transmission of gametes having *T. boeoticum* chromosome. Segregation distortions have been reported in all the interspecific or intraspecific crosses used for generating linkage maps in diploid and hexaploid wheat species. Dubcovsky et al. (1996) showed segregation distortion for 15% of the loci and most of these were in long stretches of linkage groups 1A and 7A in an inter sub-specific *T. monococcum* × *T. boeoticum* RIL population. Boyko et al. (1999) recorded 24% loci showing significant deviation in an intraspecific *Ae. tauschii* cross with greatest number of deviations on chromosome 3, 5 and 7. Segregation distortion in as many as 26% of the loci covering a region of 278 cM was reported by Cadalen et al. (1997) in an intervarietal cross of hexaploid wheat. Other studies also reported segregation distortion in intraspecific crosses (Messmer et al. 1999; Pailliard et al. 2003; Sourdille et al. 2003; Quarrie et al. 2005). The aberrant genetic segregation might be due to linkage between the loci and sterility genes, due to gametophytic selection or due to physiological and environmental effects. Xu et al. (1997) reported

segregation distortion in 6 of the 11 gametophyte gene regions and 5 of the 7 sterility gene regions in rice. Preferential transmission of one of the alleles can have both positive and negative impacts on interspecific gene transfers.

Although chromosomes of *T. boeoticum* and *T. monococcoum* show seven bivalents during meiosis (Dubcovsky et al. 1996), recombination was expected in all the chromosomes in F_6 RILs but a relatively high frequency of RILs with non-recombinant chromosomes was observed in this population. Highest frequency of RILs with non-recombinant chromosome was for chromosome $4A^m$, which is known to be involved in cyclical interchange with $5A^m$ (Devos et al. 1995) but for $5A^m$ only one RIL showed a non-recombinant chromosome. Quarrie et al. (2005) reported 19 of the 96 DH lines (20%) with one or more non-recombinant chromosome in an intraspecific cross and this varied inversely with the chromosome length.

Transferability of the markers

The SSR markers used in the present study were developed from hexaploid wheat (GWM, WMC, BARC and SWM), A genome of *T. urartu* (CFA) and the D genome of *Ae. tauschii* (CFD, GDM). *T. urartu* genome, that contributed A genome of hexaploid wheat (Dvorak et al. 1988), shows considerable differences from the A genome of *T. monococcum* or *T. boeoticum* (Johnson and Dhaliwal 1976). About 65–70% of SSR markers developed from hexaploid wheat and diploid *Ae. tauschii* showed amplification in *T. monococcum* and *T. boeoticum* with *T. monococcum* showing relatively higher transferability than *T. boeoticum*. A high level of conservation exists between A and D genomes of diploid wheat. As many as 69.1 and 58.8% of the D genome specific SSR markers showed amplification in *T. monococcum* and *T. boeoticum*, respectively, and about 68.0 and 76% of the A genome specific markers from *T. urartu* showed transferability in *T. monococcum* and *T. boeoticum*, respectively. Among the SSR markers isolated from hexaploid wheat, about 67% showed transferability in both *T. monococcum* and *T. boeoticum*. Bai et al. (2004) reported only about 50% transferability of the A genome specific makers of hexaploid wheat to diploid A genome species. The majority of the markers that did not show transferability in the study of Bai et al. (2004) did not show transferability in the present study as well, with few exceptions like GWM136, GWM614 and GWM154 that did show amplification either in *T. monococcum* or *T. boeoticum*. On the other hand, Sourdille et al. (2001) reported transferability of 93% of the SSR markers derived from hexaploid wheat on the corresponding ancestral A genome diploid species. However, they used only 12 A genome specific primers, which is much less a number than used in the present study. Guyomarc'h et al. (2002b) reported about 50% transferability of *Ae. tauschii* derived SSR markers in the diploid A genome species. Genomic relationship among A, B and D genomes of hexaploid wheat has been established by studying chromosome pairing in euhaploids having the recessive mutant allele *ph1b* and nullihaploids that lack chromosome 5B (Jauhar et al. 1991). They found that A and D genomes showed 80% association against only 11.5% between A and B and 7% between B and D. Thus, A and D genomes are much more closely related to each other than either one is to the B genome. Evolutionary relationships between A, B and D genomes of wheat and its progenitor species have been studied by gene comparisons as well (Huang et al. 2002; Petersen et al. 2006). Based on sequence homology of the genes, it was concluded that the A and D genomes are more closely related than is the S genome of sitopsis section or the B genome of tetraploid and hexaploid cultivated and wild wheats. Sitopsis section (excluding *Ae. speltoides*) is sister to *Ae. tauschii* and *Ae. speltoides* is distinct from other *Aegilops* species of sitopsis section (Petersen et al. 2006). Thus, SSR markers derived from *Aegilops* species of sitopsis section (excluding *Ae speltoides*) should also show high transferability to A genome diploid wheat.

Triticum monococcum and *T. boeoticum* harbour desirable variability, which may meet some of the future requirements for wheat improvement. Although relatively high-density linkage maps are now available in hexaploid wheat (Sourdille et al. 2003; Somers et al. 2004; Quarrie et al. 2005; Torada et al. 2006), gene cloning in hexaploid wheat is still complex due to the polyploid genome, large genome size and limited polymorphism. Diploid A and D genome species could be used for cloning orthologous genes from hexaploid wheat. *T. monococcum* is easily cultivated in the field compared to *Ae. tauschii*, which is extremely difficult to handle in the field due to shattering and hard threshing. *T. monococcum* thus has a potential for becoming a model Triticeae species for understanding wheat genomics and cloning of genes because of its relatively small genome size, higher level of polymorphism and availability of large insert genomic libraries in *T. monococcum* (Lijavetzky et al. 1999) and *T. urartu* (Akhunov et al. 2005). Compared to hexaploid wheat, higher marker polymorphism and recombination frequency are expected in diploid wheat, hence map based cloning of orthologous genes from diploid genome may be genetically simpler than cloning these from hexaploid wheat but the development of saturated linkage map of A genome diploid wheat is a pre-requisite for cloning of orthologous genes. Present study reporting an integrated map based on SSRs and RFLPs, including bin mapped ESTs, will complement the already existing maps in diploid and hexaploid wheat. The population used for generating the linkage map segregated for several disease resistance genes and for productivity

and evolutionary traits, which are being mapped and shall be reported elsewhere. Markers closely linked to these genes will be extremely useful for marker assisted introgression of desirable variability from *T. monococcum* and *T. boeoticum* to tetraploid and hexaploid wheat.

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