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ORIGINAL PAPER

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A genetic linkage map of the Durum × *Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits

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Abstract Durum wheat (*Triticum turgidum* L. var *durum*) is mainly produced and consumed in the Mediterranean region; it is used to produce several specific end-products; such as local pasta, couscous and burghul. To study the genetics of grain-milling quality traits, chromosomal locations, and interaction with the environment, a genetic linkage map of durum was constructed and the quantitative trait loci QTLs for the milling-related traits, test weight (TW) and thousand-kernel weight (TKW), were identified. The population constituted 114 recombinant inbred lines derived from the cross: Omrabi 5*/ Triticum dicoccoides 600545//* Omrabi 5. TW and TKW were analyzed over 18 environments (sites × years). Single-sequence-repeat markers (SSRs), Amplified-fragment-length-polymorphism markers (AFLPs), and seed storage proteins (SSPs) showed a high level of polymorphism (>60%). The map was constructed with 124 SSRs, 149 AFLPs and 6 SSPs; its length covered 2,288.8 cM (8.2 cM/marker). The map showed high synteny with previous wheat maps, and both SSRs and AFLPs mapped evenly across the genome, with more markers in the B genome. However, some rearrangements were observed. For TW, a high genotypic effect was detected and two QTLs with epistasic effect were identified on 7AS and 6BS, explaining 30% of the total variation. The TKW showed a significant transgressive inheritance and five QTLs were identified, explaining 32% of the total variation, out of which 25% was of a genetic nature, and showing QTL×E interaction. The major TKW-QTLs were around the centromere region of 6B. For both traits, Omrabi 5 alleles had a significant positive effect. This population will be used to determine other QTLs of interest, as its parents are likely to harbor different genes for diseases and drought tolerance.

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Introduction

Wheat is one of the most widely grown food grain crop in the world. Understanding its genetics and genome organization using molecular markers is of great scientific value to scientists and breeders. Durum wheat (*Triticum turgidum* L. var *durum*) is a tetraploid made up of A and B genomes (AABB); it is the main source of semolina for the production of pasta, couscous, burghul and other Mediterranean local end-products (Nachit 1992). Further, nutritionally, it also plays a major role for providing vitamin A in this region.

The construction of genetic linkage maps relies on the choice of the parental lines, the segregating population and markers to reveal polymorphism. They can be constructed based on F2, F3 families, backcrosses, double-haploids or recombined inbred lines (RILs). However, the RIL populations have several advantages, including reproduction, which favors the genetic analysis of quantitative traits because experiments can be replicated over years and locations; and the use of dominant markers with the same efficiency as the co-dominant ones (Saliba-Colombi et al. 2000). Maps constructed with such material could therefore facilitate the fine mapping of regions around genes of interest. Molecular linkage maps have been constructed for many organisms (Helentjaris et al. 1986; McCouch et al. 1988; Chao et al. 1989; Gill et al. 1991; Heun et al. 1991; Röder et al. 1998) and more recently for durum wheat (Blanco et al. 1998; Korzun et al. 1999; Nachit et al. 2001). The first durum map was based on 65 RILs and RFLP markers (Blanco et al. 1998); and later SSRs were integrated into this genetic map (Korzun et al. 1999). The second durum map was based on an intraspecific cross Jennah Khetifa × Cham1 with 110 RILS using RFLPs, SSRs, AFLPs, seed storage proteins and genes (Nachit et al. 2001).

Microsatellites consist of small repeat units generating repeating regions of less than 100 bp (Thomas and Scott 1993). They may be di-, tri- or tetra-nucleotides and show high levels of polymorphism (Bryan et al. 1997). The high level of polymorphism combined with a high intersper-

sion rate, makes SSRs an abundant source of genetic markers. They are highly informative and locus-specific in many species (Akkaya et al. 1992). Microsatellites are evenly distributed over the genome, are inherited in a co-dominant manner, and require only small amounts of DNA for analysis. However, due to the large genome size, the development of SSRs in wheat is time-consuming and expensive (Röder et al. 1995; Bryan et al. 1997; Röder et al. 1998). A low level of transferability was shown across the three wheat genomes and to other cereal genomes (Röder et al. 1995; Bryan et al. 1997) in contrast to results on the mammalian species (Schloetterer et al. 1991).

AFLP technique is based on the selective amplification of a limited number of DNA restriction fragments. AFLP has the great advantage that it permits simultaneous coverage of many loci in a single assay (Donini et al. 1997); but for synteny studies, it is less efficient than RFLP (Tanksley et al. 1988). AFLP may be used for studies of diversity and the fingerprinting of cloned DNA segments, like cosmids, P1 clones, Bacterial and Yeast Artificial Chromosomes (BACs, YACs) (Vos et al. 1995). Both techniques, SSRs and AFLPs, were successfully used in durum wheat genetic mapping (Nachit et al. 2001).

For durum milling and processing, a special mapping population *Triticum durum/Triticum dicoccoides//T. durum* was developed to study grain quality (Nachit 1998). The backcross to the durum variety was made in order to have RILs agronomically acceptable for multi-locational testing. The addressed grain quality traits in this paper are the test weight (TW) and the thousand-kernel weight (TKW), which are highly related to semolina yield. The TKW in durum varies greatly; from 20 to over 60 g. High TKW is desirable for easy processing, milling and semolina traits. It is affected by the environment during grain filling and by the number of heads and the number of fertile florets per spike, and has been strongly correlated with kernel length, width and volume (Schuler et al. 1994). The genetics of TKW is unknown, but it has been reported to be highly heritable with high-additive and multigenic effects (Joppa and Williams 1988; Nachit et al. 1995a).

The aim of this study was: (1) to construct a genetic linkage map of the *durum/Triticum dicoccoides//durum* population, and (2) to determine QTLs related to the milling quality parameters, kernel weight and test weight.

Material and methods

Plant material

The plant material used is the population stemming from the cross between ''Omrabi 5*/T. dicoccoides 600545//*Omrabi 5" (MDM) with the pedigree ICDMN91X-Omrabi 5*/T. dicoccoides 600545//* Omrabi 5. The population is constituted of 114 backcrossed recombinant inbred lines (RIL) developed in 1991 at the ICARDA durum breeding program as shown below. The Omrabi 5 *durum* cultivar is a cross between the landrace Haurani and the improved cultivar Jori-C69 from CIMMYT-Mexico; Omrabi 5 was developed for the Mediterranean dryland conditions (Nachit 1998). Omrabi 5 is released in Turkey, Algeria, Iran and Iraq for commercial production; it combines drought tolerance with yield and yield stability. As for *T. dicoccoides 600545*, it was collected from Jordan at 25 km west of Amman on the Amman-Dead Sea highway; it shows a high protein content, resistance to yellow rust and tolerance to drought.

Omrabi 5 × *T. dicoccoides* 600545
↓
F1 × Omrabi 5
↓
BC_1F_2
↓ Selfing using SSD during 6 generations

BC1F8 RILs (114 Progenies)

Pedigree of the Omrabi 5/*T. dicoccoides* 600545//
Omrabi 5 mapping population.

Testing environments

The RILs trial was grown in four sites (Tel Hadya, Breda, Terbol and Kfardan) for the four consecutive seasons: 1996/97 to 1999/2000. Tel Hadya is the main research station at ICARDA-HQ, located in Syria at 36°01' N latitude; 36°56' E longitude and at 284 m asl; and has a Mediterranean Continental climate with average annual precipitation of 335 mm. The double-gradient screening technique with six environments (Nachit et al. 1995b) was used (Early Planting-EP, Rainfed-Rf, Irrigated-Ir, Late Planting-LP, Summer planting-Sum and Sowing after legume crop-Inc). As for the Breda station (Br), it is located also in Syria at 35°56' N latitude, 37°10' E longitude and 300 m asl, with clayey soil, and an average annual precipitation of 260 mm, and is characterized by drought and harsh continental-climatic conditions. Terbol station is located in Lebanon in the Beka'a valley at 33°33' N latitude, 35°59' E longitude and 890 m asl, and characterized by cold winters but favorable growing conditions; it has a fine clay soil and an average annual precipitation of 524 mm. As for the Kfardan (Kf) station located also in Lebanon in Beka'a valley at 34°01' N latitude, 36°06' E longitude and 1,080 m asl, and characterized by extreme temperature fluctuations; it has a fine clay soil and an average annual precipitation of 402 mm. For the experimental design and data analysis, we used the augmented design (Federer 1956). TKW data are available for 18 year × site environments (97Ir, 97Rf, 98Br, 98EP, 98LP, 98Rf, 99Br, 99Inc, 99Kf, 99LP, 99Rf, 99Tr, 00Tr, 00Rf, 00Br, 00EP, 00Sum, 00Ir); whereas TW has 3 year × site environments (99Inc, 99Rf, 00Rf).

Molecular markers

Gliadin assay

Single seeds were crushed in a mortar. Gliadin proteins were extracted from single seeds with 1.5 M Dimethylformamide (100 μl per 20 mg of seed). After 1 h incubation at room temperature and 15 min centrifugation at 8,000 tr/min, the supernatant (5 μl) was fractionated on polyacrylamide-gel electrophoresis at 8.5% (C=2.7) and at 18°C in aluminum lactate buffer pH 3.1 (A-PAGE) according to Tkachuk and Metlish (1980, modified).

Glutenin assay

To obtain good separation of low-molecular-weight glutenin subunits (LMW) in a background free from gliadins, albumins and globulins, the glutenins were extracted with alkylation and separated using one-dimensional SDS-PAGE. The residue from the samples used for gliadin extraction was cleaned 3 times with 50% propanol-1 at 60°C to remove monomeric proteins overlapping in SDS-PAGE with LMW glutenin subunits. Further, the residue was reduced in 50% propanol-1, 80 mM Tris-HCl pH: 8.5, 2% DTT;

incubated for 30 min at 60°C, and centrifuged for 10 min at maximum speed. The 4-vinylpiridine (2.8 μl) was added to the supernatant and incubated for 30 min at 60°C. Finally, the glutenins were precipitated with 1 ml of cold acetone (−20°C), dried under a fume hood and stored at −20°C. The extracted glutenins were separated in a 8–14% gradient gel at C=1.28% in Tris-Glycine buffer (Alvarez et al. 1999), and stained with a solution of Coomasie Brilliant Blue R250 in 1% ethanol, 12% TCA.

DNA extraction

The DNA was extracted following the SDS procedure published in the ITMI wheat-mapping workshop (1994) and quantified by the spectrophotometer.

Microsatellites (SSRs)

The used Gatersleben wheat microsatellites (*gwm;* Röder et al. 1998) were amplified as in Nachit et al. (2001). The parents were screened using 195 gwm out of which 64% were polymorphic. The PCR was carried out in Perkin-Elmer 9700 Thermal Cycle. The PCR amplified fragments were either separated in 12% acrylamide gel (39:1) or a 6% denaturing acrylamide gel (19:1) depending on the number of base pairs differentiating the two parents. The gels were visualized by a quick silver-staining method: 3 min in 10% ethanol+0.5% acetic acid, 5 min in 0.2% silver nitrate, a quick rinse with ddH_2O and development in 1.5% NaOH+0.2% formaldehyde.

Amplified fragment length polymorphism analysis (AFLPs)

The AFLP method used was as described by Vos et al. (1995) using the Life Technology Kit (*Eco*RI as a rare cutter and *Mse*I as a frequent cutter). Eighty nanograms of genomic DNA were first digested in two units of *Mse*I and two units of *Eco*RI in 50 mM NaCl at 37°C for 2 h. The digestion was inactivated by incubation at 70°C for 15 min. This digested DNA was ligated to the *Eco*RI adapter: 5′-CTCGTAGACTGCGTACC//CATCTGACGCATGGT-TAA-5′ and to the *Mse*I adapter: 5′-GACGATGAGTCCTGAG// TACTCAGGACTCAT-5′ at 20°C for 2 h. The resulting ligated product was diluted five times with TE and pre-amplified in 20 μl 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, 0.8 mM dNTPs, 0.56 μM pre-amp primer *Mse*I, 0.56 μM pre-amp primer *Eco*RI, 1 unit of *Taq* DNA polymerase (Boehringer, Inc.), and 2 μl of the diluted DNA under 20 cycles: 95°C for 30 s, 56°C for 1 min, and 72°C for 1 min. The pre-amplified product was diluted five times and checked in 1% agarose gel. A final selective amplification was carried out in 10 μl of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, 0.8 mM dNTPs, 0.25 μM selective primer *Mse*I, 0.25 μM selective primer *Eco*RI, 0.75 units of *Taq* DNA polymerase (Boehringer, Inc.) and 2.5 μl of the pre-amplified DNA. The PCR program used was a touchdown from 65°C to 56°C (−0.7°C/cycle) and was carried out in a Perkin-Elmer 9700 Thermal Cycle. The amplified fragments were separated in 6% denaturing-acrylamide gel (19:1) and the fragments were visualized by silver staining. Twenty combinations were used. The polymorphic bands were scored as present/absent (1/0) using the Crosschecker program (Buntjer, Wageningen University, 1999).

Map construction

The map was constructed using MAPMAKER version 2.0 (Lander et al. 1987). Kosambi function (Kosambi 1944) was used to convert the recombination frequency to genetic distances in centimorgans (cM). The map was constructed at a LOD of 3.0, except for some segment fragments that were joined at LOD 2.5. Two-point analysis was first applied to identify groups of related markers at the maximum LOD score of at least 3.0 and the minimum recombination ratio at more than 40%. The formed groups were afterwards ordered using the "First Order" command whenever possible. Usually, the first order was aided with LOD table correlations between markers, to figure out the most-linked markers. The obtained order was then analyzed further using a Three-Point linkage analysis "Ripple" command. Other markers were added using the "Place" command and fine-tuned using again the "Ripple" command. Both chromosome assignment and centromere localization were determined by comparing the MDM map to the previously published wheat maps, especially to the Röder et al. (1998) wheat SSR map.

Quality traits

The test weight was determined with the Schopper Chondrometer, using a 500 ml container. TW was measured for three environments in Tel Hadya, two in 1999 and one in 2000. The TKW was determined with an electronic seed counter (Numigral Chopin S.A.) using 200 kernels; broken kernels and foreign material had been removed previously by handpicking. TKW was measured in 18 environments: two in 1997; four in 1998; six in 1999 and six in 2000.

QTL analysis

The QTL analysis was performed with the software package Multiple Quantitative Trait Loci (MQTLs, Tinker and Mather 1995).

Results and discussion

Molecular markers

Microsatellites

The gwm-SSRs used have-known chromosomal assignments and were previously mapped in several mapping populations, including the bread wheat population ITMI (Röder et al. 1995, 1998) and durum populations, Messapia × *T. dicoccoides MG4343* (Korzun et al. 1999) and Jennah Khetifa × Cham1 (Nachit et al. 2001). The parents Omrabi 5 and *T. dicoccoides 600545* were screened with 192 SSRs, out of which 63.5% were polymorphic. This high-rate of polymorphism found in MDM confirms the transferability of SSRs between bread and durum wheat as has been shown in a *T. durum* × *T. dicoccoides* (Korzun et al. 1999) and in a durum × durum population (Nachit et al. 2001). Polymorphic SSRs were probed across the mapping population of 114 RILs. Twenty one SSRs showed a significant deviation from the expected ratio, and they were eliminated from the mapping analysis, as they pulled the linkage groups together and/or mapped to different genome positions on several chromosomes. Molecular markers representing skewed segregation has been reported in several *Triticeae* species (Heun et al. 1991; Liu and Tsunewaki 1991; Blanco et al. 1998; Nachit et al. 2001). These distortions may be due to chromosomal rearrangements (Tanksley 1984); alleles inducing gametic or zygotic selection (Nakagarha 1986), reproductive differences between the two parents (Foolad et al. 1995), lethal genes (Blanco et

Table 1 Number of generated and mapped SSR fragments and their chromosomal assignment in the Omrabi 5/*T. dicoccoides 600545*// Omrabi 5 population in comparison with wheat published maps

SSRs	Ampl/ mapped[a]	MDM/known[b]	SSRs	Ampl/ mapped	MDM/known	SSRs	Ampl/ mapped	MDM/known
gwm 154	4/2	3BL, 7AS/5AS	gwm 234	2/2	5AS, 5BS/5BS	gwm 193	1/1	6BS/6BS
gwm 311	3/1	2AL/2A	gwm 251	2/1	4BL/4B	gwm 265	1/1	4AL/2A
gwm 63	3/1	7AL/7A	gwm 259	2/2	1BL/1B	gwm 269	1/0	/5D
gwm 88	3/2	6BS/6BL	gwm 268	2/2	1BL/1B	gwm 273	1/0	/1B
gwm 276	3/2	7AL/7A	gwm 319	2/1	2BS/2B	gwm 274	1/1	1BL/1B, 7B
gwm 554	3/3	1BL, 2AL, 5BL/5B	gwm 340	2/1	3BL/3BL	gwm 299	1/1	2BS/3B
gwm 630	3/2	2BS/2B	gwm 368	2/1	4BS/4B	gwm 302	1/0	/7BS
gwm 335	3/1	5B/5B	gwm 376	2/2	3BS/3B	gwm 332	1/1	7AL/7A
gwm 124	3/2	1BL/1B	gwm 403	2/1	1BL/1B	gwm 344	1/1	7BL/7B
gwm 149	3/2	4BL/4B	gwm 494	2/2	6A/6A	gwm 369	1/1	3AS/3A
gwm 264	3/3	1AL, 1BS, 7BS/1B, 3B	gwm 537	2/2	5BL, 7BS/7B	gwm 372	1/0	/2A
gwm 448	3/1	2AS/2A	gwm 644	2/2	1BL, 3BS/6B, 7B	gwm 374	1/1	2BS/2B, 5AS
gwm 493	3/2	3BS/3B	gwm 558	1/1	2AL/2A	gwm 375	1/1	4BL/4BL
gwm 408	2/2	5BL/5B	gwm 118	1/1	5BS/5BL	gwm 382	1/1	2AL/2A,2B
gwm 639	2/1	5BL/5A, 5B	gwm 260	1/1	7AS/7AS	gwm 400	1/1	7BS/7B
gwm 131	2/2	1BL, 7BL/7BL, 1B, 3B	gwm 626	1/1	6BL/6B	gwm 413	1/1	1B/1B
gwm 249	2/2	2AS/2A	gwm 674	1/1	3AL/3A	gwm 415	1/1	5BS/5A
gwm 282	2/2	7AL/7A	gwm 11	1/1	1B/1BL	gwm 443	1/1	5AS/5B
gwm 371	2/2	5BL/5B	gwm 30	1/1	3AL/1AS, 3A	gwm 497	1/1	1AL/1A, 2A
gwm 570	2/1	6AL/6A	gwm 46	1/0	/7BS	gwm 498	1/1	1BL/1B
gwm 219	2/1	6BL/6B	gwm 58	1/1	6BS/6BL	gwm 499	1/1	5BL/5B
gwm 2	2/1	3AS/3AS (2AS)	gwm 66	1/0	/4B, 5B	gwm 508	1/1	6BS/6B
gwm 60	2/2	7AS/7A	gwm 71	1/1	2AS/2A	gwm 512	1/1	2AS/2A
gwm 205	2/2	5AS/5A	gwm 99	1/1	1AL/1A	gwm 513	1/1	7BS/4B
gwm 33	2/2	1AS/1BL, 1AS	gwm 107	1/1	4BL/4B	gwm 518	1/1	6BS/6B
gwm 43	2/1	4AL/7BL	gwm 129	1/1	2BS/2B, 5AS	gwm 538	1/1	4BL/4B
gwm 95	2/1	2AS/2AS	gwm 122	1/1	2BS/2A	gwm 573	1/1	7BS/7A, 7B
gwm 113	2/1	4BS/4B	gwm 140	1/1	1BL/1B	gwm 582	1/1	6BL/1B
gwm 114	2/2	3BL/3B	gwm 144	1/1	3BS/3B	gwm 601	1/1	4AS/4A
gwm 136	2/1	1AS/1A	gwm 156	1/1	5BS/5A	gwm 604	1/1	5BL/5B
gwm 148	2/1	2BS/2BS	gwm 181	1/1	3BL/3B	gwm 610	1/1	4AS/4A
gwm 155	2/1	3AL/3AL	gwm 186	1/1	5AL/5AL	gwm 613	1/1	6BS/6B
gwm 165	2/2	4AS, 4BL/4BS, 4A	gwm 191	1/1	2BL/2B, 5B, 6B	gwm 614	1/1	2AS/2A
gwm 213	2/2	5BS/5B						

[a] ampl/mapped = amplified fragments/mapped
[b] MDM/known = localization in MDM population/known localization

al. 1998), sterility induced by the distant genetic parental background or selective survival of RILs caused by the single-seed descend method (Nachit et al. 2001). The current genetic map was based on the non-skewed 101 polymorphic SSRs. They generated 161 polymorphic fragments, i.e., 1.59 fragments per SSR (see Table 3) out of which 124 were mapped, 45 on the A genome and 79 on B (see Table 4).

Fifty percent of the SSRs generated only one polymorphic fragment (Table 1). Earlier results have shown that the majority of microsatellite markers are genome-specific and usually amplify only a single locus (Röder et al. 1998; Korzun et al. 1999). Nevertheless, other SSRs generated 2 to 3 fragments. Some amplifying orthologous loci (Fig. 1), such as gwm165 and gwm234 who amplified two fragments, mapped to the two homoeologous sites on the A and B genome. This confirms earlier findings for gwm165, while for gwm234 it amplified only one fragment placed on 5BS (Röder et al. 1998). In other cases, SSRs amplified non-homoeologous regions, such as gwm554, gwm264, gwm131 and gwm537 (Table 1). Gwm154 produced four polymorphic fragments; two mapped in the MDM population, one on 3BL and one on 7AS. This chromosomal localization is in disagreement with the ITMI map where gwm154 amplified just one fragment positioned on 5AS (Röder et al. 1998).

Most SSR mapping was in agreement with earlier publications. Thus, 90% of the SSRs mapped as expected, whereas 12 SSRs were found to map in other locations (Table 1). Some of these SSRs were mapped on the homoeologous chromosome, such as gwm122 (2B instead of 2A), gwm156 (5B instead of 5A), gwm415 (5B instead of 5A), gwm443 (5A instead of 5B) and gwm635 (7B instead of 7A), while gwm265 was mapped in 4A instead of 2A; gwm299 in 2B instead of 3B; gwm513 in 7B instead of 4B and gwm582 in 6B instead of 1B. This could be explained by the presence of additional loci in the wheat genome; with some differences between the AB genomes of durum and bread wheat or the occurrence of deletion. In addition, some SSRs generated fragments that mapped to different chromosomes. For instance, the gwm554a, b and c fragments were mapped in 2A, 1B and 5B, respectively. Gwm264 mapped three fragments to 1A,

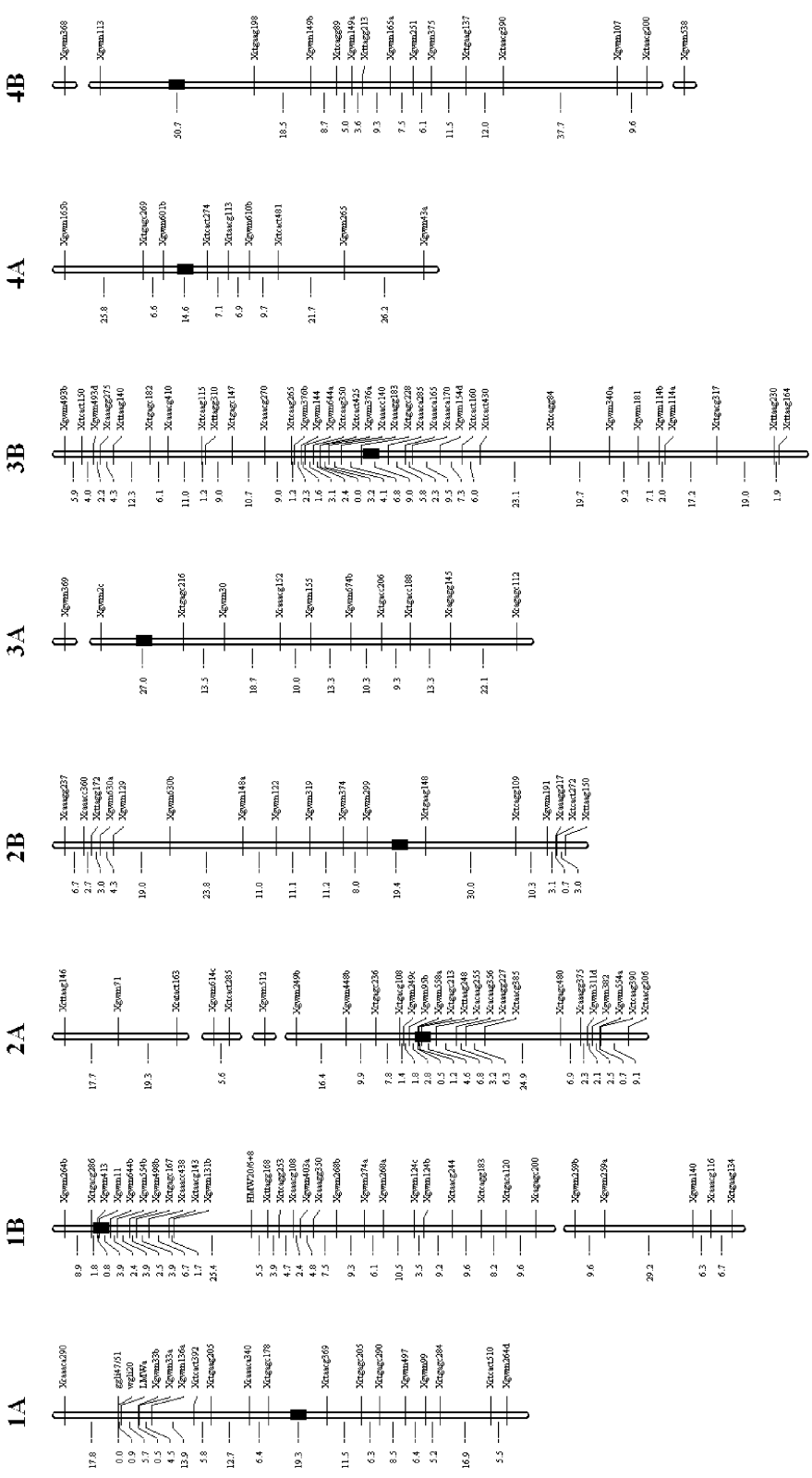
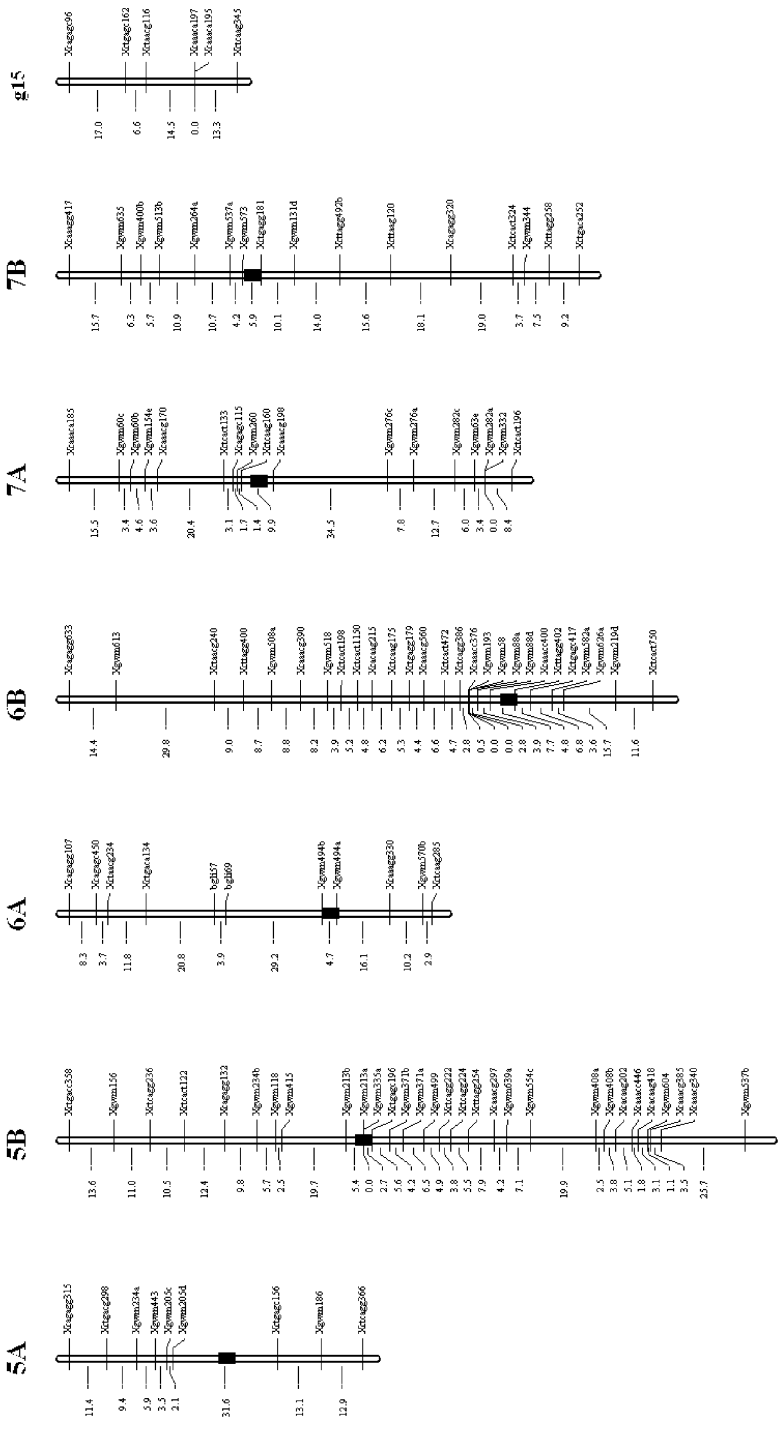


Fig. 1 Molecular linkage map of the Omrabi 5/ *T. dicoccoides 600545//* Omrabi 5 population. Short arms of chromosomes are at the top. The approximate locations of centromeres are indicated in *black* ■. On *the left* are map distances in centiMograms (cM) calculated by Kosambi function, and on *the right* are DNA-markers. Markers preceded *by X* are DNA markers of unknown function, whereas markers *without X* are genes controlling specific traits. AFLP markers are designated by six nucleotides (the first three are *Mse*I+3; and the other three are *Eco*RI+3) followed by the basepair size of the scored fragment

1B and 7B. The same was also observed for gwm131, gwm537 and gwm644 (Table 1). Similar findings were reported earlier for some SSRs, such as gwm666 (Röder et al. 1998; Korzun et al. 1999). In our population, the new localization of these SSRs should be more consistent, as the linkage groups were formed at a maximum LOD score of at least 3.0 and the minimum recombination ratio at more than 40%.

Fig. 1 (continued)



AFLPs

The 20 AFLP combinations used have generated 279 polymorphic fragments. This confirms that the AFLP technique provides an effective means to detect polymorphism at a large number of loci in a single assay (Vos et al. 1995). Of these polymorphic fragments, 54% were mapped in the MDM population. A low number of mappable AFLP markers has been also reported in rapeseed (Dreyer et al. 1999). Out of the mapped AFLPs, 36.6% mapped in the A genome and 61.3% in the B genome (see Table 4). Therefore, similar to the SSRs, more AFLP markers were mapped in the B genome than in the A genome. The B genome richness or high polymorphism in comparison to the other wheat genomes has been observed in *T. durum* and *Triticum aestivum* (Chao et al. 1989, Nelson et al. 1995; Röder et al. 1998, Nachit et al. 2001). Chao et al. (1989) suggested that the

Table 2 Scored polymorph AFLP fragments and their mapping in Omrabi 5/*T. dicoccoides 600545*//Omrabi 5 map

Combination[a]	Polymorphic fragments	Mapped fragments	Chromosomal location	% Mapped
MctgEagc	**31**	**19**	1A (4), 1B, 2A (3), 2B (2), 3A, 3B (3), 4A, 5A, 5B, 6B, g15	61.3
MctcEact	**25**	**18**	1A (2), 2A, 2B, 3B (4), 4A (2), 5B, 6B (4), 7A (2), 7B	72
McaaEacg	15	12	1B (2), 3A, 3B (2), 5B (3), 6B (2), 7A (2)	80
MctaEacg	19	11	1A, 1B (2), 2A (2), 4A, 4B (2), 6A, 6B, g15	57.9
MctcEagg	13	10	1B (2), 2B, 3B, 4B, 5A, 5B (3), 6B	76.9
McttEagg	16	9	1B, 2B, 3B, 4B, 5B, 6B (2), 7B (2)	56.3
McaaEagg	15	9	1B, 2A (2), 2B (2), 3B (2), 6A, 7B	60
McaaEaca	16	8	1A (2), 3B (3), 7A, g15 (2)	50
MctcEaag	17	8	2A, 3B (3), 6A, 6B, 7A, g15	47.1
McagEagg	18	6	3A, 5A, 5B, 6A, 6B, 7B	33.3
McaaEacc	7	6	1B, 2B, 3B, 5B, 6B (2)	85.7
McagEagc	9	5	1B, 3A, 6A, 7A, g15	55.6
McacEaag	7	5	2A (2), 5B (2), 6B	71.4
MctgEaag	9	5	1A, 1B, 2B, 4B (2),	55.6
McttEaag	13	5	2A, 3B (3), 7B	46.2
MctgEacg	**5**	4	1B, 2A, 3B, 5A	80
MctgEaca	9	**3**	1B, 6A, 7B	33.3
MctgEacc	20	**3**	3A (2), 5B	15
MctgEagg	**5**	**2**	6B, 7B	60
McatEact	10	1	2A	10
Total	279	149		54.1

[a] *M*=*Mse*I and *E*=*Eco*RI, e.g., *MctgEagc*=*Mse*I+ctg and *Eco*RI+agc

reasons are either the high mutation rate in the B genome or the high genetic variability of the B genome progenitor species. The first hypothesis is supported by the fact that B genome chromosomes contain more heterochromatin and repeated sequences than the A and D genomes, whereas the second one is supported by the fact that the B genome donor was an outbreeding species. In addition, another potential cause of polymorphism level differences between wheat genomes could be that the A genome donor was domesticated through farmers selection as a cereal grain crop, which has probably reduced its variability; in contrast the B genome was not selected for grain production.

The AFLP combinations used showed different patterns in both the number of generated polymorphic fragments and the percentage of mapped fragments (Table 2). The combinations *MctgEagc* and *MctcEact* generated the highest number of polymorphic fragments, 31 and 25 respectively, followed by *MctgEacc* with 20, whereas *MctgEagg* had generated only 5. Nevertheless, the generation of the large number of fragments is not associated with fragment mapping. For instance, only 3 fragments out of 20 (i.e. 15%) were mapped for the combination *MctgEacc*; whereas 2 out of 5 (i.e. 40%) for *MctgEagg*. Table 2 shows the chromosomal localization of different AFLP fragments derived either from the same combination or from different combinations. The majority of AFLP fragments were mapped in several chromosomes. These map findings consolidated earlier suggestions to use the AFLP technique as DNA fingerprints or for studies of diversity, since this technique provides simultaneous coverage of many loci in a single assay (Donini et al. 1997). Sixteen combinations out of the 20 used were mapped in both A and B genomes. Only 3 combinations were specific to the B genome (Table 2). The AFLPs markers were reported as locus-specific at the species level (Rouppe et al. 1997), and corresponding to unique positions on the genomes (Vos et al. 1995). The *MctgEagc* combination generated 31 polymorphic fragments; 19 were mapped: 4 in 1A, one in 1B, 3 in 2A, 2 in 2B, one in 3A, 3 in 3B, one in 4A, one in 5A, one in 5B, one in 6B and one in the unassigned group15. These results demonstrate that the AFLP technique is useful for random amplification across the whole genome as well as for map saturation (Becker et al. 1995; Nachit et al. 2001).

Seed storage proteins

The two parents of the RIL population have different patterns for three fractions of gliadin proteins: β, γ and ω subunits. The first ω-20 subunit (*Gli-A1*) was mapped on 1AS, concurring with previous mapping of the *Gli-A1* loci (Payne et al. 1984). It is of interest to notice that the two parents have γ-45 reported to be tightly and positively linked to gluten strength (Pogna et al. 1990); whereas they showed other polymorphic γ-gs fragments: γ-47 and γ-51 which co-segregated and mapped at the same location as ω-20 on 1AS (*Gli-A1*). On the other hand, the polymorphic α-gs were both skewed but did map very well on 6AS (*Gli-A2*), 3.9 cM apart from each other.

For glutenin proteins, the two parents presented different patterns for both LMW and HMW glutenin subunits. For HMWgs, both parents showed null alleles at the *Glu-A1x* and *Glu-A1y* loci. However, they were polymorphic for the *Glu-B1* loci. Durum cultivars present high null-allele frequency at the *Glu-A1* loci (DuCros

Table 3 Distribution of molecular markers, assignment and cM coverage across the 14 durum chromosomes in the A and B genomes and g15

Chromosome	SSR	AFLPs	Genes	Markers		Size (cM)	cM/marker
				No.	%		
1A	6	10	3	19	6.8	147.8	7.8
1B	**16**	14	1	31	11.1	208.6	6.7
2A	11	15	0	26	9.3	153.9	**5.9**
2B	9	8	0	17	6.1	167.3	9.8
3A	5	6	0	11	3.9	137.4	12.5
3B	11	**24**	0	**35**	12.5	**239.4**	6.8
4A	5	**4**	0	**9**	3.2	118.6	**13.2**
4B	9	6	0	15	5.4	180.1	12.0
5A	5	**4**	0	**9**	3.2	**90.0**	10.0
5B	**16**	14	0	30	10.8	209.4	7.0
6A	**3**	6	2	11	3.9	111.7	10.2
6B	10	17	0	27	9.7	180.2	6.7
7A	10	7	0	17	6.1	136.5	8.0
7B	8	8	0	16	5.7	156.5	9.8
g15	0	6	0	6	2.2	51.4	8.6
Total	124	149	6	279	100	2,288.8	8.2

1987; Branlard et al. 1989), whereas *T. dicoccoides* had high variability at these loci (Nachit et al. 1995b), but the accession used in our population showed null alleles. As for LMW, the known LMW-models are lately recognized to be a mixture of subunits controlled by different alleles (Carrillo et al. 1990; Impiglia et al. 1995; Nieto-Taladriz 1997). Therefore, instead of scoring the B-LMW as a pattern, they were scored as separate fragments. The LMWa (*Glu-A3*) showed a Mendelian distribution and mapped to 1AS at 0.9 cM far from the *Gli-A1* locus. The *Glu-3* loci are known to be localized on the short arm of the chromosomal group 1 (Lafiandra et al. 1984; Nachit et al. 2001). In our population, the genetic distance (0.9 cM) between *Glu-A3* and *Gli-A1* was closer than the estimated distance between *Glu-B3* and *Gli-B1* (2 cM) reported by Pogna et al. (1990). The second scored subunit, LMWb, was skewed and has not been assigned to any linkage group.

The genetic linkage map

The primary genetic map is an essential prerequisite to detail the genetic studies in any organism. Furthermore, dense and saturated genetic maps provide geneticists and breeders with essential tools for genetic studies like positional gene cloning, quantitative trait mapping and marker-assisted selection. The parents Omrabi 5 and *T. dicoccoides 600545* showed a high level of polymorphism (63.5% of SSRs). This may be due to the genetic difference between *T. durum* and *T. dicoccoides*. The Omrabi 5/*T. dicoccoides 600545*//Omrabi 5 map was constructed using 478 markers (192 SSRs, 279 AFLPs and 7 SSPs). Two hundred and seventy nine markers (124 SSRs, 149 AFLPs and 6 SSPs) were mapped and formed 15 linkage groups; 14 durum chromosomes plus an unknown group, named g15, which has not been assigned to any chromosome (Fig. 1). This group is composed of only six AFLP fragments. The mapped markers were evenly distributed with no significant clustering except in the centromere regions of some chromosomes, such as 1B, 2A, 3B, 5B and 6B (Fig. 1). The length of the map was 2,288.8 cM, i.e., an average marker each of 8.2 cM. The longest chromosomes were 3B, 5B, 1B and 6B, with 239.4, 209.4, 208.6 and 180.2 cM, respectively, whereas the smallest chromosome was 5A with 90 cM (Table 3). The centromeres were positioned at the midpoint between the most-proximal SSRs on the short and long arms according to previously published maps: namely, the ITMI SSR map (Röder et al. 1998), the Messapia × MG4343 map (Korzun et al. 1999), and the Jennah Khetifa × Cham1 map (Nachit et al. 2001).

Table 3 shows the distribution of mapped molecular markers, their assignment, and their cM coverage across the 15 formed linkage groups. Chromosome 3B, 1B and 5B showed the highest number of mapped markers: 35, 31 and 30 markers, respectively. Indeed, chromosome 5B and 1B showed the highest number of mapped SSRs, 16 each, and 3B showed the highest number of mapped AFLPs. The 3B, 1B and 5B chromosomes had also a very good coverage with 6.8, 6.7 and 7.0 cM/marker, respectively (Table 3). The lowest number of mapped markers was noticed in chromosomes 4A and 5A.

Chromosomal linkage groups

Chromosome 1A was constructed with 6 SSRs, 10 AFLPs and 3 SSPs (*Gli-A1*: γ-47+ω-20 and *Glu-A3*). The two gliadin subunits co-segregated and mapped at 0.9 cM from the *Glu-A3* locus. Chromosome 1A shows a good agreement with earlier reported marker orders (Röder et al. 1998; Korzun et al. 1999); nevertheless, relocation was revealed involving gwm33 and gwm136. In the ITMI, gwm33 amplified three fragments mapped to the three homoeologous sites (Röder et al. 1998), whereas in MDM it amplified two fragments mapped to 1AS at 0.5 cM from each other. Only one RIL (84) showed no co-segregation. The homoeologous chromosome 1B was well-formed and showed a full-order agreement with the ITMI, Messapia ×

MG4343, and the Jennah Khetifa × Cham1 maps (Röder et al. 1998; Korzun et al. 1999; Nachit et al. 2001). On the long arm, three SSRs (gwm268, 124 and 259) each amplified two fragments, whereas, both earlier published maps reported only one fragment for each (Röder et al. 1998; Korzun et al. 1999). The *Glu-B1* gene was mapped as expected on 1BL, at 45.5 cM apart from the centromere.

Chromosome 2A presented one marker per 5.9 cM; nevertheless, it showed three gaps. The centromere and the long arm showed a good agreement with the ITMI SSR map (Röder et al. 1998); however, several rearrangements were noticed on the short arm. Gwm249 was reported to map to the two homoeologous sites on 2A and 2D (Röder et al. 1998), while in the MDM the two fragments mapped both to 2A at 35.5 cM. Similarly, gwm630 mapped on 2BS. On the other hand, gwm382 in Jennah Khetifa × Cham1 generated two fragments that mapped on the telomeric region of 2AL (Nachit et al. 2001), and in the ITMI three fragments mapped on the three homoeologous long arm telomeres of chromosomal group 2 (Röder et al. 1998); whereas, in our population only one fragment was mapped on the 2AL telomeric region. Chromosome 2B was constructed with 17 markers (9 SSRs and 8 AFLPs) (Table 3). In comparison to the ITMI map, 2BS showed few rearrangements involving gwm129, 630, 148, 319 and 374. It seems like a double inversion involving *Xgwm374-319* on one hand and *Xgwm148-630* on the other hand; although it should be mentioned that in the ITMI map these SSRs were just assigned to the most likely interval on the RFLP framework map (Röder et al. 1998).

Chromosome 3A showed a relatively loose construction (one marker per 12.5 cM). The long arm showed rearrangement involving gwm155 and 674 (Röder et al. 1998; Korzun et al. 1999; Nachit et al. 2001). In contrast, chromosome 3B was well-saturated and showed a full order-agreement with the ITMI SSR map, except for the telomere region of the long arm where a reorganization including gwm340, 181 and 114 was revealed (Röder et al. 1998). Gwm376 amplified two fragments spaced by four other markers covering 9.4 cM. The assignment is in agreement with the Messapia × MG4343 map where two gwm376 fragments were reported to map at 1.9 cM apart from each other (Korzun et al. 1999). Gwm493 on the short arm and gwm114 on the long arm amplified two fragments each, which mapped at 9.9 and 2 cM from each other, respectively. In the ITMI population, gwm114 amplified two fragments that mapped to 3BL and 3DS (Röder et al. 1998).

Chromosome 4A was the linkage group with the least marker-coverage (one marker per 13.2 cM). This chromosome constituted of 5 SSRs and 4 AFLPs. Four SSRs showed the expected order (gwm165, 601, 610 and 265), whereas gwm43 was mapped on 4A instead of 7B. Gwm265 mapped to 4AL, which is in agreement with the Messapia × MG4343 map (Korzun et al. 1999); but not with the ITMI map where it was reported to map to 2AL (Röder et al. 1998). Using the bread-wheat nullisomic-tetrasomic lines, Korzun et al. (1999) showed that gwm265 has loci on 2A, 4A and 4D. In the MDM, chromosome 4A was constructed at LOD 3.5 except for *Xgwm165b* on the short arm that was joined at LOD 1.8. In this mapping population, gwm165 amplified two fragments that mapped to 4AS and 4BL, and similar results were reported (Röder et al. 1998). Chromosome 4B displays some gaps on both arms. This linkage group was constructed at a high LOD (>3), whereas the telomeres were linked at a lower LOD value (<1.5).

The short arms of chromosome 5B and 5A showed a translocation-inversion rearrangement (Fig. 1). Gwm156 and gwm415 mapped was on 5BS instead of 5AS, whereas gwm443 was on 5AS instead of 5BS. The SSR gwm234 amplified two fragments a and b, one mapped to 5AS and the other one to 5BS. The remaining parts of chromosome 5B (the centromeric and long arm) showed a perfect agreement with the ITMI and Messapia × MG4343 maps (Röder et al. 1998; Korzun et al. 1999). Gwm639 amplified two fragments, out of which only one was mapped on 5BL; however, this SSR was reported to generate 3 or 2 fragments that mapped on the homoeologous sites of chromosome 5 (Röder et al. 1998; Korzun et al. 1999). This could be explained by the short size of MDM chromosome 5A in comparison with other wheat maps (Röder et al. 1998; Korzun et al. 1999; Nachit et al. 2001). In the centromeric region, *Xgwm213a* and *Xgwm335a* mapped at a 0 cM distance from each other. These two SSRs were reported to co-segregate and to consist of almost identical sequences (Röder et al. 1998). On 5BL, *Xgwm554c* was flanked by *Xgwm408a* and *Xgwm639c*. This microsatellite, in contrast to the ITMI and Messapia × MG4343 maps, has amplified three fragments in MDM, which mapped to three different chromosomes: 1B, 2A and 5B (Fig. 1). Further, on 5AS, two fragments of gwm205 were mapped at 2.1 cM far from each other, whereas two loci were reported to map on 5AS and 5DS (Röder et al. 1998). The centromere of chromosome 5A was positioned at the midpoint between *Xgwm205d* and *Xgwm186*, according to the ITMI map (Röder et al. 1998); however, there is only a 0.37 recombination fraction between these two SSRs.

Chromosome 6B was well-saturated and showed a good order-agreement with the ITMI SSR map (Fig. 1). Three SSRs co-segregated in the centromeric region of 6B (*Xgwm193*, *58* and *88b*). Gwm193 and 88 were assigned to the most-likely interval on the ITMI RFLP framework map (Röder et al. 1998). As for 6A, few markers were mapped on it. Gwm494 amplified two fragments that mapped closely to each other. The centromere was positioned on gwm494 as reported by Röder et al. (1998).

On 7AL, gwm282 amplified two fragments (a and b) that flanked *Xgwm63e* at 9.4 cM far from each other. *Xgwm282a* and gwm332 co-segregated, which was not reported earlier. In general, chromosome 7A showed a good agreement to previous published maps (Röder et al. 1998; Korzun et al. 1999; Nachit et al. 2001). A new microsatellite (*Xgwm154e*) was incorporated in to 7AS. In the MDM, this SSR amplified two fragments mapped to

Table 4 Assigned markers on the A and the B genome

Genome	SSRs	AFLPs	Genes	Markers		Size (cM)	cM/marker
				No.	%		
A	45	52	5	**102**	36.6	859.9	**8.4**
B	79	91	1	**171**	61.3	1,341.5	**7.8**

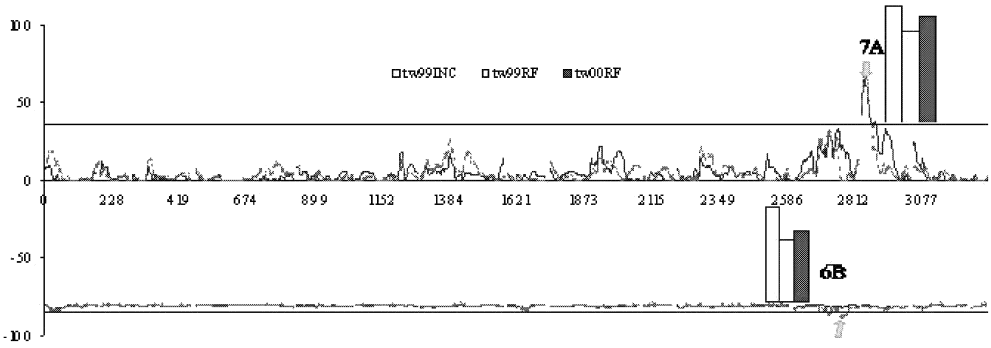


Fig. 2 TW scan of a test statistic for SIM (*solid red lines*) and sCIM using few background markers (*broken blue lines*) for the QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with the short arm). *Horizontal lines* show thresholds for testing SIM with 5,000 permutations. *Arrows* show positions of selected QTLs. *Bars* present the relative magnitude of identified QTLs in each environment

3BL and 7AS; however, in the ITMI it was positioned on 5AS (Röder et al. 1998). In 7B, two new SSR-fragments *Xgwm264a* and *Xgwm537a* were mapped. In the MDM, Gwm264 was mapped to 1AL, 1BS and 7BS, and gwm537 to 7BS and 5BL; however, the first mapped in ITMI to 1BS and 3BS, and the latter to 7BS (Röder et al. 1998). As for gwm635, it mapped on the MDM 7BS, while in the ITMI population it mapped to 7AS and 7DS (Röder et al. 1998).

In general, SSRs mapped more frequently than AFLP markers (77% versus 53.4%). Indeed, more markers were mapped in the B genome compared to the A genome (Table 4). This was true for SSRs and AFLPs; both markers showed preference mapping to the B genome. There is one marker for each 7.8-cM in the B genome while one was for 8.4-cM in the A genome (Table 4). Thus, 63.7% of the total mapped SSRs and 61.1% of the total mapped AFLPs were localized on the B genome, with only 39.2 and 34.7% on the A genome. This is in agreement with earlier published maps (Röder et al. 1998; Nachit et al. 2001). Probably, it reflects the amount of revealed polymorphism within the A and B genomes of the MDM population. Further, it is of interest to note that new SSR fragments were mapped on MDM in different chromosomal groups than on the ITMI map. This may indicate some differences between the AB genomes of bread and durum wheat. Further studies should be conducted to confirm or refute this hypothesis. In addition, the population requires further saturation with new SSRs; this work is ongoing. This population will be first used for the detection of grain quality QTLs and later for drought tolerance, as the two parents are showing different behavior for adaptation to water-stressed conditions. Using this population a major yellow pigment QTL was identified on the telomeric region of chromosome 7AL, explaining 53% of the total variation (Elouafi et al. 2001).

Milling quality QTLs

Test weight (TW)

The test-weight mean for the RILs was 72.4 ranging from 66.6 to 77.6, and for the parents (Omrabi 5 and *T. dicoccoides 600545*) 79.2 and 70.6, respectively. These results demonstrate that the RILs had equal or lower TWs than the durum parent. All environments have shown a good fit to the normal distribution. The test statistic scan for SIM analysis was simple and showed only few peaks on the main effect (Fig. 2). No interaction effect was shown across the whole genome, suggesting a high genotypic effect on this milling-related trait. The sCIM analysis confirmed the first high peak on chromosome 7A and obliterated the second low peak on the same chromosome; and revealed two new peaks on 6B with only the QTL×E effect (Fig. 2). Across this genomic region, a regression model was applied using either one of the two detected peaks, or both of them. Consequently, *Xgwm88a* on 6BS was selected as a QTL, as adding the second QTL did not contribute further to the total variation.

Consequently, two QTLs were identified for the TW trait; *Xgwm60c* on 7AS, explaining 17% of the total variation, and *Xgwm88a* on 6BS, explaining 9%. The total contribution was of a genetic nature, and no interaction effect was revealed. These findings are in agreement with earlier studies in which the genotypic effect on TW can

Table 5 Detected QTLs for TW and TKW and their contributions

Trait	Chromosomal localization	QTL marker	cM	V_g/V_{ph}[a]	$Vg+V_{QTL\times E}/V_{ph}$[b]
TW	6BS	*Xgwm88a*	0	9	9
	7AS	*Xgwm60c*	0	17	17
Total explanation				29%	**30%**
TKW	3BS	*XMctcEaag350*	0	3	5
	3BL	*XMctcEagg84*	0	3	5
	4BL	*XMcttEagg213*	5	2	3
	6BS	*Xgwm518*	0	12	13
	6BL	*Xgwm582a*	5	20	22
Total explanation				25%	**32%**

[a] V_g = genetic variance / V_{ph} = phenotypic variance
[b] $V_g+V_{QTL\times E}$ = QTL×E variance/V_{ph}

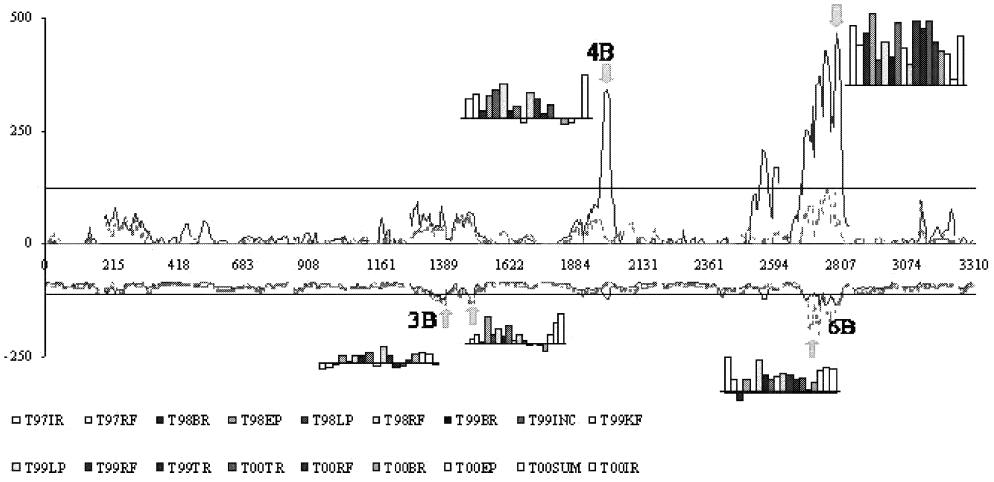


Fig. 3 TKW scan of a test statistic for SIM (*solid red lines*) and sCIM using few background markers (*broken blue lines*) for the QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with the short arm). *Horizontal lines* show thresholds for testing SIM with 5,000 permutations. *Arrows* show positions of selected QTLs. *Bars* presented relative magnitude of identified QTLs in each environment

explain up to 80% of the total variation (Nachit et al. 1995a). In other studies, a medium to high environmental effect on test weight was found (Roth et al. 1984; Schuler et al. 1994). Furthermore, the two identified QTLs *Xgwm88a* and *Xgwm60c* indicate a possible epitasis effect; as their individual effect explained just 9 and 17%, while together the explanation rate was found to be higher (30%) (Table 5). The difference between the lines comprising p1p1 (74.5) and p1p2 (71.9), compared with the difference between p2p1 (72.2) and p2p2 (70.9), was large. However, using MQTL epistasis analysis no epistasis was detected.

Omrabi 5 alleles had a significant positive and consistent effect on the TW trait with small magnitude changes between different environments (Fig. 2). This positive effect on the TW of Omrabi 5 alleles was expected, as the *T. dicoccoides* is known to hold smaller and shriveled grain in comparison with cultivated wheat.

Thousand-kernel weight (TKW)

The RILs TKW mean over the 18 studied environments was 29.9 g, and for the parents (Omrabi 5 and *T. dicoccoides 600545*) was 32.1 and 28.6 g, respectively. The average minimum of the RILs over all sites × years was 22.5 g and the maximum was 38.4 g. Indeed, 10 environments out of 18 produced maximum values higher than 40 g. This result shows the effect of transgressive inheritance. In addition, broad-sense heritability estimation in 16 environments show a mean of 0.60 with a maximum up to 0.96. Most of the environments show heritabilities higher than 0.5, except for some extreme-stressed environments with respect to drought and low temperatures. The test statistic of the SIM analysis showed significant peaks in chromosomes 4B, 6A and 6B with main and QTL×E interaction effects (Fig. 3). The major peak on 6B was wide (approximately 50 cM) with five prominent peaks; four out of them were taken as background markers for sCIM analysis. Therefore, on chromosome 6B, *Xgwm518* on the short arm and *Xgwm582a* on the long arm, were selected for their main

effect (detected by SIM) and their interaction effects (as indicated by sCIM). Yet, their explanation was mainly of a genetic nature (Table 5). In other studies, *Xgwm518* affected also protein content and gluten strength in an opposing way (Elouafi et al., under press). On chromosome 4B, *XMcttEagg213* was selected while the peaks detected on 6A by SIM were discarded, because the total explanation was not enhanced. Using regression models on the interaction peaks in 3B, two markers *XMctcEaag350* on the short arm and *XMctcEagg84* on the long arm were selected for being the best contributing markers.

The five detected TKW-QTLs explained 32% of the total variation, of which 25% was of genetic variation origin. The major QTLs were around the centromeric region of 6B (28%), whereas the other QTLs on 3BS, 3BL and 4BL showed additive minor effects (Table 5). These findings are in agreement with earlier studies, in which intermediate to high heritabilities and high additive genetic effect were reported (Nachit et al. 1995a).

Similar to the test weight trait, the Omrabi 5 alleles had also a significant positive effect on the TKW trait (Fig. 3). In general, this positive effect was consistent across environments, especially through the QTLs on 6B. The interaction effects of *Xgwm582a* and *Xgwm518* were mainly due to changes in magnitude, while QTL×E interactions of *XMctcEaag350*, *XMctcEagg84* and *XMcttEagg213* were due to cross-over interactions.

Conclusion

This work has allowed us to construct a saturated genetic linkage map of durum. We have confirmed the usefulness of the two employed techniques (SSRs and AFLPs) and their complementarities. A high level of polymorphism was detected between the *T. dicoccoides* accession and the variety Omrabi 5 suggesting a large genetic distance. The constructed map will be the base for further QTL determination. In the present study, QTLs associated with grain-milling quality traits were localized and, in the future, this population will be used for identification of QTLs linked to other grain quality traits; agronomic performance, and abiotic and biotic stress resistance. It may also help in assessing the environmental impact on trait expression, as it is already genetically stable.

For TW and TKW, the determined QTLs will be validated on diverse durum genetic backgrounds and used in marker-assisted breeding. Consequently, a selection at the genetic level could take place, rather than relying only on the phenotypic expression.

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