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Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat

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Abstract Seventy nine microsatellite markers from hexaploid bread wheat (*T. aestivum* L.) were integrated into a genetic linkage map of durum wheat (*T. turgidum* ssp. *durum* (Desf.) Huns.) created by RFLP segregation data from a population of 65 recombinant inbred lines. The results indicate a relatively even distribution of microsatellite loci and demonstrate that microsatellite markers from hexaploid wheat provide an excellent source of molecular markers for use in the genetics and breeding of durum wheat.

Key words Wheat microsatellites · Linkage map · RFLP · *T. turgidum* ssp. *durum*

Introduction

Previous work in bread wheat has demonstrated a higher level of polymorphism with microsatellites than with any other marker system (Plaschke et al. 1995; Röder et al. 1995; Bryan et al. 1997). Recently, wheat microsatellites (Röder et al. 1998) have been used for mapping genes in bread wheat (Korzun et al. 1997 a, 1998), for verifying the identity of genetic stocks (Korzun et al. 1997 b), studying the genetic diversity of bread wheat and related species (Plaschke et al. 1995; Fahima et al. 1998), and to address questions concerning the genetic diversity and evolutionary history of single genes in bread wheat (Worland et al. 1998).

Durum wheat (*Triticum turgidum* ssp. *durum* Desf) is a tetraploid species (genome AABB) which has attracted little genetic attention; indeed it was only recently that the first linkage map of the chromosomes of durum wheat, based on RFLP markers, was reported (Blanco et al. 1998). This map comprises 213 loci and spans a total length of 1352 cM. No microsatellite markers developed from durum wheat are currently available. The previous work of Plaschke et al. (1995), Fahima et al. (1998) and Korzun (unpublished data) indicated that it is possible to use microsatellites developed in bread wheat in closely related species such as diploid and tetraploid wheats. The objective of the study described here was to determine the map location of microsatellites from hexaploid wheat in the durum wheat genome.

Materials and methods

Plant material

Sixty five recombinant inbred lines were developed at the Institute of Plant Breeding, University of Bari (Bari, Italy) from a cross between the cultivar Messapia (*T. turgidum* ssp. *durum*) and the accession MG4343 of *T. turgidum* ssp. *dicoccoides* (Blanco et al. 1996). DNA from these lines was used for the mapping of microsatellite markers.

Microsatellites analysis

The development of the microsatellite markers was described in Röder et al. (1995, 1998) and Plaschke et al. (1995). Ninety eight primer pairs of wheat microsatellites (WMS) representing all

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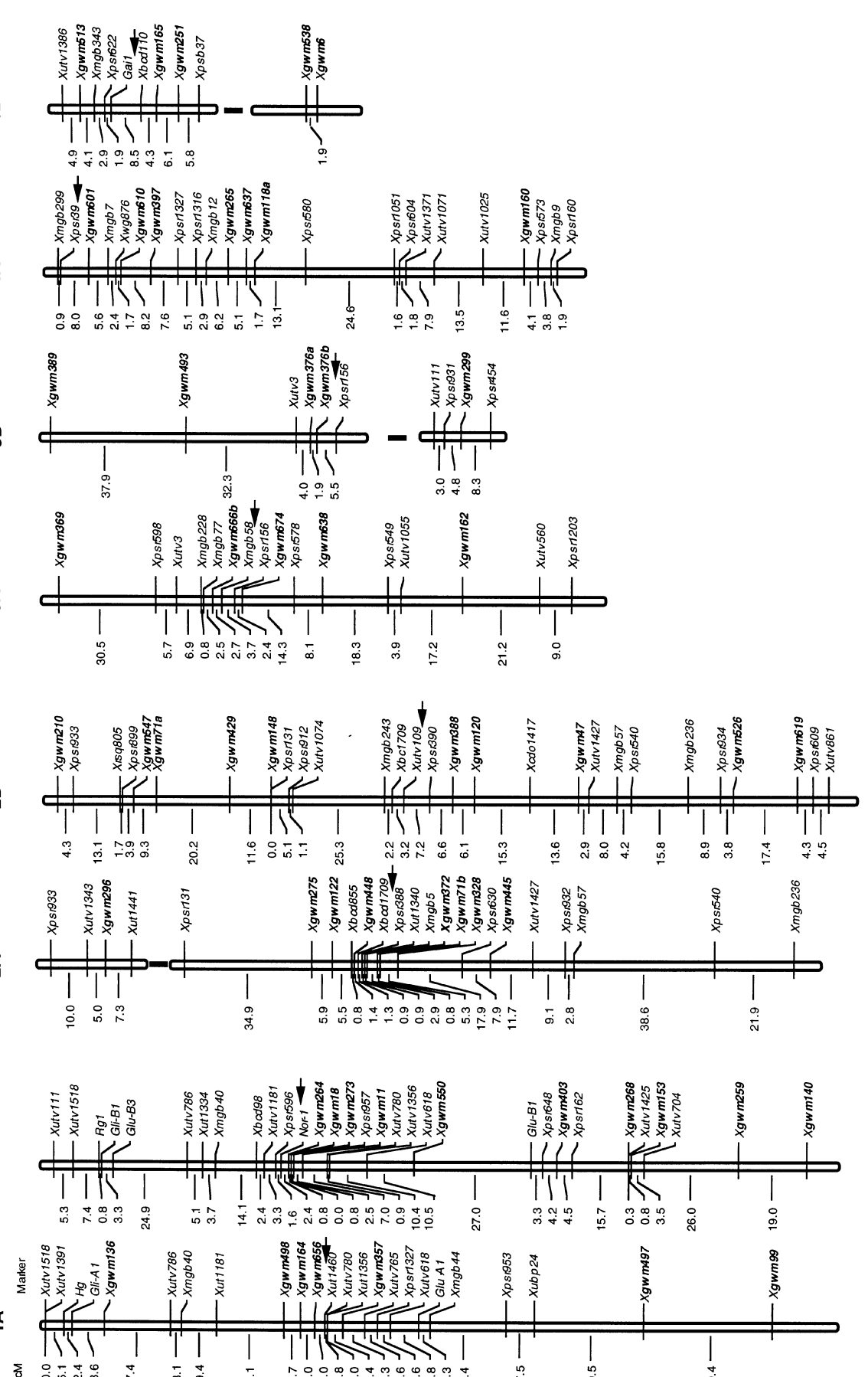

Fig. 1 Combined linkage map of durum wheat including the map position of RFLP and microsatellite loci (*in bold*). Genetic distances are given in centiMorgans (cM). Short arms of the chromosomes are at the top, positions of the centromeres are indicated by *arrows*

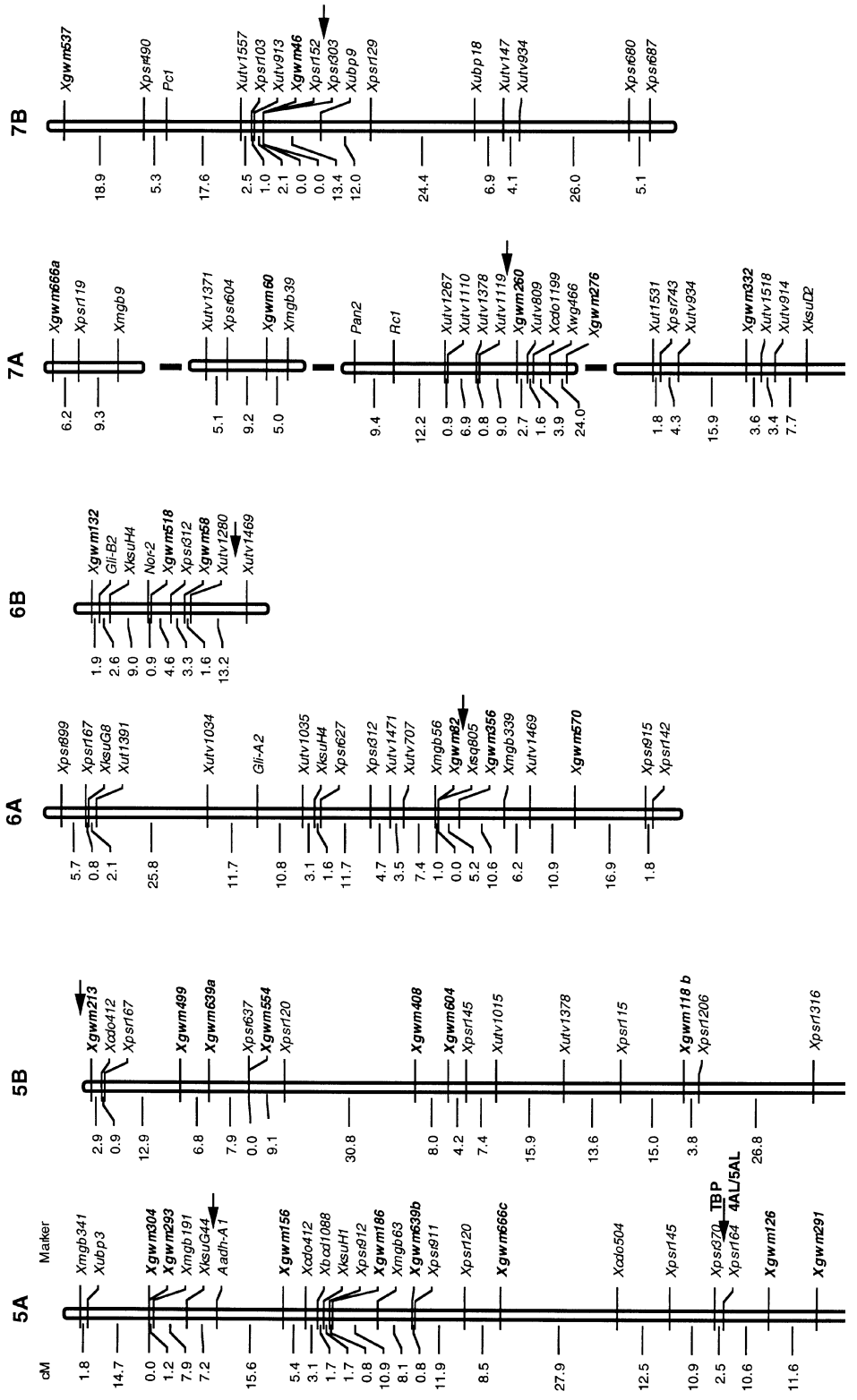

Fig. 1 Continued

chromosomes of the A- and B-genomes of hexaploid wheat were chosen for analysis. WMS designation, primer sequences, chromosome location of the amplified loci and the annealing temperature for most of primers employed in this study are presented by Röder et al. (1998). PCR reactions and fragment detection were performed as described by Plaschke et al. (1995) and Röder et al. (1995). Fragment sizes were calculated using the computer program Fragment Manager Version 1.2 (Pharmacia) by comparison with internal size standards.

Analysis of data

Segregation was scored, and markers were integrated into the existing RFLP map of durum wheat (Blanco et al. 1998), using the 'PLACE' and 'TRY' commands of the program MAPMAKER 2.0 (Lander et al. 1987). CentiMorgan units were calculated using the Kosambi mapping function.

Mapped wheat microsatellite loci were designated *Xgwm* for 'Gatersleben wheat microsatellite'.

Results and discussion

Out of 96 microsatellites chosen for analysis, 81 (84.4%) detected polymorphism between the parental cultivar Messapia (*T. turgidum* ssp. *durum*) and the accession MG4343 of *T. turgidum* ssp. *dicoccoides*. Eighty five loci amplified by 79 microsatellite primers could be placed on the RFLP map of durum wheat (Fig. 1). Most of the microsatellite markers are distributed fairly evenly throughout the linkage maps of the chromosomes, but a few clusters were detected on chromosomes 1A, 1B and 2A.

The level of polymorphism detected by the microsatellites in durum wheat was similar to that of RFLP markers (70.1%) on the same material (Blanco et al. 1998). This can be explained by the wide cross between *T. turgidum* ssp. *durum* × *T. turgidum* ssp. *dicoccoides* that has been used for mapping. The advantage of microsatellites versus RFLPs is that high levels of polymorphism are found in crosses between closely related varieties and germplasm (Plaschke et al. 1995; Bryan et al. 1997).

Seventy four microsatellite markers constituted genome-specific markers for durum wheat and only five (WMS71, WMS118, WMS376, WMS639 and WMS666) out of the 79 microsatellites identified more than one locus. The highest number of loci was detected by WMS666 with three loci, mapped on 3A, 5A and 7A, respectively. A few microsatellites (WMS58, WMS82, WMS638 and WMS656) were successfully integrated in the map of durum wheat, but these markers were monomorphic in the bread wheat mapping population employed (Röder et al. 1998). Seventeen microsatellite markers could not be mapped on to the durum map due to a lack of polymorphism and other factors, although these markers were integrated into the bread wheat map.

The original RFLP framework was extended by microsatellite markers on the ends of chromosomes 1A, 1B, 2B, 3A, 3B, 4B, 5A, 5B, 6B, 7A and 7B. The largest extensions of the genetical length of chromosomes by two linked microsatellite markers was for chromosomes 1A, 1B, 3B, 4B and 5A. The microsatellite loci *Xgwm497* and *Xgwm99* (chromosome 1A), *Xgwm259* and *Xgwm140* (chromosome 1B), *Xgwm369* (chromosome 3A), *Xgwm389* and *Xgwm493* (chromosome 3B), *Xgwm538* and *Xgwm6* (chromosome 4B) that were only weakly linked to their respective linkage groups were also assigned to their relevant chromosomes by comparing the position of these markers on the bread wheat map and/or their location on particular chromosome arms by using aneuploid wheat lines. For chromosome 5A, two microsatellite markers, *Xgwm126* and *Xgwm291*, and the RFLP locus *Xpsr164* were mapped onto a 4A/5A translocation, which is in agreement with the RFLP data from bread wheat (Devos et al. 1995; Nelson et al. 1995).

With a few exceptions (WMS118, WMS265, WMS356 and WMS498) all microsatellites were mapped in the same order compared to the microsatellite map of hexaploid wheat published by Röder et al. (1998). Inconsistency in map position for these microsatellites could be explained by the presence of additional loci in the wheat genome. For example, microsatellite WMS265 was mapped on chromosome 2A of bread wheat and chromosome 4A of durum wheat. However, the additional analysis of chromosome locations using nullisomic-tetrasomic lines of wheat has shown that this microsatellite has loci on chromosomes 2A, 4A and 4D of bread wheat.

Comparative maps for chromosome 5A and 5B of hexaploid and durum wheat are shown in Fig. 2. Eight microsatellites and one RFLP marker are common for 5A, and six microsatellites are common to the 5B map.

This study shows that microsatellites developed for hexaploid bread wheat can also be used in tetraploid durum wheat. The use of microsatellites from bread wheat for the genetic study of related species depends on the genetic relationship between these species and hexaploid wheat. For example, nearly all microsatellite markers selected from the A and B genomes of hexaploid bread wheat were useful in this study. Thus, 25 out of 30 (83.3%) microsatellites from the A genome of hexaploid wheat were useful for studying the genetic diversity among diploid wheat accessions (Korzun et al., in preparation), but only a few wheat microsatellites were useful for genetic mapping in barley and rye (Röder et al., 1995; Korzun, unpublished data).

The integration of microsatellites from hexaploid bread wheat into a genetic linkage map of durum will accelerate the transfer of knowledge from bread wheat to durum wheat and facilitate the development of new varieties of durum wheat.

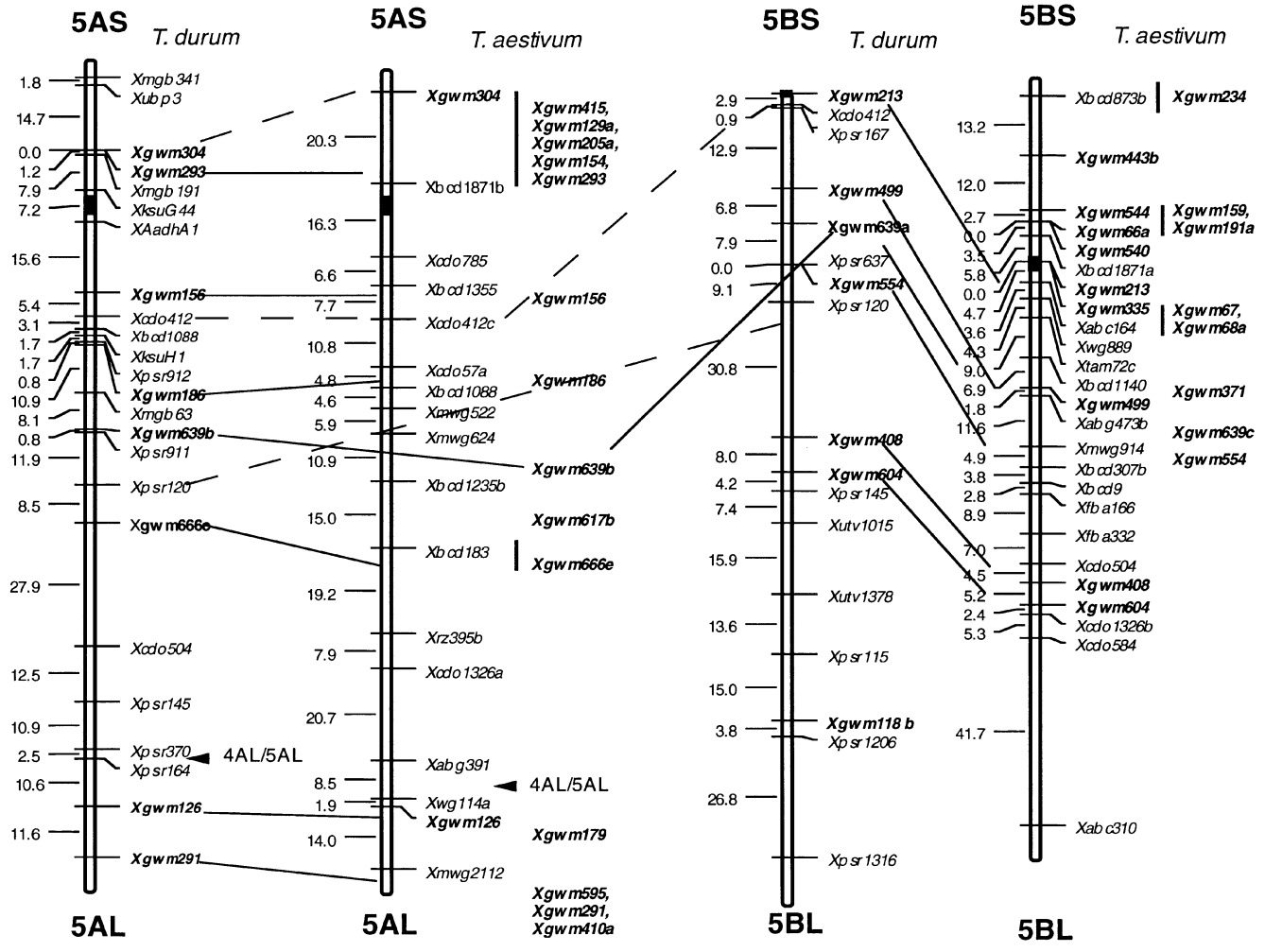

Fig. 2 Comparison between the maps of the homoeologous group-5 chromosomes of hexaploid bread wheat (Röder et al. 1998) and durum wheat. The location of the 4AL/5AL translocation break point is indicated; map distances are given in cM

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