Theor Appl Genet (2002) 105:413–422
DOI 10.1007/s00122-002-0865-9

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Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat

Received: 2 July 2001 / Accepted: 8 October 2001 / Published online: 19 June 2002
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Communicated by P. Langridge

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Abstract In hexaploid bread wheat (*Triticum aestivum* L. em. Thell), ten members of the IWMMN (*International Wheat Microsatellites Mapping Network*) collaborated in extending the microsatellite (SSR = simple sequence repeat) genetic map. Among a much larger number of microsatellite primer pairs developed as a part of the WMC (*Wheat Microsatellite Consortium*), 58 out of 176 primer pairs tested were found to be polymorphic between the parents of the ITMI (*International Triticeae Mapping Initiative*) mapping population W7984 × Opata 85 (ITMI*pop*). This population was used earlier for the construction of RFLP (*Restriction Fragment Length Polymorphism*) maps in bread wheat (ITMI*map*). Using the ITMI*pop* and a framework map (having 266 anchor markers) prepared for this purpose, a total of 66 microsatellite loci were mapped, which were distributed on 20 of the 21 chromosomes (no marker on chromosome 6D). These 66 mapped microsatellite (SSR) loci add to the existing 384 microsatellite loci earlier mapped in bread wheat.

Keywords *Triticum aestivum* · Bread wheat · Molecular genetic maps · Microsatellites · SSRs

Introduction

Bread wheat is one of the most important world food crops. It is a hexaploid, having three closely related genomes (A, B, D), each with seven chromosomes. The 21 wheat chromosomes are arranged in a two-way classification with seven homoeologous groups, each group having three chromosomes, one from each of the three genomes (for a review see Gupta 1991). It also has a large genome of 16 × 10^9 bp (Gupta et al. 1991; Bennett and Leitch 1995), of which more than 80% is repetitive DNA. This makes bread wheat a difficult material for genome-wide studies. Despite this, detailed RFLP genetic maps with more than 1,500 mapped loci spanning a genetic distance of over 3,700 cM (a density of 4.4 Mb per cM) are now available for bread wheat, due to concerted efforts made by several laboratories, coordinated by the ITMI (see

Gupta et al. 1999 for a review). Physical maps for all 21 chromosomes involving a sizable proportion of the genetically mapped loci are also available (Gill et al. 1993; Kota et al. 1993; Hohmann et al. 1994; Ogihara et al. 1994; Delaney et al. 1995a, b; Mickelson-Young et al. 1995; Varshney et al. 2001). These genetic and physical maps, however, are still far from the ultimate objective of getting a map as saturated as those available for the tomato and rice genomes. Therefore, the addition of more markers on these maps is a valuable objective for the wheat research community. The preparation of microsatellite or SSR (simple sequence repeat) maps was one such attempt, where a total of 279 and 55 microsatellite loci were mapped in Germany by Röder et al. (1998) and Pestsova et al. (2000) respectively, and 50 microsatellite loci were mapped in UK by Stephenson et al. (1998). There are other published or unpublished reports of additional mapped microsatellite loci, which include 144 loci (beloning to 137 microsatellites) mapped in Australia (Harker et al. 2001), 205 loci mapped in Canada (personal communication, D. Somers) and 337 loci mapped in France (Sourdille et al. 2001). However, only the recent maps from Australia and Canada include some *wmc* loci from a total of 396 microsatellites developed through the efforts of the WMC, which was started in the year 1996. The WMC was an international effort to develop and use these second-generation microsatellite markers, since development of these markers by individual laboratories was considered time-consuming and expensive. Microsatellite markers were considered superior to markers mapped earlier, the majority of which were RFLP markers. The microsatellite markers are locus-specific and PCR-based, thus making them attractive. Although the initial development of microsatellite markers may be costly, once developed they are cost effective and convenient for a variety of purposes including QTL analysis and marker-assisted selection. To aid this, an International Wheat Microsatellites Mapping Network (IWMMN) was created in February 1997, with the main objective to map additional microsatellite loci (particularly the WMC primers) on the bread wheat ITMI*map*. For this purpose the ITMI population W7984 × Opata 85 consisting of approximately 130 recombinant inbred lines (RILs) was available, of which 70 RILs were considered adequate for subsequent mapping work (Röder et al. 1998). IWMMN is a world-wide goodwill network that depends essentially on collaboration among all its members and provides for a mechanism, whereby researchers at the international level can release and exchange among themselves valuable data concerning the mapping of wheat microsatellites. The results of the first mapping efforts of the IWMMN are reported here.

Materials and methods

Genomic clones and development of microsatellites

Several genomic libraries enriched for a number of microsatellites were prepared by K. Edwards (Edwards et al. 1996) and 48 clones were supplied by Agrogene (coordinator of the WMC) to each member of the WMC for the purpose of sequencing. The sequence data was collated into a database by Agrogene who designed primer sets using the software PrimerPick (in-house software of Agrogene). Primers were synthesized by each member of WMC from their corresponding sequence data and were then pooled by Agrogene, which distributed primer aliquots to all members of WMC, for characterization and genetic mapping.

Chromosome assignment

In some cases, the markers were assigned to specific chromosomes using Chinese Spring nullisomic-tetrasomic lines and to specific arms of chromosomes using Chinese Spring ditelocentric lines.

DNA extraction and distribution

The ITMI population W7984 × Opata 85 (ITMI*pop*), used earlier (Nelson et al. 1995a) for the preparation of RFLP and microsatellite maps, was utilized for mapping in the present study. At the UMR INRA-UBP ASP of Clermont-Ferrand (Institut National de la Recherche Agronomique), France, total DNA was extracted and purified as described by Lu et al. (1994). After lyophilization, the same samples of total DNA extracted and purified from the 70 RILs were distributed to the ten members of the IWMMN for mapping analysis. These 70 RILs are a subset (following data from RFLP and cytogenetic analysis, data not shown) of 115 RILs (F7 progeny) of the ITMI*pop* used previously to build the ITMI*map* (Nelson et al. 1995a, b, c; Marino et al. 1996; Röder et al. 1998; Pestsova et al. 2000). Segregating data were collated into a database at the INRA of Clermont-Ferrand for mapping analysis.

Genetic mapping

A reference ITMI*map* consisting of 266 anchor markers (mainly RFLPs; Leroy et al. 1997) was prepared using MAPMAKER/Exp Version 3.0b (Lander et al. 1987) and marker data from 70 RILs were used for mapping each of the new microsatellite markers. Linkage groups were established by using a maximum recombination fraction of 0.35 and a minimum LOD score of 3.0. Markers with minimal missing data or segregation distortion were selected to build a skeleton map for each chromosome. The order was then refined using the MAPMAKER 'ripple' command. Other markers were assigned to intervals between the anchor markers using the MAPMAKER 'assign' command at LOD 3.0 and recombination fraction 0.35. The markers were also mapped using the MAPMAKER 'map' command. The loci on the skeleton map were checked using the MAPMAKER 'links any' command. Segregation distortion was calculated using an in-house S+ program. Map distance (cM) values were calculated using Haldane mapping function (Haldane1919). In some cases, the "assigned values" differed from the "mapped values". Only mapped values were used for integrating the mapped microsatellite markers into the skeleton map.

Results

Functional and polymorphic primer pairs

A total of 396 microsatellite primer pairs, the "*wmc* series", were developed by the WMC, which had 38 members in November 1998. Of the 396 *wmc* primer pairs thus designed by WMC, during the present study a total of 176 primer pairs were used by ten members of the IWMMN, to detect polymorphism between synthetic

Table 1 List of microsatellite markers analyzed by individual members of the IWMMN

Laboratory	wmc microsatellites	Number of microsatellites analyzed
UMR INRA-UBP ASP, France	wmc163, wmc166, wmc167, wmc168, wmc169, wmc173, wmc175, wmc177, wmc181, wmc182	10
Monsanto, USA	wmc147, wmc149, wmc153, wmc154, wmc156, wmc157, wmc161, wmc213	8
CCS University, India	wmc254, wmc256, wmc257, wmc261, wmc262, wmc264, wmc265	7
University of California, USA	wmc41, wmc43, wmc44, wmc47, wmc48, wmc49, wmc51, wmc52	8
CIMMYT, Mexico	wmc322, wmc326, wmc327, wmc329, wmc331	5
Agriculture and Agri-Food, Canada	wmc94, wmc95, wmc96, wmc97, wmc104, wmc105	6
University of Sydney, Australia	wmc232, wmc233, wmc238, wmc243, wmc245	5
University of Zurich, Switzerland	wmc272, wmc273, wmc276	3
USDA-ARS, Utah, USA	wmc24, wmc25, wmc27	3
Benoist Ets, France	wmc215, wmc216, wmc219	3
	Total	58

W7984 and the wheat variety Opata 85, the parents of the ITMI*pop*. Fifty eight (58) primer pairs (33%) were found to identify polymorphism, and were therefore used for genotyping the set of 70 RILs from the ITMI*pop*. These 70 RILs have been found to be adequate for adding new molecular markers on the existing map and are the same which were earlier used successfully for genetic mapping of 279 microsatellite loci (Röder et al. 1998). Microsatellites used for genotyping by different members of the IWMMN during the present study are listed in Table 1 and the primer sequences of these microsatellites are given in Table 2.

Mapping of microsatellite loci

A reference skeleton framework ITMI*map*, consisting of 266 anchor loci, was prepared for mapping purposes, and the genotyping data of the ITMI*pop* were used for gene-tic mapping on this framework map. Fifty eight (58) primer pairs amplified 66 mappable loci. For 20 of the 58 primer pairs, the loci amplified were also assigned to specific chromosomes or their specific arms, utilising Chinese Spring nullisomic-tetrasomic lines and the ditelocentric lines (Table 2). The genetic map showing the loci mapped during the present study along with the reference loci is shown in Fig. 1. In this figure, the chromosomal centromeric positions on the genetic map are approximate and are based on earlier mapping analyses conducted using the ITMI*pop* (Nelson et al. 1995a, b, c; Marino et al. 1996; Röder et al. 1998; Pestsova et al. 2000) and recent comparative mapping conducted using deletion maps (Cadalen, personal communication; Langridge, personal communication). The linear order of marker loci for each chromosome skeleton was also verified from the reference ITMI*map* prepared in these earlier studies. The results of genetic mapping were in agreement with the results of the chromosome assignment undertaken using nullisomic-tetrasomic lines and ditelocentric lines in 15 of the 20 cases (Table 2).

Distribution of mapped loci on genomes and homoeologous groups

The B genome had a maximum of 27 from the 66 loci mapped, while A and D genomes had 20 and 19 loci respectively (Fig. 1). Such a predominance of mapped microsatellites on the B genome was also observed in the map constructed earlier by Röder et al. (1998). Among the seven homoeologous groups also, the microsatellites were not uniformly distributed. A maximum of 18 loci were mapped on the three chromosomes of homoeologous group 2, a minimum of six loci each were available on chromosomes of groups 6 and 7, and no locus was mapped on chromosome 6D. There was no consistent pattern in the distribution of loci in individual linkage groups; in some cases the loci were restricted to the distal regions, while in other cases loci mapped near the centromere or were evenly distributed.

Microsatellites having multiple loci (homoeologous vs non-homoeologous)

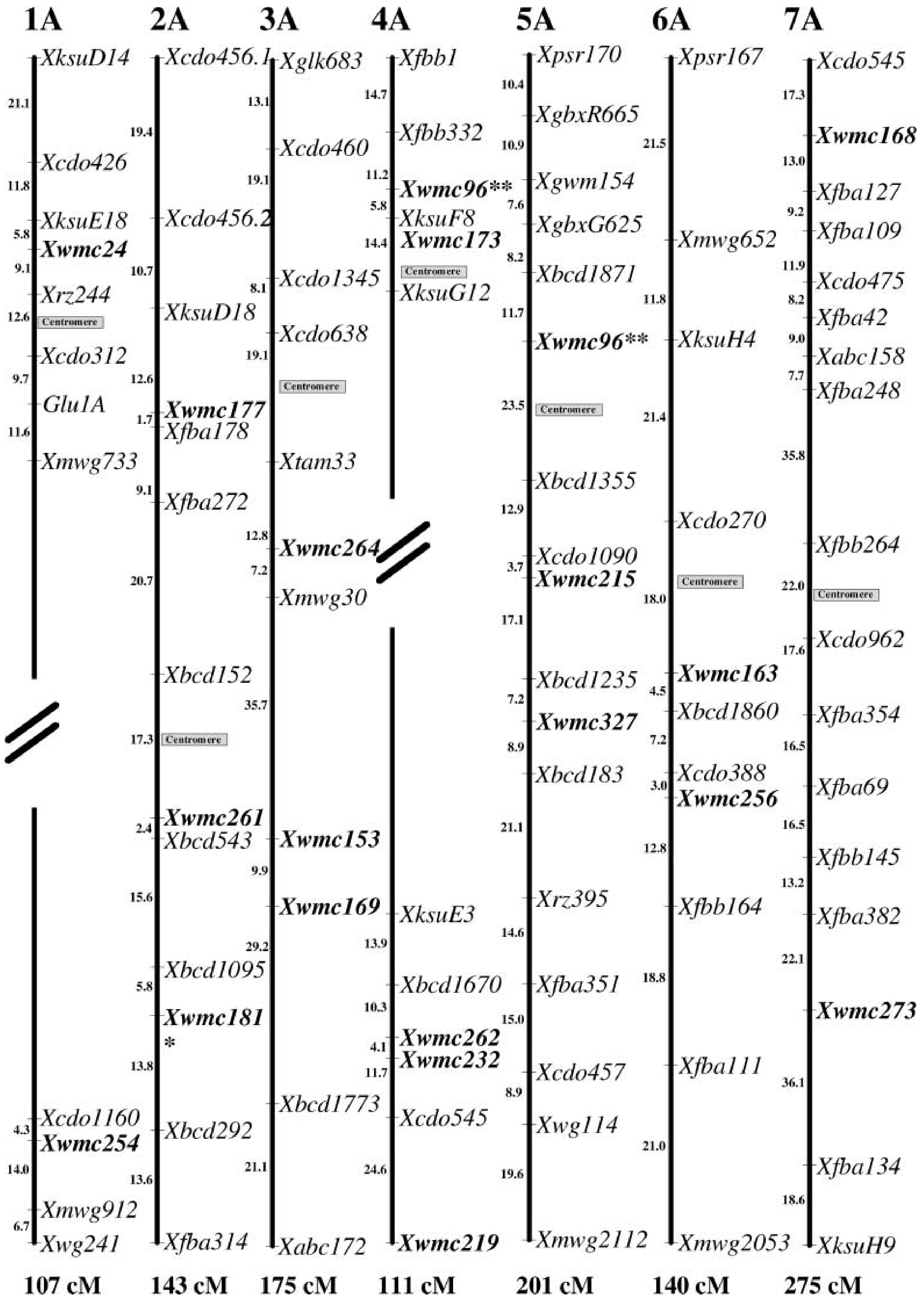
Out of the 58 primer pairs used, only 12 amplified more than one mappable locus, so that the majority of markers mapped in this study were chromosome specific. This is true for the majority of microsatellites in general, since in an earlier study, out of 15 functional microsatellite primer pairs examined in this manner, only five amplified more than one locus suggesting that only about 20–30% microsatellites have homoeoloci (Varshney et al. 2000). These results are also in agreement with those obtained with the 'gwm' set of microsatellites, where only 20% of the markers detected more than one locus (Röder et al. 1998). It is possible that homoeoloci for individual microsatellite loci in two other genomes carry null alleles thus allowing no amplification. Only sequencing of the corresponding regions in homoeologous chromosomes could resolve this question. In the present study, however, homoeoloci were detected by only 6 out of the 58 pairs of primers, of which six primer pairs

Table 2 Primer sequences of 58 microsatellites used for mapping

Microsatellites	Forward primer (5′→3′)	Reverse primer (5′→3′)	Chromosome/arm[a]
wmc24	GTGAGCAATTTTGATTATACTG	TACCCTGATGCTGTAATATGTG	2A
wmc25	TCTGGCCAGGATCAATATTACT	TAAGATACATAGATCCAACACC	2AS, 2BS, 2DS
wmc27	AATAGAAACAGGTCACCATCCG	TAGAGCTGGAGTAGGGCCAAAG	–
wmc41	TCCCTCTTCCAAGCGCGGATAG	GGAGGAAGATCTCCCGGAGCAG	–
wmc43	TAGCTCAACCACCACCCTACTG	ACTTCAACATCCAAACTGACCG	–
wmc44	GGTCTTCTGGGCTTTGATCCTG	TGTTGCTAGGGACCCGTAGTGG	1B
wmc47	GAAACAGGGTTAACCATGCCAA	ATGGTGCTGCCAACAACATACA	7A
wmc48	GAGGGTTCTGAAATGTTTTGCC	ACGTGCTAGGGAGGTATCTTGC	–
wmc49	CTCATGAGTATATCACCGCACA	GACGCGAAACGAATATTCAAGT	–
wmc51	TTATCTTGGTGTCTCATGTCAG	TCGCAAGATCATCAGAACAGTA	–
wmc52	TCCAATCAATCAGGGAGGAGTA	GAACGCATCAAGGCATGAAGTA	–
wmc94	TTCTAAAATGTTTGAAACGCTC	GCATTTCGATATGTTGAAGTAA	–
wmc95	GTTTTTGTGATCCCGGGTTT	CATGCGTCAGTTCAAGTTTT	–
wmc96	TAGCAGCCATGCTTAGCATCAA	GTTTCAGTCTTTCACGAACACG	–
wmc97	GTCCATATATGCAAGGAGTC	GTACTCTATCGCAAAACACA	–
wmc104	TCTCCCTCATTAGAGTTGTCCA	ATGCAAGTTTAGAGCAACACCA	6BS
wmc105	AATGTCATGCGTGTAGTAGCCA	AAGCGCACTTAACAGAAGAGGG	–
wmc147	AGAACGAAAGAAGCGCGCTGAG	ATGTGTTTCTTATCCTGCGGGC	–
wmc149	ACAGACTTGGTTGGTGCCGAGC	ATGGGCGGGGGTGTAGAGTTTG	2B
wmc153	ATGAGGACTCGAAGCTTGGC	CTGAGCTTTTGCGCGTTGAC	–
wmc154	ATGCTCGTCAGTGTCATGTTTG	AAACGGAACCTACCTCACTCTT	–
wmc156	GCCTCTAGGGAGAAAACTAACA	TCAAGATCATATCCTCCCCAAC	–
wmc157	CTTGATCCAAGTGGTTCTTTCC	TCCAAATGTTTGCGAAACCTGA	–
wmc161	ACCTTCTTTGGGATGGAAGTAA	GTACTGAACCACTTGTAACGCA	–
wmc163	TTACACCCATCAGGGTGGTCTT	GTCTATCCATACGACAAA	–
wmc166	ATAAAGCTGTCTCTTTAGTTCG	GTTTTAACACATATGCATACCT	–
wmc167	AGTGGTAATGAGGTGAAAGAAG	TCGGTCGTATATGCATGTAAAG	2D
wmc168	AACACAAAAGATCCAACGACAC	CAGTATAGAAGGATTTTGAGAG	–
wmc169	TACCCGAATCTGGAAAATCAAT	TGGAAGCTTGCTAACTTTGGAG	3A
wmc173	TGCAGTTGCGGATCCTTGA	TAACCAAGCAGCACGTATT	–
wmc175	GCTCAGTCAAACCGCTACTTCT	CACTACTCCAATCTATCGCCGT	–
wmc177	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	–
wmc181	TCCTTGACCCCTTGCACTAACT	ATGGTTGGGAGCACTAGCTTGG	–
wmc182	GTATCTCACGAGCATAACACAA	GAAAGTGTATGGATCATTAGGC	–
wmc213	ATTTTCTCAAACACACCCCG	TAGCAGATGTTGACAATGGA	–
wmc215	CATGCATGGTTGCAAGCAAAAG	CATCCCGGTGCAACATCTGAAA	–
wmc216	ACGTATCCAGACACTGTGGTAA	TAATGGTGGATCCATGATAGCC	7B
wmc219	TGCTAGTTTGTCATCCGGGCGA	CAATCCCGTTCTACAAGTTCCA	–
wmc232	GAGATTTGTTCATTTCATCTTCGCA	TATATTAAAGGTTAGAGGTAGTCAG	4AL
wmc233	GACGTCAAGAATCTTCGTCGGA	ATCTGCTGAGCAGATCGTGGTT	5DS
wmc238	TCTTCCTGCTTACCCAAACACA	TACTGGGGGATCGTGGATGACA	–
wmc243	CGTCATTTCCTCAAACACACCT	ACCGGCAGATGTTGACAATAGT	2BL
wmc245	GCTCAGATCATCCACCAACTTC	AGATGCTCTGGGAGAGTCCTTA	2AS, 2BS, 2DS
wmc254	AGTAATCTGGTCCTCTCTTCTTCT	AGGTAATCTCCGAGTGCACTTCAT	1A
wmc256	CCAAATCTTCGAACAAGAACCC	ACCGATCGATGGTGTATACTGA	6A, 6D
wmc257	GGCTACACATGCATACCTCT	CGTAGTGGGTGAATTTCGGA	2B
wmc261	GATGTGCATGTGAATCTCAAAAGTA	AAAGAGGGTCACAGAATAACCTAAA	2A
wmc262	GCTTTAACAAAGATCCAAGTGGCAT	GTAAACATCCAAACAAAGTCGAACG	4A, 5B
wmc264	CTCCATCTATTGAGCGAAGGTT	CAAGATGAAGCTCATGCAAGTG	3A
wmc265	GTGGATAACATCATGGTCAAC	TACTTCGCACTAGATGAGCCT	2B
wmc272	TCAGGCCATGTATTATGCAGTA	ACGACCAGGATAGCCAATTCAA	–
wmc273	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	–
wmc276	GACATGTGCACCAGAATAGC	AGAAGAACTATTCGACTCCT	–
wmc322	CGCCCCACTATGCTTTG	CCCAGTCCAGCTAGCCTCC	–
wmc326	GGAGCATCGCAGGACAGA	GGACGAGGACGCCTGAAT	–
wmc327	TGCGGTACAGGCAAGGCT	TAGAACGCCCTCGTCGGA	–
wmc329	ACAAAGGTGCATTCGTAGA	AACACGCATCAGTTTCAGT	–
wmc331	CCTGTTGCATACTTGACCTTTTT	GGAGTTCAATCTTTCATCACCAT	–

[a] Assigned using nullisomic-tetrasomics and ditelocentrics

Fig. 1 Molecular linkage map of bread wheat showing the positions of 66 new microsatellite loci. For each chromosome, markers are shown on the right and genetic distances in cM (centimorgans) are shown on the left. The new wmc (**w**heat **m**icrosatellite **c**onsortium) microsatellite loci that were mapped during the present study are shown in ***bold italics***. Primer pairs that amplify more than one locus on homoeologous chromosomes are marked *, and markers with more than one locus on non-homoeologous chromosomes are marked **. The approximate position of the centromere on each chromosome is shown by a *box* and the approximate length in cM (Haldane) is given at the bottom of each chromosome ▶


1A
XksuD14
21.1
Xcdo426
11.8
XksuE18
5.8
Xwmc24
9.1
Xrz244
12.6
Centromere
Xcdo312
9.7
Glu1A
11.6
Xmwg733
Xcdo1160
4.3
Xwmc254
14.0
Xmwg912
6.7
Xwg241
107 cM
2A
Xcdo456.1
19.4
Xcdo456.2
10.7
XksuD18
12.6
Xwmc177
1.7
Xfba178
9.1
Xfba272
20.7
Xbcd152
17.3
Centromere
Xwmc261
2.4
Xbcd543
15.6
Xbcd1095
5.8
Xwmc181
*
13.8
Xbcd292
13.6
Xfba314
143 cM
3A
Xglk683
13.1
Xcdo460
19.1
Xcdo1345
8.1
Xcdo638
19.1
Centromere
Xtam33
12.8
Xwmc264
7.2
Xmwg30
35.7
Xwmc153
9.9
Xwmc169
29.2
Xbcd1773
21.1
Xabc172
175 cM
4A
Xfbb1
14.7
Xfbb332
11.2
Xwmc96**
5.8
XksuF8
14.4
Xwmc173
Centromere
XksuG12
XksuE3
13.9
Xbcd1670
10.3
Xwmc262
4.1
Xwmc232
11.7
Xcdo545
24.6
Xwmc219
111 cM
5A
Xpsr170
10.4
XgbxR665
10.9
Xgwm154
7.6
XgbxG625
8.2
Xbcd1871
11.7
Xwmc96**
23.5
Centromere
Xbcd1355
12.9
Xcdo1090
3.7
Xwmc215
17.1
Xbcd1235
7.2
Xwmc327
8.9
Xbcd183
21.1
Xrz395
14.6
Xfba351
15.0
Xcdo457
8.9
Xwg114
19.6
Xmwg2112
201 cM
6A
Xpsr167
21.5
Xmwg652
11.8
XksuH4
21.4
Xcdo270
Centromere
18.0
Xwmc163
4.5
Xbcd1860
7.2
Xcdo388
3.0
Xwmc256
12.8
Xfbb164
18.8
Xfba111
21.0
Xmwg2053
140 cM
7A
Xcdo545
17.3
Xwmc168
13.0
Xfba127
9.2
Xfba109
11.9
Xcdo475
8.2
Xfba42
9.0
Xabc158
7.7
Xfba248
35.8
Xfbb264
22.0
Centromere
Xcdo962
17.6
Xfba354
16.5
Xfba69
16.5
Xfbb145
13.2
Xfba382
22.1
Xwmc273
36.1
Xfba134
18.6
XksuH9
275 cM

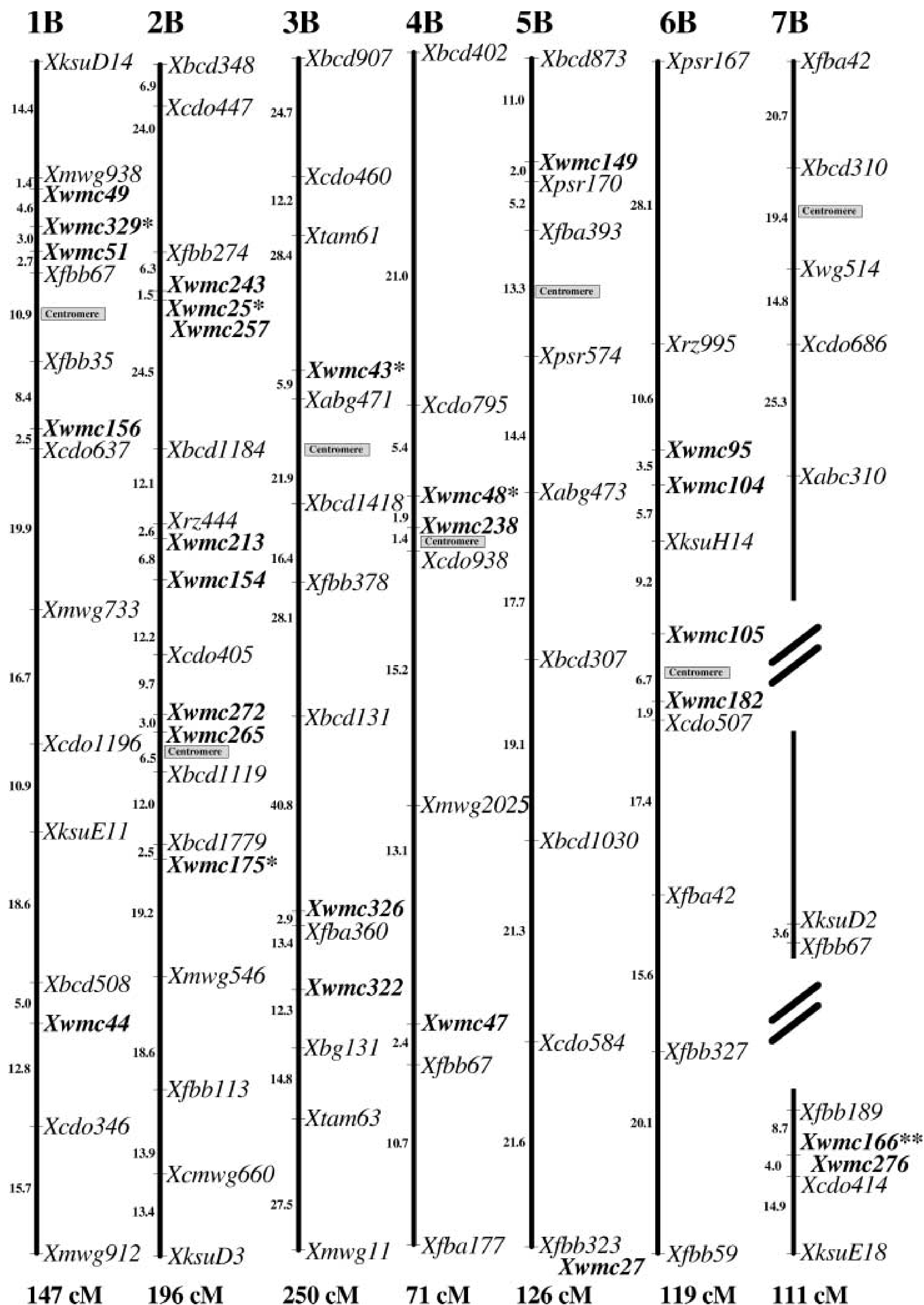


Fig. 1 (continued)

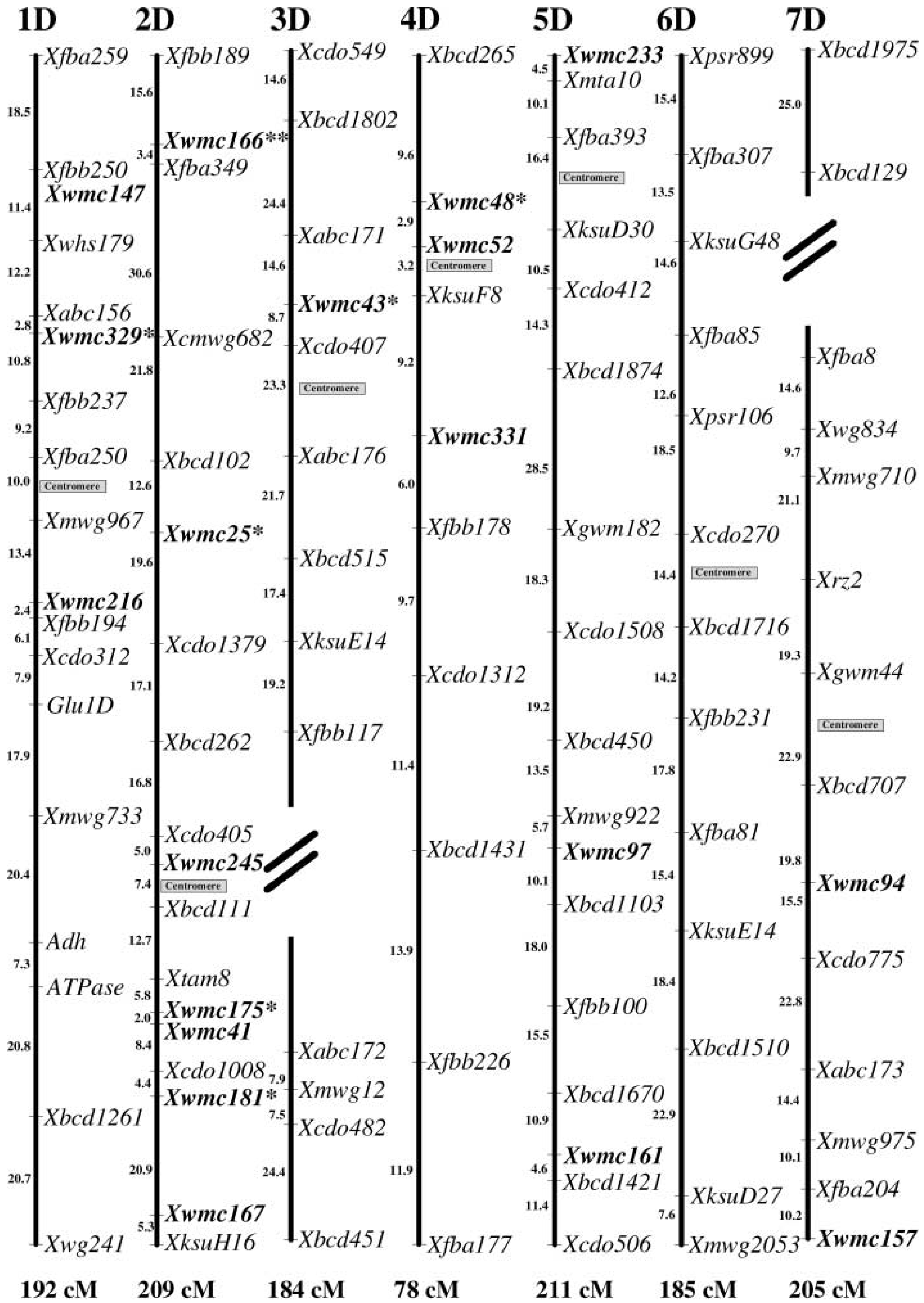


Fig. 1 (continued)

Table 3 Homoeoloci among 66 microsatellite loci assigned on a skeleton map using the ITMI*pop*

Homoeologous group	Genome and chromosome arm (S = short arm; L = long arm)					
	AS	AL	BS	BL	DS	DL
1	–	–	*wmc329*	–	*wmc329*	–
2	–	*wmc181*	*wmc25*	–	*wmc25*	*wmc181*
	–	–	–	*wmc175*	–	*wmc175*
3	–	–	*wmc43*	–	*wmc43*	–
4	–	–	*wmc48*	–	*wmc48*	–

(wmc25, wmc43, wmc48, wmc175, wmc181, wmc329) each detected two homoeoloci (Table 3). In at least two cases (wmc96, wmc166), the multiple loci amplified by the same primer pair belonged to non-homoeologous chromosomes; in four other cases (wmc169, wmc173, wmc322, wmc323) multiple loci were available but could not be assigned to specific chromosomes.

Discussion

The present study adds to the repertoire of molecular markers, so far used for the construction of molecular maps in bread wheat. These maps were extensively used for comparative genomics, although all classes of mapped markers were not found to be equally useful for this purpose (Devos and Gale 1997). It is also known that the resolution available in the existing molecular genetic maps in bread wheat is not satisfactory either for map-based cloning or for gene tagging that is required for marker-aided selection. Therefore, there is a need to add more molecular marker loci to the available maps. The present study is an effort in this direction.

The results of genetic mapping in the present study were largely in agreement with those of chromosome assignment done using nullisomic-tetrasomic and ditelocentric lines, although this exercise of chromosome assignment could not be undertaken for all the microsatellite primer pairs used in the present study (only 20 primer pairs could be used for chromosome assignment; see Table 2). For some of the microsatellite markers, the mapping results of the present study involving the ITMI*pop* were also confirmed either by using another mapping population (at CIMMYT by M. Khairallah, personal communication) or in another independent study using ITMI*pop* (at Agriculture Canada, in Winnipeg by D. Somers, personal communication). For two markers, namely wmc41 (associated with grain protein content) and wmc104 (associated with pre-harvest sprouting), the results of physical mapping obtained using deletion stocks (Endo and Gill 1996) were also in agreement with the present results of genetic mapping (Varshney et al. 2001). Hopefully, physical mapping for all other SSR loci mapped during the present study will certainly be undertaken in the future with the help of available deletion lines.

In bread wheat, molecular markers that have been used for mapping can be broadly classified into three groups: (1) those having triplicate homoeoloci, one locus each on three chromosomes of a homoeologous group, (2) those having multiple loci, but not on homoeologous chromosomes, and (3) those which are chromosome specific, each with a single locus. RFLPs can also be of two types, those derived from cDNA probes (based on conserved expressed sequences) generally having triplicate homoeoloci, and others derived from genomic DNA, which may or may not have triplicate homoeoloci. In sharp contrast to these RFLPs, microsatellite primers usually amplify a specific locus each, and therefore they generally belong to the third category. In the present study, only a small proportion of microsatellite primer pairs (12 out of 58) amplified more than one locus, and only 50% of these multilocus microsatellite primers amplified homoeoloci (Table 3). Other multilocus microsatellites amplified loci on non-homoeologous or unknown chromosomes. The microsatellite wmc96 amplified two loci, on non-homoeologous chromosome arms 4AS and 5AS; similarly wmc166 amplified two loci on non-homoeologous chromosome arms 2DS and 7BL. If it is assumed that multiple loci in bread wheat should be either homoeoloci or duplicated loci, then the loci for the same microsatellite on non-homoeologous chromosomes may be either due to translocations or to duplications between non-homoeologous chromosomes. Many such translocations and duplications were actually detected in plants, using the approach of comparative genomics involving use of heterologous probes for molecular mapping. In wheat also, a locus-containing gene encoding a receptor-like kinase was shown to be duplicated on chromosomes 3S and 1S. The duplication on 1S was found to be specifically occurring in the whole Triticeae (Feuillet and Keller 1999). In any case, the microsatellite markers may not prove very useful for comparative genomics to resolve the conservation of colinearity. However, they are very useful for gene tagging and QTL analysis.

Efforts are being made worldwide to develop and map additional microsatellite primers, so that within a few years at least 1,000 microsatellite loci will be available on the map. For instance, recently, M. Röder and her colleagues at Gatersleben (Germany) have developed a set of 55 *gdm* microsatellite markers for the D genome of bread wheat (Pestsova et al. 2000), and in France an additional set of 337 microsatellite loci were mapped on all the three genomes (Sourdille et al. 2001). Other sets of microsatellites including some *wmc* loci are being mapped in Australia (Harker et al. 2001) and Canada

(personal communication, D. Somers; no details were made available). Thus the remaining wmc and other additional microsatellite markers will be mapped in due course of time.

The WMC has already initiated the development of another set of *wmc* markers in its second phase with fewer members, and it may also undertake designing a set of anchored wmc primers from sequences that were earlier considered unsuitable for primer designing (P. Isaac, personal communication). In future, microsatellites will also be developed through searches of genomic and EST (expressed sequence tag) sequence resources, that are becoming freely available from the ITEC (International Triticeae EST Consortium, http://wheat.pw.usda.gov/genome/index.html), the USDA/ARS wheat endosperm EST project, and its joint NSF project "The Structure and Function of the Expressed Portion of the Wheat Genomes" (http://wheat.pw.usda.gov/NSF/htmlversion.html). In addition, microsatellites will be searched for in a large number of proprietary ESTs available from the private sector. The new microsatellite markers, which will thus become available, will also be eventually mapped giving a fairly saturated map to be used for map-based cloning and gene tagging in bread wheat. The ITMI is also making new coordinating efforts towards the development of functional microsatellites and SNPs (Single Nucleotide Polymorphisms), and it is proposed that a set of 150 core microsatellites spanning the whole genome be developed and a core germplasm for characterization of these microsatellites may be assembled for future users (ITMI meeting at San Diego, California, January 13, 2001, during the Plant and Animal Genome IX meeting).

Molecular genetic maps in bread wheat, in the past, as well as in the present study, have been prepared with the joint effort of several laboratories. In such an exercise, it is necessary to ensure that different laboratories use the same mapping population. Further, while adding new markers to an existing map jointly by different laboratories, as being done in the present study, it is important to ensure that not only the same mapping population be used by different laboratories, but also that this population does not differ from the one used for preparing the original map that is being extended. In bread wheat, a mapping population (ITMI*pop*) consisting of 130 RILs, was initially prepared and a set of 115 RILs was used by different laboratories for preparing the genetic maps. However, subsequently it was realized that a smaller subset of 70 RILs along with a framework map may actually be adequate for adding new markers to the existing map. This subset of 70 RILs was also earlier utilized successfully by Röder et al. (1998) in their study involving the mapping of 279 SSR loci. In the present study, the same sample of DNA from such a single set of 70 RILs of the ITMI*pop* was used by each of the ten laboratories for mapping 66 microsatellite loci. However, when different subsets of the ITMI*pop* are used, or seeds of the ITMI*pop* are obtained from different sources, no major differences were actually observed (Mark Sorrells, personal communication), thus indirectly validating the mapping of 66 microsatellite loci in the present study. Despite this, since there may be additional problems with the identity of RILs due to chromosome rearrangements, and other errors due to handling, it is recommended that in future mapping efforts, fresh seed should always be obtained from the same source. It is also suggested that the subset of the ITMI*pop* being used for mapping be validated by genotyping them using a subset of mapped representative markers from all the 21 chromosomes, to ensure that no alterations of any kind that may interfere with mapping have occurred over time. One should, however, also realize that even if different subsets are used by different laboratories engaged in mapping, eventually rare mistakes will be sorted out and standard high-density integrated maps with a variety of molecular markers should become available to be used for a variety of purposes including marker-aided selection and map-based cloning of genes. Wheat workers worldwide look forward to further developments for the saturation of genetic maps with new molecular markers including SSRs and SNPs.

Acknowledgements Thanks are due to B. Charef (INRA at Clermont-Ferrand) and Joy K. Roy (Meerut, India) for their help in the preparation of the manuscript, and to Dr. D. Somers (AAFC, Winnipeg) and Dr. M. Sorrells (Cornell University) for useful discussion and advice.

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