Theor Appl Genet (1998) 96: 138–146

A. Peil · V. Korzun · V. Schubert · E. Schumann
W. E. Weber · M. S. Röder

The application of wheat microsatellites to identify disomic *Triticum aestivum-Aegilops markgrafii* addition lines

Received: 29 May 1997 / Accepted: 10 September 1997

Abstract We describe the use of wheat microsatellites for the discrimination of *Aegilops markgrafii* chromosomes. Twenty out of eighty eight wheat microsatellites (WMS) tested were able to distinguish *Triticum aestivum-Ae. markgrafii* addition lines. Six, three, three, one and six of 18 WMS can be used as markers for single *Ae. markgrafii* chromosomes B, C, D, F and G, respectively. Addition line A is not available but additional bands, appearing only in *Ae. markgrafii* and the *T. aestivum-Ae. markgrafii* amphiploid and not in any of the available addition lines, indicate that three WMS detect markers for *Ae. markgrafii* chromosomes A. Addition line E could not be detected by any of the WMS markers applied, although the 20 WMS represented all the homologous groups of wheat. All three WMS located on the short arm of group-2 chromosomes were located on *Ae. markgrafii* chromosome B; three of four WMS, located on the long arm of wheat group-2 chromosomes, were specific to *Ae. markgrafii* chromosome G and three of four WMS, specific to group-5 chromosomes, were markers for *Ae. markgrafii* chromosome C, indicating the homoeology of these wheat chromosome arms with the respective *Ae. markgrafii* chromosomes.

Communicated by J. W. Snape

A. Peil (✉) · V. Schubert · E. Schumann · W. E. Weber
Institut für Pflanzenzüchtung und Pflanzenschutz,
Martin-Luther-Universität Halle-Wittenberg, Berlinerstrasse 2,
D-06188 Hohenthurm, Germany
Fax: 034602154617
E-mail: peil@landw.uni-halle.de

V. Korzun · M. S. Röder
Institut für Pflanzengenetik und Kulturpflanzenforschung,
Corrensstrasse 3, D-06466 Gatersleben, Germany

Key words *Aegilops markgrafii* · *Triticum aestivum* · Addition lines · Chromosome markers · Homoeology · Wheat · Wheat microsatellites

Introduction

Aegilops markgrafii (genome CC) is one of the wild wheat species which can serve as a genetic resource for resistance against powdery mildew and rust (Schubert et al. 1995). To use *Ae. markgrafii* efficiently in breeding programmes, detailed knowledge of the homoeology between hexaploid wheat and *Ae. markgrafii* would be helpful. Molecular markers for *Ae. markgrafii* chromosomes have been developed using random amplified polymorphic DNAs (RAPDs) (Peil et al. 1997). In spite of their rapid development, ease of use and low costs, RAPDs have the disadvantage of limited reproducibility. RFLPs are an alternative, but non-radioactive methods are difficult to use in wheat and their application is very time-consuming. As a second alternative we tried to use microsatellites (MS) as molecular markers for *Ae. markgrafii* chromosomes. MS have been described for several plant species, for example: *Arabidopsis thaliana* (Bell and Ecker 1994), barley (Saghai Maroof et al. 1994; Becker and Heun 1995), *Brassica napus* (Kresovich et al. 1995), maize (Senior and Heun 1993), rice (Zhao and Kochert 1993), soybean (Maughan et al. 1995; Rongwen et al. 1995), tomato (Smulders et al. 1997) and wheat (Plaschke et al. 1995; Röder et al. 1995). In wheat, MS are more convenient than RFLPs because they detect higher levels of polymorphism (Röder et al. 1995). Closely related bread-wheat cultivars and lines can be differentiated (Plaschke et al. 1995). Röder et al. (1995) showed that some WMS can be used for the detection of polymorphisms in rye or barley. In the present study, we describe the application of WMS to the wild species *Ae. markgrafii*, their use for identifying *Ae. markgrafii* chromosomes and for detecting polymorphisms between two *Ae. markgrafii* accessions.

Materials and methods

Plant material and DNA extraction

The wheat cultivar 'Alcedo'; *Ae. markgrafii* (Greuter) Hammer var. *markgrafii* accessions 'S740-69' and 'AE110'; the amphiploid *Triticum aestivum* cv 'Alcedo'-*Ae. markgrafii* acc. 'S740-69'; six derived disomic addition lines carrying the *Ae. markgrafii* chromosomes B, C, D, E, F and G, respectively; a disomic addition line containing a chromosome pair F with a deletion (F_{del}) and a (1D)1C substitution line were used. So far, no addition line with *Ae. markgrafii* chromosome A has been developed. The disomic addition lines and the amphiploid *T. aestivum* cv 'Alcedo'-*Ae. markgrafii* acc. 'S740-69' were developed at the Institute of Plant Breeding and Plant Protection of the Martin-Luther-University Halle-Wittenberg (Schubert and Blüthner 1992, 1995).

The addition line F_{del} containing a pair of deleted F chromosomes was identified on the basis of C-banding analysis (Friebe et al. 1992, unpublished data) and evidence of the morphological trait 'brittle rachis' (Schubert and Blüthner 1995).

The (1D)1C *T. aestivum-Ae. markgrafii* substitution line produced by Kihara (1958) was supplied by F. Zeller (Technical University Munich-Weihenstephan, Germany). The 1C chromosome of this line is claimed to be homologous to chromosome A of accession 'S740-69'. Extraction of total genomic DNA was modified from the CTAB procedure outlined by Saghai Maroof et al. (1984).

Microsatellites, primers and PCR-amplification

Microsatellites (dinucleotide repeats) for application to *T. aestivum-Ae. markgrafii* addition lines were selected from a pool of WMS established for wheat at the IPK Gatersleben (Plaschke et al. 1995, 1996; Röder et al. 1995; Korzun et al. 1997; unpublished data).

PCR reactions were performed mainly as described in Plaschke et al. (1996) and Röder et al. (1995). One primer of each primer pair was 5′ labelled with fluorescein to allow the detection of PCR-amplified fragments on an automated laser fluorescence (A.L.F.) sequencer (Pharmacia, Uppsala, Sweden). From each PCR reaction, 0.5–1.5 µl were separated on denaturing polyacrylamide gels (0.35-mm thick) for 60–120 min. Running conditions were 600 V, 40 mA and 50 W with a sampling interval of 0.84 s and 2 mW laser power. The running buffer was 1 × TBE. Gels were re-used up to five times. Fragment sizes were calculated using the computer program 'Fragment Manager 1.1' (Pharmacia, Uppsala, Sweden) in relation to internal size standards, which were added to each lane in the loading buffer.

Screening for polymorphisms

In the first screening, 88 WMS were applied to 'Alcedo' and 'S740-69' to detect polymorphisms between 'Alcedo' and 'S740-69'. 'Chinese Spring' was also tested to check the PCR for the expected fragments. Sixty five out of the eighty eight WMS were additionally analyzed with the amphiploid *T. aestivum* cv 'Alcedo'-*Ae. markgrafii* acc. 'S740-69' to verify the polymorphic fragment generated by *Ae. markgrafii* acc. 'S740-69'. To determine whether polymorphism exists between the two *Ae. markgrafii* accessions, 'S740-69' and 'AE110' 39 of the WMS were applied to 'AE110'.

Those WMS which appeared to be useful as markers were analyzed on 'Alcedo', 'S740-69', and two plants of the amphiploid, each addition line, and the substitution line (1D)IC. A WMS was considered as a marker for an *Ae. markgrafii* chromosome if the WMS produced a specific polymorphic PCR product in *Ae. markgrafii* acc. 'S740-69', the *T. aestivum* cv 'Alcedo'-*Ae. markgrafii* acc. 'S740-69' amphiploid, and in both plants of one *T. aestivum-Ae. markgrafii* addition line. If a WMS generated a polymorphic fragment in 'S740-69' and the amphiploid, but not in any of the six addition lines (i.e. added chromosomes B, C, D, E, F, G), the respective WMS is a possible marker for *Ae. markgrafii* chromosome A, for which no addition line exists.

Results

Eighty eight WMS were tested. Thirty eight of them showed polymorphisms between 'Alcedo' and 'S740-69' and were therefore applied to the whole set of plants. The number of WMS specific to the respective homologous wheat group is given in Table 1. Only 20 of these 38 WMS, i.e. 23% of the 88 WMS tested, were useful for distinguishing *Ae. markgrafii* chromosomes. The other 18 WMS did not amplify the polymorphic fragments obtained with 'S740-69' in the amphiploid or in any of the addition lines. The results shown in Table 2 were used for information about the homoeology between the C-genome of *Ae. markgrafii* and the wheat genome.

Figure 1 shows the pattern created by WMS 135 which amplifies a MS located on the long arm of wheat chromosome 1A. It generated a distinct PCR product in *Ae. markgrafii* and the two amphiploid plants but not in any of the six addition lines, indicating that it might be a marker for *Ae. markgrafii* chromosome A, the unavailable addition line. The specific product was also absent in substitution line (1D)1C.

All three WMS specific to the short arm of group-2 chromosomes were markers for *Ae. markgrafii* chromosome B, whereas three of four WMS specific to the long arm of that group resulted in distinct fragments with *Ae. markgrafii* chromosome G. The fourth WMS, located on wheat chromosome 2DL, amplified a product from addition line D and the same-sized product from substitution line (1D)1C.

Three out of four WSM specific to group-5 chromosomes were markers for *Ae. markgrafii* chromosome C,

Table 1 Number of WMS tested in the first screening to 'Alcedo', 'S740-69' and 'Chinese Spring', and tested in the second screening to 'Alcedo', 'S740-69', the amphiploid, the addition lines B–G and substitution line (1D)1C, and their specificity to the seven homoeologous wheat groups (Plaschke et al. 1995, 1996; Röder et al. 1995; Korzun et al. 1997, unpublished data)

Wheat homoeologous group	WMS 1st screening	WMS 2nd screening
1	7	2
2	21	11
3	20	2
4	8	3
5	7	7
6	13	6
7	12	7

Table 2 WMS applied to *T. aestivum* cv 'Alcedo', *Ae. markgrafii* acc. 'S740-69', the amphiploid, *T. aestivum-Ae. markgrafii* addition lines and substitution line (1D) 1C. x, y, z: fragments specific for an *Ae. markgrafii* chromosome; – no chromosome-specific fragment

WMS	Group	Sequence 5′ → 3′	Ann. temp. °C	Alcedo	S740-69	Amphiploid	Added *Ae. markgrafii* chromosome								(1D) 1C
							A[a]	B	C	D	E	F	G	F_{del}	
135	1AL	TGT CAA CAT CGT TTT GAA AAG G ACA CTG TCA ACC TGG CAA TG	55	–	x	x	x[b]	–	–	–	–	–	–	–	–
512	2AS	AGC CAC CAT CAG CAA AAA TT GAA CAT GAG CAG TTT GGC AC	60	–	x	x	–	x	–	–	–	–	–	–	–
210	2AS/DS	TGC ATC AAG AAT AGT GTG GAA G TGA GAG CAA GGC TCA CAC CT	60	–	x	x	–	x	–	–	–	–	–	–	–
319	2BS	CGT TGC TGT ACA AGT GTT CAC G CGG GTG CTG TGT GTA ATG AC	55	–	x	x	–	x	–	–	–	–	–	–	–
382	2AL/DL	GTC AGA TAA CGC CGT CCA AT CTA CGT GCA CCA CCA TTT TG	60	–	x	x	–	–	–	–	–	–	x	–	–
30	2DL	ATC TTA GCA TAG AAG GGA GTG GG TTC TGC ACC CTG GGT GAT	60	–	x	x	–	–	–	–	–	–	x	–	–
301	2DL	GAG GAG TAA GAC ACA TGC CC GTG GCT GGA GAT TCA GGT TC	55	–	x	x	–	–	–	–	–	–	x	–	–
349	2DL	GGC TTC CAG AAA ACA ACA GG ATC GGT GCG TAC CAT CCT AC	55	–	x	x	–	–	–	x	–	–	–	–	x
3	3DL	GCA GCG GCA CTG GTA CAT TT AAT ATC GCA TCA CTA TCC CA	55	–	x	x	–	–	–	–	–	–	x	–	–
314	3DL	AGG AGC TCC TCT GTG CCA C TTC GGG ACT CTC TTC CCT G	55	–	x	x	–	–	–	–	–	x	–	x	–
165	4AS/BL/DL	TGC AGT GGT CAG ATG TTT CC CTT TTC TTT CAG ATT GCG CC	60	–	x	x	–	x	–	–	–	–	–	–	–
205	5AS/DS	CGA CCC GGT TCA CTT CAG AGT CGC CGT TGT ATA GTG CC	60	–	x	x	–	–	x	–	–	–	–	–	–
179	5AL	AAG TTG AGT TGA TGC GGG AG CCA TGA CCA GCA TCC ACT C	55	–	x	x	–	x	–	–	–	–	–	–	–
186	5AL	GCA GAG CCT GGT TCA AAA AG CGC CTC TAG CGA GAG CTA TG	60	–	x	x	–	–	x	–	–	–	–	–	–
335	5BL	CGT ACT CCA CTC CAC ACG G CGG TCC AAG TGC TAC CTT TC	55	–	x	x	–	–	x	–	–	–	–	–	–
508	6BS	GTT ATA GTA GCA TAT AAT GGC C GTG CTG CCA TGA TAT TT	50	–	x	x	–	–	–	x	–	–	–	–	–
219	6BL	GAT GAG CGA CAC CTA CCC TC GGG GTC CGA GTC CAC AAC	60	–	x	x	x[b]	–	–	–	–	–	–	–	–
260	7AS	GCC CCC TTG CAC AAA TC CGC AGC TAC AGG CC	55	–	x	x	–	–	–	–	–	–	x	–	–
46	7BS	GCA CGT GAA TGG ATT GGA C TGA CCC AAT AGT GGT GGT CA	60	–	xyz	xyz	x[b]	y	–	–	–	–	z	–	–
332	7AL	AGC CAG CAA GTC ACC AAA AC AGT GCT GGA AAG AGT AGT GAA GC	60	–	x	x	–	–	–	x	–	–	–	–	–

[a] Addition line A is not available

[b] Fragments evident in 'S740-69' and the amphiploid, but not in any of the available addition lines, indicate that this WMS is presumed to be a marker for *Ae. markgrafii* chromosome A

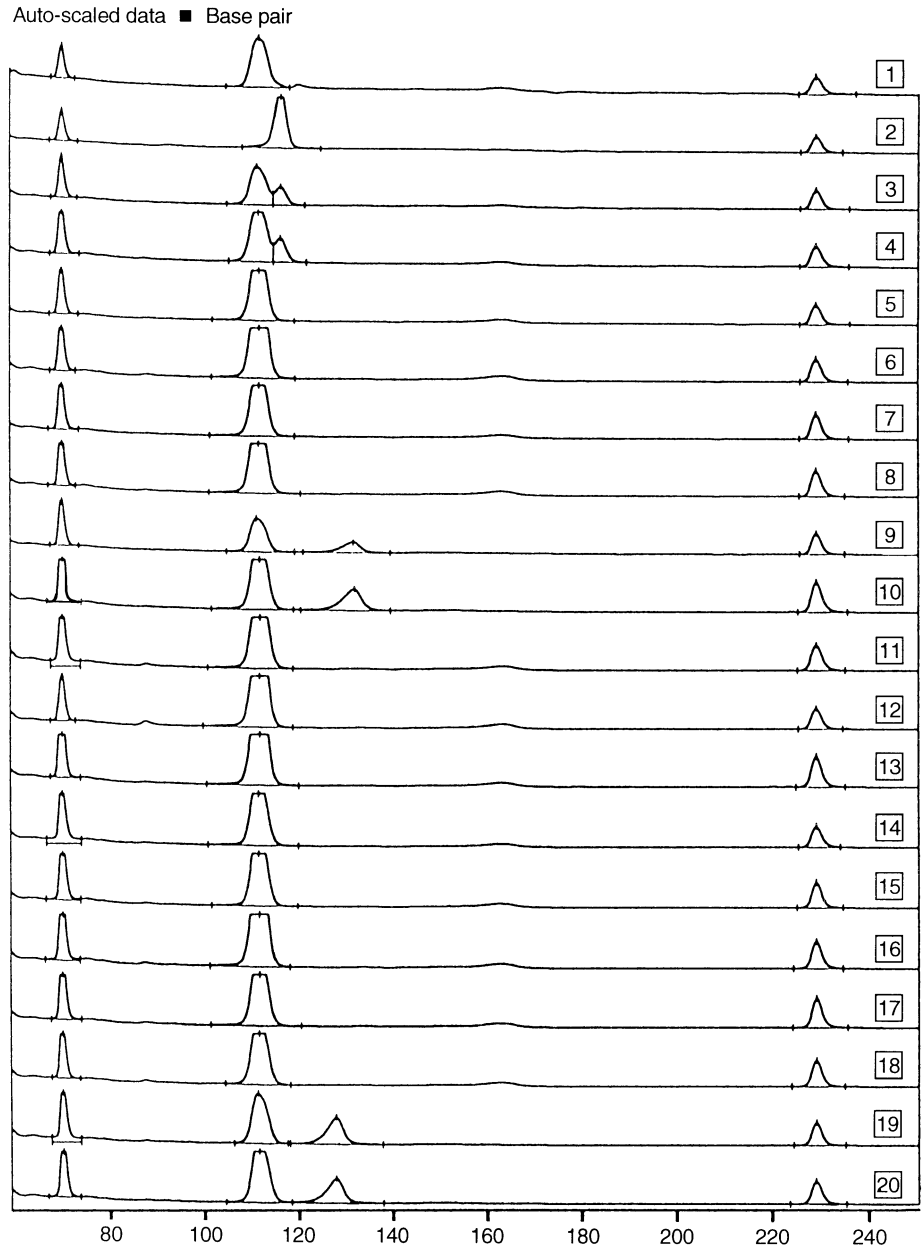

Fig. 1 WMS applied to 'Alcedo' (*1*), *Ae. markgrafii* (*2*), amphiploid (*3*, *4*) addition lines B (*5*, *6*), C(*7*, *8*), D(*9*, *10*), E (*11*, *12*), F (*13*, *14*), G (*15*, *16*), F_{del} (*17*, *18*) and substitution line (1D)1C (*19*, *20*). Number of lanes in brackets ( ). The peaks at 73 bp and 234 bp in all lanes represent the internal size standards which were used for the calculation of fragment sizes. WMS135 produced a specific fragment (116 bp) with *Ae. markgrafii* and the amphiploids only, indicating it to be a marker for chromosome A, and additional fragments with addition line D (132 bp) and substitution line (1D)1C (128 bp)

as shown for WMS205 in Fig. 2. The fourth, WMS179, located on the distal part of the long arm of wheat chromosome 5A, which carries the 4AL/5AL translocation (Devos et al. 1995; Nelson et al. 1995), is evident in addition line B. WMS165, a marker for wheat group-4 chromosomes, is also evident in addition line B.

These results suggest the homoeology of the short arm of group-2 chromosomes and the long arm of group-4 chromosomes with *Ae. markgrafii* chromosome B, of group-5 chromosomes with *Ae. markgrafii* chromosome C, and the long arm of group-2 chromosomes with *Ae. markgrafii* chromosome G. The results for other groups/arms are not sufficient to give indications about homoeology. *Ae. markgrafii* chromosome E did not show any specific fragment with the WMS tested.

WMS46 was the only MS which produced polymorphic bands for more than one *Ae. markgrafii* chromosome (Fig. 3), i.e. chromosomes B, G and probably A; the fragment sizes are 216 bp, 129 bp and 80 bp, respectively.

An interesting peak pattern was generated by WMS350 (wheat chromosome 7AS): a polymorphic peak 129 bp in size appeared with *Ae. markgrafii* but not in one of the two amphiploids, in both plants of the line with an added deleted chromosome pair F_{del} and in only one plant of addition line B, whereas line F,

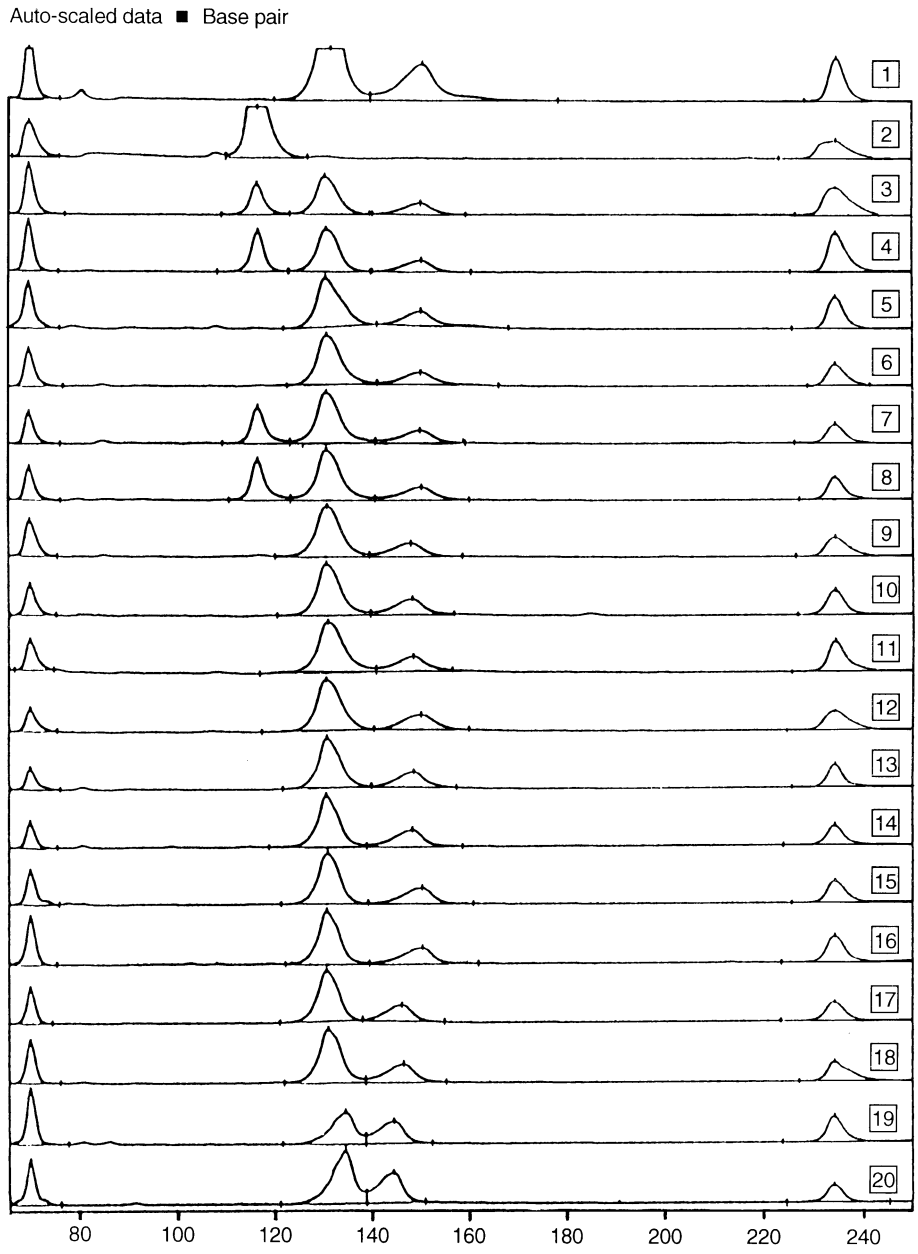

Fig. 2 WMS205, produced a specific fragment (119 bp) with *Ae. markgrafii*, the amphiploids and addition line C, and fragments of different size with addition line F_{del} and substitution line (1D)1C. Other data as in Fig. 1

with the complete chromosome F added, did not exhibit this peak; an additional peak of 153 bp was identified only from the one plant of addition line B exhibiting the 129-bp fragment and both plants of addition line F_{del}.

A general problem is the lower amplification of *Ae. markgrafii*-specifc fragments in both the amphiploids and the addition lines compared with the parent *Ae. markgrafii* acc. 'S740-69' (Figs. 1, 2 and 3). This was often the reason for the rejection of a WMS producing good polymorphisms in the first screening. For example, WMS46 (Fig. 3) shows two large peaks (80 and 129 bp in size) and three weaker peaks (216, 243 and 255 pb in size) in 'S740-69', whereas the amphiploids and the respective addition line show smaller peaks (80, 129 and 216 bp fragments) or no peaks at all (243- and 255-bp fragments). Interestingly, some lines showed peak losses, additional peaks or polymorphic peaks when compared with the parents. This was expected for substitution line (1D)1C, generated from another background for both *T. aestivum* and *Ae. markgrafii*. Changes in band pattern were also observed for all *T. aestivum*-*Ae. markgrafii* addition lines. The only exception was addition line C. Addition lines F and F_{del}, in particular, produced different band patterns for 8 out of 38 WMS tested. WMS135 shows additional peaks with addition line D (132 bp) and the substitution line

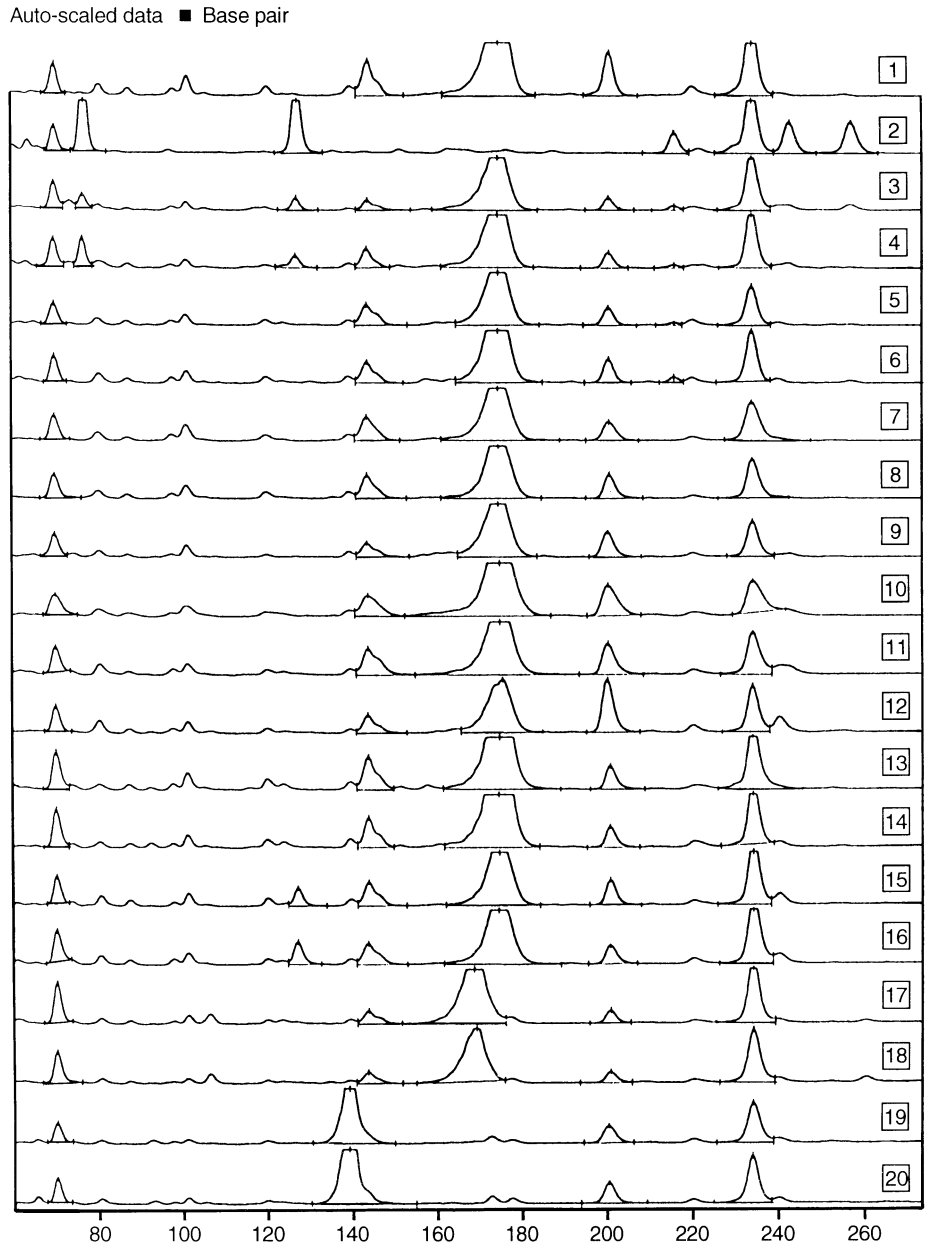

Fig. 3 WMS46, produced a specific fragment (80 bp) with *Ae. markgrafii* and the amphiploids only, indicating it to be a marker for chromosome A, specific fragment (129 bp) with *Ae. markgrafii*, the amphiploids and addition line G, and another specific fragment (216 bp) with *Ae. markgrafii*, the amphiploids and addition line B. Addition line F_{del} and substitution line (1D)1C showed fragments of different size. Other data as in Fig. 1

(1D)1C (128 bp) (Fig. 1). WMS205 showed a slightly different peak pattern for line (1D)1C (Fig. 2) and WMS46 shows changed patterns for addition line F_{del} and substitution line (1D)1C (Fig. 3) as well. Polymorphisms between the two *Ae. markgrafii* accessions 'S740-69' and 'AE110' were produced by 14 out of 39 WMS tested, i.e. 36%.

Discussion

MS as molecular markers have been employed for the differentiation of cultivars such as grapevine (Thomas and Scott 1993), barley (Saghai Maroof et al. 1994) or wheat (Plaschke et al. 1995) and can supplement genetic maps (Becker and Heun 1995; Grandillo and Tanksley 1996). MS generated for cereals such as wheat or barley were successfully applied to other *Gramineae*. Röder et al. (1995) found that 7 and 6 out of 15 WMS produced polymorphisms in different barley and rye accessions, respectively. However, in only one case, respectively, did the polymorphic fragments in barley and rye contain a microsatellite sequence. Saghai Maroof et al. (1994) applied four barley MS to six disomic wheat-barley addition lines, but only one of them produced additional fragments in the wheat background. We applied 88 WMS to *Ae. markgrafii*, 69 of them

produced fragments and 38 of these WMS showed polymorphism to wheat.

Only 20 out of the 88 WMS tested yielded useful polymorphisms and could be employed as molecular markers for *Ae. markgrafii* chromosomes. A possible reason why only 20 out of the 38 WMS were useful as markers may be a competition effect for primers between hexaploid wheat and *Ae. markgrafii*. Presumably, the sequences in hexaploid wheat may be better matched to the primer sequences than are the equivalent loci in *Ae. markgrafii* and, due to the competition effect, *Ae. markgrafii*-specific fragments were not detected as well in the amphiploid and addition lines as in the *Ae. markgrafii* parent.

The effectiveness of RAPDs was much lower in this respect (Peil et al. 1997). Only 18 of 238 primers were useful as molecular markers for *Ae. markgrafii* chromosomes, but it should be borne in mind that only those MS have been used which were successfully applied to wheat. The *Ae. markgrafii* chromosomes B, C, D, F and G were detected by six, three, three, one and six WMS, including the WMS which marked at least two different *Ae. markgrafii* chromosomes (B, G) and probably chromosome A, the unavailable addition line. Although WMS for all wheat chromosome arms were tested, no WMS was detected which was specific for chromosome E. Chromosome A was probably recognized by two additional WMS. We assume that the amplification of specific *Ae. markgrafii* fragments in the amphiploids, but not in one of the available addition lines, i.e. chromosomes B to G, is a sufficient indication that a WMS is a marker for chromosome A, as described previously for RAPDs (Peil et al. 1997). Another point of interest is the lack of the assumed chromosome A peaks in the substitution line (1D)1C, thought to be an *Ae. markgrafii* line containing chromosome A. It should be noted that for both *T. aestivum* and *Ae. markgrafii* the genetic background of that substitution line differs from our lines. Thirty six per cent of the WMS tested to two different *Ae. markgrafii* accessions 'S740-69' and 'AE110' showed polymorphisms between these two lines. This is comparable to results from RAPDs (Peil et al. 1997): 41% of 180 primers tested to these accessions showed polymorphisms. Peil et al. (1997) reported that one of two presumed RAPD markers for chromosome A generated the specific band with substitution line (1D)1C. Nevertheless, WMS349 produced the specific *Ae. markgrafii* fragment when applied to the line (1D)1C, but WMS349 generated this fragment with addition line D. This may be due to chromosomal rearrangements existing between these different *Ae. markgrafii* accessions or occurring during the development of the substitution line.

Senior and Heun (1993) reported the amplification of MS fragments longer than expected for barley and they showed that these fragments did not contain the targeted repeat. The amplification of fragments on non-homoeologous chromosomes in wheat with MS primer pairs was shown by Plascke et al. (1996). For the use of WMS as markers for *Ae. markgrafii* chromosomes, it is not necessary that the specific WMS peak is located on a homoeologous chromosome. However, the phenomenon of amplified fragments located on non-homoeologous chromosomes for WMS raises difficulties in assessing the homoeology of genome C and the wheat genome. Nevertheless, from the results of isozyme tests (Schmidt et al. 1993) and chromosome structure we have obtained at least some information about the homoeology for some chromosomes. Wheat homoeologous groups 1, 5 and 6 contain nucleolus organizer regions (McIntosh 1988) visible as secondary constrictions on chromosomes 1B and 6B. *Ae. markgrafii* chromosomes A and C also contain secondary constrictions (Schubert et al. 1987). Additionally, peroxidase and glutenin genes, located on wheat homoeologous group 1 (Hart 1983; Schubert and Blüthner 1992), seem to be located on chromosome A because they were found only in 'S740-69' and the amphiploid but in none of the available addition lines (Schubert and Blüthner 1992; Schmidt et al. 1993). WMS135, located on group 1 in wheat, is probably a marker for chromosome A. These results indicate the homoeology of wheat group-1 chromosomes with *Ae. markgrafii* chromosome A. The aromatic alcohol dehydrogenase (NADP-AADH) gene, located on *Ae. markgrafii* chromosome C (Schmidt et al. 1993), is located on the long arm of group-5 chromosomes. Also, three out of four WMS (group-5) are located on chromosome C whereas the fourth WMS in located on chromosome B, but in wheat it is located on the 4AL/5AL translocation. These data, taken together, provide strong evidence that chromosome C is homoeologous to homoeologous group-5, *Ae. markgrafii* chromosome B seems to be homoeologous to the short arm of homoeologous group-2 and the long arm of homoeologous group-4. Three WMS specific to the short arm of homoeologous group-2 are located on chromosome B. WMS165 (4AS/BL/DL) is located on chromosome B and WMS179 is located on the 4AL/5AL translocation of 5A. This agrees with the presence of the β-amylase gene on wheat chromosome 4DL (Hart 1983) and chromosome B in *Ae. markgrafii* (Schmidt et al. 1993) as well as the location of RFLP-clone BCD1709, located on 2S in wheat, on chromosome B (unpublished data). The location of three out of four WMS, on the long arm of homoeologous group-2 on chromosome G, indicates their homoeology. Aspartate aminotransferase (3AL/BL/DL; Hart 1983) is on chromosome F (Schmidt et al. 1993) and WMS314 (3D), a marker for chromosome F, indicates the homoeology of *Ae. markgrafii* chromosome F with wheat homoeologous group-3. Nevertheless, Schmidt et al. (1993) located an esterase (3AL/BL/DL; Hart 1983) on chromosome G; and WMS3, specific to chromosome G, is located on the long arm of homoeologous group-3, but more distal

than WMS314 (unpublished data). Leucine aminopeptidase (6 AS/BS/DS; Hart 1983) is present on chromosome D (Schmidt et al. 1993) and WMS508 (6BS), too, but WMS219 is found on chromosome A. The correspondence of most WMS with the results of isozyme analysis and chromosome morphology traits with regard to homoeology indicate that they detect homoeologous loci, although some results did not fit with these homoeologies. WMS349 (2DL), WMS219 (6BL) and WMS332 (7AL) are present on chromosomes D, A and D, respectively. This may be due to the amplification of non-homoeologous loci or to chromosomal rearrangements. The data observed indicate that there are rearrangements between the wheat genomes (A, B, D) and the *Ae. markgrafii* genome (C).

Some WMS showed additional bands, band losses, and band changes in some addition lines and the amphiploids, compared with the fragment pattern of parents, 'Alcedo' and 'S740-69'. A different peak pattern was expected for the substitution line, because of its different background for both parents. Changes in peak pattern for the addition lines or the amphiploid may be due to the observed chromosomal instability of the amphiploid and the spontaneous alien introgression into the euploid segregants (Blüthner et al. 1988). These chromosomal instabilities could also be caused by the gametocidal action of alien chromosomes from different *Aegilops* species introduced into wheat. The C genomes of *Ae. markgrafii*, *Ae. triuncialis* and *Ae. cylindrica*, in particular, are known to induce chromosomal rearrangements in backcrossing and selfing progenies from crosses with common wheat (Endo and Katayama 1978; Endo 1988; Tsujimoto and Noda 1988). Peak pattern changes could also be caused by changes in the wheat background over the several generations needed to produce the addition lines. It should be noted that this work was carried out to find robust markers for *Ae. markgrafii* chromosomes. Polymorphic WMS can only give hints of the homoeologous relationships between the A, B, D and C genomes.

Acknowledgements This work was supported by the 'Deutsche Forschungsgemeinschaft' (DFG, grant no. WE647/8-1/2).

References

Becker J, Heun M (1995) Barley microsatellites: allele variation and mapping. Plant Mol Biol 27 : 835–845

Bell CJ, Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19 : 137–144

Blüthner WD, Schubert V, Mettin D (1988) Instability in amphiploids and backcross derivatives of a *Triticum aestivum* × *Aegilops caudata* cross. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Cambridge. Institute of Plant Science Research, Cambridge Laboratory, Trumpington, pp 209–213

Devos KM, Dubcovsky J, Dvořák J, Chinoy CN, Gale MD (1995) Structural evolution of wheat chromosomes 4A, 5A and 7B and its impact on recombination. Theor Appl Genet 91 : 282–288

Endo TR (1988) Chromosome mutations induced by gametocidal chromosomes in common wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Cambridge. Institute of Plant Science Research, Cambridge Laboratory, Trumpington, pp 259–265

Endo TR, Katayama Y (1978) Finding of a selectively retained chromosome of *Aegilops caudata* L. in common wheat. Wheat Inf Service 47/48 : 32–35

Friebe B, Schubert V, Blüthner WD, Hammer K (1992) C-banding pattern and polymorphism of *Aegilops caudata* and chromosomal constitutions of the amphiploid *T. aestivum* × *Ae. caudata* and six derived chromosome addition lines. Theor Appl Genet 83 : 589–596

Grandillo S, Tanksley SD (1996) Genetic analysis of RFLPs, GATA microsatellites and RAPDs in a cross between *L. esculentum* and *L. pimpinellifolium*. Theor Appl Genet 92 : 957–965

Hart GE (1983) Hexaploid wheat (*Triticum aestivum* L. em Thell). In: Tanksley ED, Orton TJ (eds) Isozymes in plant genetics and breeding. Elsevier Science Publishers B.V, Part B, Amsterdam, pp 35–56

Kihara H (1958) Fertilty and morphological variation in the substitution and restoration backcrosses of the hybrids *Triticum vulgare* × *Aegilops caudata*. In: Proc 10th Int Congr Genet 1, McGill University, Montreal. University of Toronto Press, Ontario, pp 142–171

Korzun V, Börner A, Worland AJ, Law CN, Röder MS (1997) Application of microsatellite markers to distinguish inter-varietal chromosome substitution lines of wheat (*Triticum aestivum* L.). Euphytica 95 : 149–155

Kresovich S, Szewc-McFadden AK, Bliek SM, McFerson JR (1995) Abundance and characterization of simple-sequence repeats (SSRs) isolated from a size-fractionated genomic library of *Brassica napus* L. (rapeseed). Theor Appl Genet 91 : 206–211

Maughan PJ, Saghai Maroof MA, Buss GR (1995) Microsatellite and amplified sequence length polymorphisms in cultivated and wild soybean. Genome 38 : 715–723

McIntosh RA (1988) Catalogue of gene symbols for wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Cambridge. Institute of Plant Science Research, Cambridge Laboratory, Trumpington, pp 1225–1323

Nelson JC, Sorrells ME, van Deyzne AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141 : 721–731

Peil A, Schubert V, Schumann E, Weber WE (1997) RAPDs as molecular markers for the detection of *Aegilops markgrafii* chromatin in addition and euploid introgression lines of hexaploid wheat. Theor Appl Genet 94 : 934–940

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91 : 1001–1007

Plaschke J, Börner A, Wendehake K, Ganal MW, Röder MS (1996) The use of wheat aneuploids for the chromosomal assignment of microsatellite loci. Euphytica 89 : 33–40

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246 : 327–333

Rongwen J, Akkaya MS, Bhagwat AA, Lavi U, Cregan PB (1995) The use of microsatellite DNA markers for soybean genotype identification. Theor Appl Genet 90 : 43–48

Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81 : 8014–8018

Saghai Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA 91 : 5466–5470

Schmidt J-C, Schubert V, Blüthner WD (1993) Use of isozymes to characterize *Triticum aestivum-Aegilops markgrafii* addition lines. Biochem Physiol Pflanzen 188 : 385–392

Schubert V, Blüthner WD (1992) Zerlegung des Genoms von *Aegilops markgrafii* mit Hilfe von chromosomalen Additionslinien. Kühnarchiv 86 : 38–46

Schubert V, Blüthner WD (1995) *Triticum aestivum-Aegilops markgrafii* addition lines: production and morphology. In: Li ZS, Xin ZY (eds) Proc 8th Wheat Int Genet Symp, Beijing. China Agricultural Scientech Press, pp 421–425

Schubert V, Blüthner WD, Schlegel R (1987) N-banding and feulgen karyogram of *Aegilops markgrafii* (Greuter) HAMMER var. *markgraffi*. Cereal Res Commun 15 : 317–320

Schubert V, Junghanns W, Weidner A, Oertel C, Blüthner WD (1995) Powdery mildew and leaf rust resistance from *Aegilops markgrafii*. In: Börner A, Worland AJ (eds) Proc 9th EWAC Conf, Gatersleben-Wernigerode, IPK Gatersleben, pp 77–79

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36 : 884–889

Smulders MJM, Bredemeijer G, Rus-Kortekaas W, Arens P, Vosman B (1997) Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor Appl Genet 94 : 264–272

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor Appl Genet 86 : 985–990

Tsujimoto H, Noda K (1988) Chromosome breakage in wheat induced by the gametocidal gene of *Aegilops triuncialis* L.: its utilization for genetics and breeding. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Cambridge. Institute of Plant Science Research, Cambridge Laboratory, Trumpington, pp 435–460

Zhao X, Kochert G (1993) Phylogenetic distribution and genetic mapping of a $(GGC)_n$ microsatellite from rice (*Oryza sativa* L.). Plant Mol Biol 21 : 607–614