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Repetitive, genome-specific probes in wheat (*Triticum aestivum* L. em Thell) amplified with minisatellite core sequences

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Abstract The detection and analysis of DNA polymorphisms in crops is an essential component of marker-assisted selection and cultivar identification in plant breeding. We have explored the direct amplification of minisatellite DNA by PCR (DAMD-PCR) as a means for generating DNA probes that are useful for detecting DNA polymorphisms and DNA fingerprinting in wheat. This technique was facilitated by high-stringency PCR with known plant and animal minisatellite core sequences as primers on wheat genomic DNA. The products of DAMD-PCR from *Triticum aestivum, T. durum, T. monococcum, T. speltoides and T. tauschii* showed a high degree of polymorphism and the various genomes could be identified. Cloning of the DAMD-PCR products and subsequent Southern hybridization frequently revealed polymorphic probes showing a good degree of genome specificity. In addition, polymorphic, single locus, and moderately dispersed PCR products were cloned that may have a potential for DNA fingerprinting. Our experiments were limited primarily to diploid wheats and the results indicated that DAMD-PCR may isolate genome-specific probes from wild diploid wheat species that could be used to monitor genome introgression into hexaploid wheat.

Key words Genome-specific · DAMD · Minisatellite · PCR · *Triticum* · Wheat

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Introduction

The general lack of detectable DNA polymorphism in wheat (*Triticum aestivum* L. em Thell) in respect of conventional restriction fragment length polymorphisms (RFLPs) (10% of genomic clones, M. D. Gale, personal communication) has limited cultivar identification and DNA fingerprinting in this species. The ability to efficiently identify hexaploid wheat cultivars or wild-species genomes is important in current breeding practices which make use of wide crosses or alien chromosome introgression. Recent advances in rice (*Oryza sativa* L.) repetitive DNA research has resulted in the development of minisatellite DNA probes that can identify related US rice cultivars (Zhou and Gustafson 1995). These probes are the rice derivatives (Winberg et al. 1993) of the human minisatellite probe first introduced by Jeffreys et al. (1985a, b). DNA fingerprinting in rice can be performed with a single minisatellite DNA probe and a single restriction enzyme, which greatly reduces the time and financial investment involved in cultivar identification. Therefore, repetitive DNA can be a valuable source of genetic polymorphism for genome and variety identification, in contrast to single-locus RFLP markers.

Two types of repeated sequence, polymorphic DNA include short poly-nucleotide tandem repeats [microsatellites or simple sequence repeats (SSRs, 2–5 bp) (Roder et al. 1995) and minisatellites (10–60 bp) as defined by Jeffreys et al. (1985a)]. These repetitive DNA regions normally contain tandem repeats that vary in the number of

repeat units between genotypes and thus are referred to as variable number tandem repeats (VNTRs) or hypervariable regions (HVRs). Identification of microsatellites has led to the development of polymorphic, site-tagged sequence markers in wheat (Devos et al. 1995).

Minisatellite DNA regions contain a VNTR where each repeat itself includes a short, highly conserved core sequence. This form of HVR is found at numerous loci within the human genome and displays high levels of polymorphism between individuals (Jeffreys et al. 1985a). The core sequences of several reported human minisatellites were recently used as primers in PCR reactions that efficiently amplify regions rich in minisatellites and VNTR loci in fish (Heath et al. 1993). This PCR strategy is referred to as the direct amplification of minisatellite DNA (DAMD-PCR) and overcomes the difficulty of searching for VNTR loci with a heterologous probe in a genome, like that of wheat, which contains a considerable amount of repeated DNA.

Our interest was focused on amplifying repetitive DNA present in wheat genomes at or near minisatellite loci in order to develop highly polymorphic, DNA fingerprinting probes. We were searching for repetitive DNA that was moderately dispersed at 10–30 loci, so as to be resolved on conventional genomic DNA Southern blots, and was hypervariable. These probes could potentially be used in the cultivar identification of wheats, if sufficient polymorphism was detected, or possibly to follow the introgression of wild-species chromosome segments into wheat. It is more efficient to develop a probe of this nature through DAMD-PCR as opposed to randomly searching a genomic library for informative DNA-fingerprinting probes.

The strategy used for the isolation and cloning of DNA fingerprinting probes and the types of repetitive DNA probes isolated is presented here. Surprisingly, in addition to moderately dispersed, potential DNA-fingerprinting probes, genome-specific probes were isolated with high efficiency. Examples of repetitive, genome-specific probes have been reported in rye (McIntyre et al. 1990) and in *Agilops squarrosa* (Rayburn and Gill 1987). The genome-specific probes isolated with DAMD-PCR may be equally useful in identifying alien wheat DNA in a hexaploid wheat genetic background.

Methods and materials

Germ plasm

T. aestivum L. (cv Chinese Spring) (ABD genomes), *T. durum* L. (AB), *T. monococcum* L. (A^m), *T. speltoides* Tausch (S), *T. tauschii* Coss. (D), *T. urartu* L. (A^u) and *T. timopheevii* Zhuk. (AG), were all acquired from the E.R. Sears, USDA-ARS germ plasm collection at the University of Missouri.

DNA isolation and PCR

All of the genotypes were greenhouse-grown in Missouri and high-molecular-weight genomic DNA was isolated from leaf tissue according to Saghai-Maroof et al. (1984). PCR reactions had a final volume of 20 μL and included: 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 80 ng genomic DNA template, 1 U *Taq* DNA polymerase (Gibco BRL), 200 μM of each dNTP and 44 pmol of a single primer. The primer synthesis (Genosys Biotechnologies Inc., Woodlands Tex.) was based on published plant and animal minisatellite core sequences (see Table 1). The parameters for PCR amplification were 95°C, 2 min then 35 cycles of 95°C, 1 min –55°C, 1 min –72°C, 1.5 min then 72°C, 5 min for one cycle, using a Perkin-Elmer DNA thermocycler.

Gel electrophoresis, Southern hybridization and cloning

The DAMD-PCR products were electrophoresed in agarose gels (2% w/v) in 1 × TAE (40 mM Tris-acetate, 1 mM EDTA) for 4 h at 100 V and were then visualized by ethidium bromide staining. Small gel samples of PCR products of interest were plugged from the gel and stored at –20°C. Alternatively, the DAMD-PCR products were Southern blotted onto a nylon membrane (Hybond-N^+, Amersham). PCR products were re-amplified as above by stabbing the stored gel plugs with a needle and agitating the needle in a PCR reaction containing the appropriate primer (Bjourson and Cooper 1992). The re-amplified PCR products were subsequently blunt ended with T_4 DNA polymerase and T_4 polynucleotide kinase (Wang et al. 1994) and electrophoresed in low-melting-point agarose gels (1.5% w/v) in 1 × TAE. The re-amplified PCR products were then purified from gel slices, using a beta-agarase treatment (New England Biolabs) and ethanol precipitation, for use in Southern hybridizations or cloning.

High-molecular-weight genomic DNA was digested with *Dra*I or *Hin*dIII and electrophoresed for 48 h in agarose gels (0.8% w/v) in 1 × TBE (89 mM Tris-borate, 89 mM borate, 2 mM EDTA) until the 1000-bp fragments of DNA were near the bottom of the 25-cm gel. The electrophoresed DNA was then Southern blotted onto a nylon membrane (Hybond-N^+, Amersham). The re-amplified PCR products were screened for informativness by southern hybridization prior to cloning. We were searching for probe characteristics such as moderate dispersal and good RFLPs. The re-amplified PCR products were labeled by random primer extension (Feinberg and Vogelstein 1983) with alpha-^{32}P-dCTP and hybridized to the genomic DNA digests.

Informative PCR fragments were cloned into the *Sma*I site of pBluescript II (SK+) (Stratagene). The cloned PCR products were labeled by random primer extension (Feinberg and Vogelstein 1983) with alpha-^{32}P-dCTP and hybridized to genomic-DNA digests. Hybridizations were performed in 250 mM NaH_2PO_4 (pH 6.5), 7% (w/v) SDS and 1% (w/v) BSA at 65°C. Southern blots were washed at low stringency [2×30 min, 65°C, 1×SSC/0.1% (w/v) SDS] or subsequently at high-stringency [2×30 min, 65°C, 0.1×SSC/0.1% (w/v) SDS]. Autoradiograms were produced by exposing hybridized Southern blots to Kodak X-Omat film for 6 h–4 days.

DNA sequencing

Double-stranded DNA from the cloned PCR products was sequenced in both directions using T_3 and T_7 primers with the DyeDeoxy™ Terminator-Cycle Sequencing Kit (Perkin Elmer). DNA sequences were then compared to the DNA databases using 'Basic Local Alignment Search Tool' (BLAST) (Altschul et al. 1990).

Results and discussion

DAMD-PCR profiles

The DAMD-PCR reactions were annealed at 55°C, which was higher than the melting temperature of most of the primers used (Table 1). Reproducibility was checked by

Table 1 Minisatellite core sequences used as primers for PCR amplification of DNA in wheat

Primer[a]	Sequence (5′–3′)	Length (bp)	Melt temp. (°C)	Reference
14C2	GGCAGGATTGAAGC	14	52	Vergnaud (1989)
HBV-3	GGTGAAGCACAGGTG	15	53	Nakamura et al. (1987)
HBV-5	GGTGTAGAGAGGGGT	15	49	Nakamura et al. (1987)
FVIIex8	ATGCACACACACAGG	15	51	Murray et al. (1988)
FVIIex8-C	TACGTGTGTGTGTCC	15	47	Murray et al. (1988)
YNZ-22	CTCTGGGTGTGGTGC	15	56	Nakamura et al. (1987)
M13	GAGGGTGGCGGCTCT	15	63	Vassart et al. (1987)
33.6	AGGGCTGGAGG	11	48	Jeffreys et al. (1985a)
Rice HVR (–)	CCCTCCTCCTCCTTC	15	50	Winberg et al (1993)
Rice HVR (+)	AGGAGGAGGGGAAGG	15	50	Winberg et al. (1993)

[a] Primers are a core sequence from a plant or animal minisatellite array

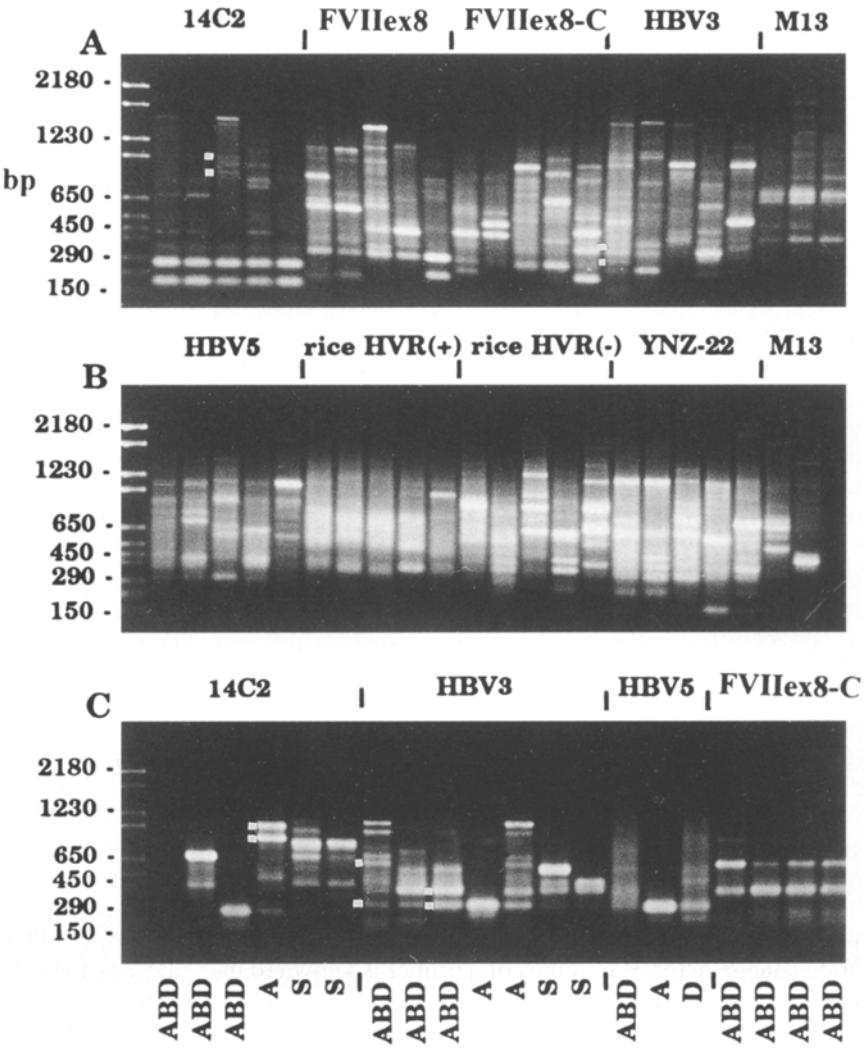

Fig. 1A–C Direct amplification of minisatellite DNA by PCR in five genotypes of wheat **A, B** Minisatellite core sequences were used as primers in PCR reactions annealed stringently at 55°C and the PCR products were separated in agarose gels. Within each primer, the wheat genotypes are left to right: *T. aestivum*, *T. durum*, *T. monococcum*, *T. speltoides* and *T. tauschii*. **C** Re-amplified DAMD-PCR products by 'band stabbing'. The genomic DNA that served as template for the original PCR fragments were *T. aestivum* (*ABD*), *T. monococcum* (A^m), *T. speltoides* (*S*) and *T. tauschii*. (*D*). The DAMD-PCR primers are listed in Table 1 and are indicated above the lanes. *White dots* to the left of bands are referred to in the text

performing DAMD-PCR on DNA from two separate DNA extracts from each of three individual plants (data not shown). The DAMD-PCR profiles were always consistent, primarily due to the high annealing temperature. This strategy was in contrast to random amplified polymorphic DNA (RAPD) analysis, where the annealing temperature is typically 36–42°C and lower than the melting temperature of the primers used. The DAMD-PCR reaction also used a relatively high quantity of primer (44 pmol) which was optimal for amplifying repetitive DNA regions from multiple loci. Lower primer quantities (5–20 pmol) are typically used in the amplification of single-locus PCR products but could be exhausted when amplifying repetitive DNA.

The DAMD-PCR profiles indicated that a large amount of polymorphism was detectable among the five wheat genotypes, and each primer amplified a unique set of products (Fig. 1A, B). There were bands of major and minor abundance within the DAMD-PCR profiles. Six primers which amplified discrete PCR products were informative, but primers rice HVR(+), rice HVR(−) and YNZ-22 generally amplified a smear of products, as did one additional primer (33.6, not shown in Fig. 1A,B) (Table 1). This less-informative smearing pattern was still evident at annealing temperatures of 60 and 62°C (data not shown).

Since single primers were used in the PCR reaction, two copies of the priming sequence must exist in an inverted orientation to produce a PCR product. Heath et al. (1993) suggests that amplification is possible due to an inversion present in the minisatellite region that creates the amplicon. There is no direct evidence for this hypothesis in our data, or in the data of Heath et al. (1993). The minisatellite core sequences at one locus normally occur as direct tandem repeats, thus an inversion that included a minisatellite core sequence could include some flanking DNA. Heath et al. (1993) reported the isolation of a single-locus VNTR sequence which most likely contained a unique sequence, flanking DNA.

A high degree of polymorphism was detected among the five wheat genotypes with each of the six informative primers (14C2, FVIIex8, FVIIex8-C, HBV-3, HBV-5 and M13). The A, S and D genomes (lanes 3, 4, 5 respectively within each primer) (Fig. 1A, B) were easily identified by distinct band profiles for each of these primers. There were numerous bands in tetraploid *T. durum* that were not present in hexaploid *T. aestivum*. Also, there were no significant differences in the number of bands amplified between the three wheat ploidy levels (Fig. 1A, B).

These results suggested that there was a large degree of genome specificity to the DAMD-PCR reactions. Since *T. aestivum* did not show all the products of the diploid species combined, the data suggested that the DNA of the diploid species was partially unrelated to *T. aestivum* DNA (Fig. 1A, B). The A, D and B genomes in *T. aestivum* are thought to have originated from the three diploid species *T. monococcum, T. tauschii* and *T. speltoides* respectively, yet our data show that there has been some degree of sequence divergence since the genomes combined. The polymorphisms could also result from very little sequence divergence among the species. For example, a single nucleotide change in the 3′ terminus of a primer is known to inhibit the amplification of microsatellite loci in wheat (Roder et al. 1995). Therefore, it is possible that the PCR products observed are those from loci that match the exact primer sequence and that subtle sequence variation between core sequences can influence the final DAMD-PCR profile.

Re-amplified DAMD-PCR products

Figure 1C is included to demonstrate the results of re-amplification of the DAMD-PCR products via the band-stab method (Bjourson and Cooper 1992). Each lane in Fig. 1C shows the PCR products derived from stabbing a gel plug from a band present in gels similar to Fig. 1A and B. Occasionally, a re-amplification reaction would not yield any fragments (Fig. 1C, 14C2, lane 1 ABD genomes) or commonly would yield fragments of smaller size (Fig. 1C, HBV-3, lane 1 ABD genomes). Faint fragments also re-amplified to a greater extent; for example, the fragments indicated in Fig. 1C, 14C2, lane 4, A^m genome, are re-amplified from those indicated in Fig. 1A, 14C2, lane 3, *T. monococcum*. This increased the number of original DAMD-PCR products that could be screened by the selection of even low-quantity DAMD-PCR products. In addition, bands from less informative DAMD-PCR profiles (e.g., Fig. 1A, HBV-3, lane 1, *T. aestivum*) re-amplified with sufficient resolution and abundance (Fig. 1C, HBV-3, lane 3 ABD genomes), which further increased the number of DAMD-PCR bands to be screened (Fig. 1C). A finite number of minisatellite primers could be used in DAMD-PCR, so mechanisms of re-amplification that increased the number of DAMD-PCR products to be screened were vital to the overall strategy.

Primer-specific DAMD-PCR products

There were only two occurrences where all five wheat species displayed a common, monomorphic PCR product at equal intensity (i.e., primer 14C2 at 270 bp and 180 bp) (Fig. 1A). The vast majority of the PCR products were polymorphic between the five wheat species. Two pairs of primers were complementary, FVIIex8/FVIIex8-C and rice HVR(−)/rice HVR(+). The former are directly complementary over the 15-bp length and the latter are complementary for 10 bp internally and possess 5-bp 3′ tails which complete the complementarity (Table 1). Each member of these primer pairs amplified unique products. The rice HVR primers showed similar smears of PCR products in contrast to the FVIIex8 primers (Fig. 1A, B). The different banding patterns derived from different primers, and particularly complementary primers, suggested that the DAMD-PCR reactions were amplifying unrelated regions of DNA. A probe derived by DAMD-PCR with primer FVIIex8 showed homology for other FVIIex8-derived PCR products only (Fig. 2). The probe was a 800-bp DAMD-PCR fragment from *T. aestivum* DNA and it did not hybridize to any other PCR products amplified with the other ten primers, including PCR products amplified from the complementary primer FVIIex8-C (Fig. 2). Therefore, it appeared that each primer may have the potential to amplify a primer-specific family of products

Repetitive, genome-specific probes

A few different types of repetitive DNA probes were isolated by DAMD-PCR. Clone pTs19.3 (Table 2), amplified from *T. speltoides*, hybridized to all five wheat species.

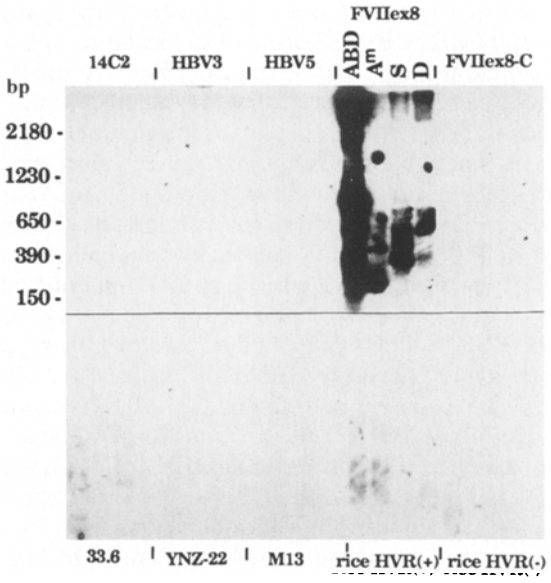

Fig. 2 Southern hybridization of DAMD-PCR products with a DAMD-PCR product. Minisatellite core sequences served as primers for PCRs annealed stringently at 55°C to wheat genomic DNA. PCR products were separated in agarose gels and Southern blotted. An 800-bp PCR fragment derived from *T. aestivum* using primer FVIIex8 was hybridized and washed at 1× SSC/0.1% SDS, 65°C. Within each primer, the genomic DNA template for the PCR products on the blot are left to right: *T. aestivum* (*ABD*), *T. monococcum* (A^m), *T. speltoides* (*S*) and *T. tauschii*, (*D*). The primers are listed in Table 1 and are indicated above or below the lanes

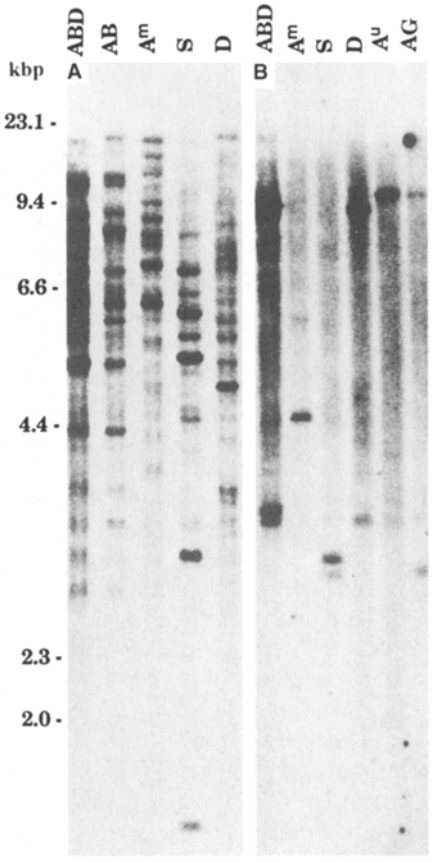

Fig. 3A, B Southern hybridization of wheat genomic-DNA digests. Genomic DNA was digested with *Dra*I and was hybridized with DAMD-PCR clones pTs19.3 (**A**) and pTs3.6 (**B**). The wheat genotypes are indicated above the lanes: *T. aestivum* (*ABD*), *T. durum* (*AB*), *T. monococcum* (A^m), *T. speltoides* (*S*), *T. tauschii* (*D*), *T. urartu* (A^u) and *T. timopheevii* (*AG*)

Table 2 DAMD-PCR clones used

Clone	Template DNA	Primer[a]	Size (bp)
pTm2.3.1	*T. monococcum*	14C2	277
pTm2.3.2	*T. monococcum*	14C2	542
pTs3.6	*T. speltoides*	14C2	480
pTs3.7	*T. speltoides*	14C2	300
pTs19.3	*T. speltoides*	14C2	460

[a] Primer 14C2 is a core sequence from a human minisatellite array

Multiple loci were detected and the three diploid wheats showed clear species-specific DNA fingerprint patterns (Fig. 3A). Many RFLPs found in the diploid species were not detected in *T. aestivum* and *T. durum*, which was consistent with observations from the DAMD-PCR profiles (Fig. 1). The RFLP pattern of *T. aestivum* (ABD genomes) and *T. durum* (AB genomes) were qualitatively similar, which could be related to *T. speltoides* (the putative B genome) being the source of the clone and this cloned sequence not having diverged since the emergence of *T. aestivum* (Fig. 3A).

Clone pTs3.6 (Table 2), amplified from *T. speltoides*, hybridized to several fragments in *T. aestivum* and one or two fragments in the diploid wheats. This appeared to be a one- or two-locus hybridization pattern (Fig. 3B) which contrasted with the multi-locus profile of pTs19.3 (Fig. 3A). Clone pTs3.6 could distinguish between *T. monococcum* and *T. urartu* (both A genome species) and showed that *T. speltoides* and *T. timopheevii* shared a unique band. The frequency of single-locus clones was 2 out of 19 DAMD-PCR products cloned, with the remainder being multi-locus or repetitive in nature. The frequency of clones that were potentially useful for DNA fingerprinting, e.g., pTs19.3, was also 2 out of 19.

Two clones were isolated from a single ligation of a single re-amplified DAMD-PCR fragment from *T. monococcum* (pTm2.3.1 and pTm2.3.2) (Table 2). Clone pTm2.3.1 was 2-fold larger than pTm2.3.2 and, except for the S genome, hybridized to similar fragments on Southern blots (Fig. 4A, B). A similar result from a single ligation was also observed in a concurrent study in rice. Clones pTm2.3.1 and pTm2.3.2 showed strong homology to the A^m genome of *T. monococcum* and the fragments they identified appeared to be widely dispersed (based on a smeared hybridization pattern) with several high-copy number fragments detected, particularly by pTm2.3.2. Both clones showed weaker homology to *T. tauschii* DNA and less dis-

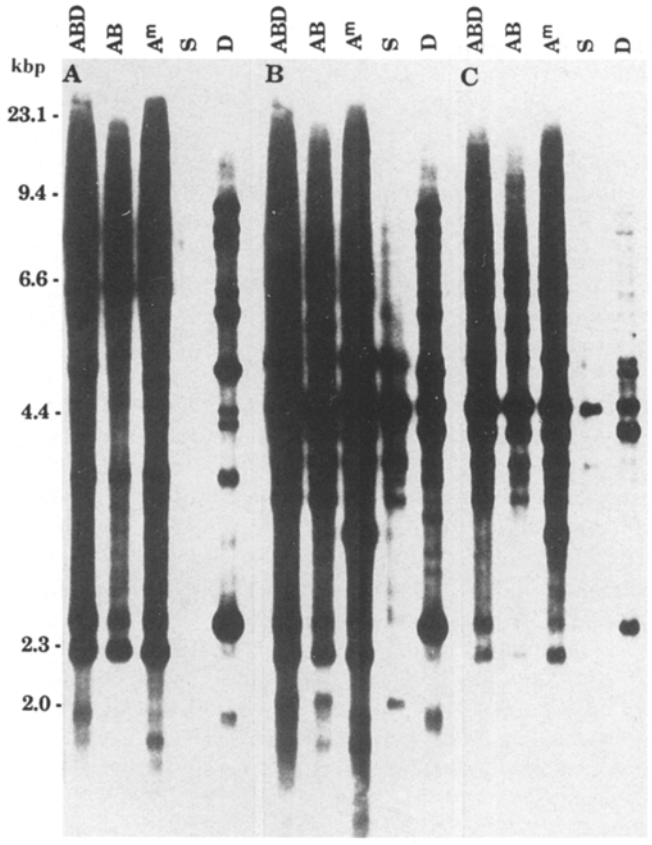

Fig. 4A–C Southern hybridization of wheat genomic DNA. Genomic DNA was digested with *DraI* and was hybridized with DAMD-PCR clones pTm2.3.1 (**A**) and pTm2 3.2 (**B, C**). Blots **A** and **B** were washed at 1×SSC/0.1% SDS, 65°C and blot **C** was washed at 0.1×SSC/0.1% SDS, 65°C. The wheat genotypes are indicated above the lanes. *T. aestivum* (*ABD*), *T. durum* (*AB*), *T. monococcum* (A^m), *T. speltoides* (*S*) and *T. tauschii* (*D*)

persal than *T. monococcum*. The detection of fragments in *T. speltoides* DNA by pTm2.3.2 was unique compared to pTm2.3.1 with only a 4.4-kbp fragment showing strong homology after washing at high-stringency (Fig. 4C). The fragments detected by these two probes in *T. tauschii* were generally qualitatively similar at low stringency (Fig. 4A, B). This was good evidence that clones showing some degree of genome specificity could be isolated from wheat using DAMD-PCR. In this case, pTm2.3.2 included additional DNA that showed homology to *T. speltoides* DNA (Fig. 4A, B).

Clones pTm2.3.1 and pTm2.3.2 were sequenced and neither showed any significant homology to DNA sequences in databases (Altschul et al. 1990). The entire sequence in Fig. 5 represents pTm2.3.2, and the sequence from nucleotides 266–542 represents pTm2.3.1. The original DAMD-PCR product selected for cloning was approximately 270 bp. pTm2.3.2 appeared to be an unexpected product of the ligation and included ligated PCR fragments from two different loci, based on the different sequences defined by the DAMD-PCR priming sites (Fig. 5, underlined and with open arrowheads). The sequence data showed that DAMD-PCR could amplify similar-sized PCR products (e.g., 266 bp and 276 bp) from different locations and that these products could be ligated into a single plasmid. This demonstrated that each original DAMD-PCR band was potentially a population of fragments from different loci. There are additional, potentially informative clones to be isolated from each DAMD-PCR product; thus many transformed bacterial colonies from a single ligation should be screened to make full use of this strategy.

An analysis of the sequence data also revealed several repetitive DNA elements, including three with high homology to the DAMD-PCR primer 14C2 sequence (Fig. 5, dashed arrows). In addition, sequencing revealed four pairs

Fig. 5 Nucleotide sequence of clones pTm2.3.1 (nucleotides 266–542) and pTm2.3.2 (nucleotides 1–542). Both clones originated from *T. monococcum* and are the products of a single ligation reaction involving DAMD-PCR fragments approximately 270 bp in length. pTm2.3.2 showed two inverse DAMD-PCR priming sites centered at nucleotide 266. DAMD-PCR priming sites (*underlined open-arrowhead*), homologous DAMD-PCR priming sites (*dashed arrow*); homologous, direct repeat pairs (*number and bracket*)

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1   GGCAGGATTGAAGCTATACACATACTACTGGCCTATGNAANTCANCATAACATNCTCCTG   60
61  TNTCAAATGGATGTGAAGAGTGCCTTTCTCAATGGNAAGATTGANGAAGAAGTGTNTGTT  120
121 GCACAACCTCCTGGCTTGGANGATCCAAAACATCATGACATGGTTTACAGCTCAACAAGG  180
181 CACTGTNTGGCCTCAAACAAGCCCCTCGTTGCTTGGTATGACACACTCAAGGACTTCTTG  240
241 AAGATCCAAAGGCTTCAATCCTGCCGGCAGGATTGAAGCCCATGTCAGGTGCGCTGCACA  300
301 GTCACGACGAGCATGACGTAACTCTGCCGTCGGACAATATTGGGAGATCGCGTCTGCAAC  360
361 TACTCCGGCCGTCATCTGGAGCAAATCGCGCCATCCGAGAGCGGAGGGATAGACCCCGCC  420
421 ATGGAGGAAGCACTCTCAGTGGCGATAGAGTAACATACTGACATTCACCCCTTACGAGAG  480
481 CCGTGTCACCGAACCACTGGATTCACCTCCGGCCACGGACTCCGAGCCGCTTCAATCCTG  540
541 CC                                                            542
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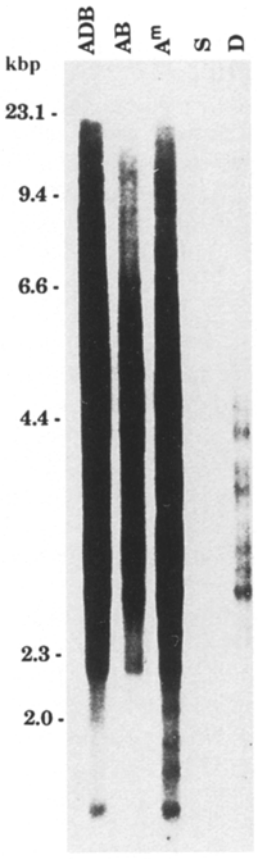

Fig. 6 Southern hybridization of wheat genomic DNA. Genomic DNA was digested with *Dra*I, hybridized with DAMD-PCR clones pTs3.7 and washed at 1 × SSC/0.1% SDS, 65 °C. The wheat genotypes are indicated above the lanes *T. aestivum* (*ABD*), *T. durum* (*AB*), *T. monococcum* (A^m), *T. speltoides* (*S*) and *T. tauschii* (*D*)

of direct repeats showing significant homology (68–93%) (Fig. 5, brackets). The sequences of clones pTm2.3.1 and pTm2.3.2 demonstrated that DAMD-PCR products can originate from regions of abundantly repetitive elements which may also possess additional, highly homologous DAMD-PCR priming sites. The similar hybridization pattern between these clones (Fig. 4A, B) can be explained by the identical sequences found in both clones. Although pTm2.3.2 originates from *T. monococcum*, the sequence from nucleotides 1–265 contained DNA homologous to a high-copy number sequence in *T. speltoides*.

Finally, clone pTs3.7 (Table 2), was amplified from *T. speltoides* but did not hybridize to *T. speltoides* DNA on Southern blots washed at low stringency (1 × SSC, 65°C). The genomic sequences homologous to pTs3.7 appear to be highly dispersed and in high-copy number in *T. monococcum* and show very little cross hybridization to *T. tauschii* (Fig. 6). Following a longer autoradiogram exposure, a single band at 4.8 kbp was seen in the *T. speltoides* lane (data not shown). This *T. speltoides* fragment may be the source of the original DAMD-PCR product that was cloned. The data suggest that a single-copy locus, homologous to pTs3.7 in *T. speltoides*, may have evolved as a highly dispersed and high-copy number locus in *T. monococcum* but with very little presence in *T. tauschii* (Fig. 6). We have no immediate explanation for the nature of this sequence among the genotypes tested.

The genome-specific probes were very prevalent and were easily detected among those DAMD-PCR fragments screened on genomic-DNA digests. Many of the 30 DAMD-PCR fragments that were hybridized showed specific homology to two of the three A, S and D genomes. Some level of genome specificity was detected in 10 out of 19 clones generated in this study (data not shown). Definitive genome specificity was not demonstrated in this study by hybridization of cloned DAMD-PCR products to several accessions of one species. These experiments are intended in the future and the genome-specific probes should be considered putative.

Hybridization patterns were observed from PCR fragments that originated as monomorphic DAMD-PCR bands. For example, three DAMD-PCR bands (primer 14C2), that were monomorphic among the three diploid wheats, showed some degree of genome specificity to each of the three respective diploid wheat genomes (data not shown). Therefore, monomorphic bands should not be ignored when searching for probes to detect DNA polymorphisms using DAMD-PCR.

In summary, the most frequent result of performing DAMD-PCR in wheat was the isolation of wheat genome-specific probes. Less frequent was the isolation of probes that were moderately dispersed and useful for DNA fingerprinting wheat cultivars. Both types of probes are very desirable.

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