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PCR primed with minisatellite core sequences yields DNA fingerprinting probes in wheat

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Abstract Four minisatellite core sequences were used as primers in a polymerase chain reaction (PCR) technique, known as the directed amplification of minisatellite-region DNA (DAMD), to detect polymorphisms in three pairs of hexaploid/tetraploid wheat cultivars. In each pair, the tetraploid cultivar (genomic formula AABB) was extracted from its corresponding hexaploid (genomic formula AABBDD) parent. Reproducible profiles of the amplified products revealed characteristic bands that were present only in the hexaploid wheats but not in their extracted tetraploids. Some polymorphisms were observed among the hexaploid cultivars. Twenty-three DAMD-PCR amplified fragments were isolated and screened as molecular probes on the genomic DNA of wild wheat species, hexaploid wheat and triticale cultivars. Subsequently, 8 of the fragments were cloned and sequenced. The DAMD-PCR clones revealed various degrees of polymorphism among different wild and cultivated wheats. Two clones yielded individual-specific DNA fingerprinting patterns which could be used for species differentiation and cultivar identification. The results demonstrated the use of DAMD-PCR as a tool for the isolation of informative molecular probes for DNA fingerprinting in wheat cultivars and species.

Key words PCR · Minisatellite · DNA fingerprinting · Wheat · Triticale

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Introduction

The development of plant molecular techniques is providing a base for the development of DNA-based diagnostics in plant science (Rafalski and Tingey 1993). DNA-based markers can be extremely useful in studying genetic diversity and taxonomy, and as tools in plant breeding cultivar and selection programs. These markers can be used to identify any DNA sequence polymorphism between individuals and to tag genes of agronomic importance. In utilizing DNA diagnostics in plants, molecular markers which can detect high levels of polymorphism, such as hypervariable region (HVR) DNA, will be of great value. Hypervariable regions of a genome are those containing minisatellites, or a variable number of tandem repeats (VNTRs), which are tandemly repeated DNA sequences that generally consist of 10- to 60-bp motifs, known as core sequences (Jeffreys et al. 1985a; Nakamura et al. 1987). The variation in core-sequence copy number at a locus results in a high level of minisatellite variability (Jeffreys et al. 1990), thus making them useful for DNA fingerprinting (Jeffreys et al. 1985b). Minisatellite sequences are abundant in human (Jeffreys et al. 1985a), animal and plant genomes (Jeffreys and Morton 1987;

Georges et al. 1991; Broun and Tanksley 1993; Zhou and Gustafson 1995).

When minisatellite core sequences are utilized as single primers in an amplified polymorphic DNA polymerase chain reaction (PCR), the DNA amplification can be directed to hypervariable regions (Heath et al. 1993). This technique, named the directed amplification of minisatellite-region DNA (DAMD), has been used by Heath et al. (1993) in fish, birds and human, by Zhou et al. (1997) in rice (*Oryza*) species and by Somers et al. (1996) in wild wheat (*Triticum*) species to detect polymorphism among different species. Utilization of minisatellite region-specific primers makes it possible to employ high annealing temperatures to overcome the problem of repeatability that has been observed in conventional random amplified polymorphic DNA (RAPD) analysis and is due to a low reaction stringency. DAMD-PCR has yielded species-specific banding patterns in wild wheats and in rice species (Somers et al. 1996; Zhou et al. 1997). The isolated DAMD-PCR fragments have the potential of being used for DNA fingerprinting in wild and cultivated rice (Zhou et al. 1997). DAMD-PCR can also be valuable in generating genome-specific probes in both rice (Zhou et al. 1997) and wheat (Somers et al. 1995).

The D genome of hexaploid wheat (*Triticum aestivum* L. em Thell.) is very important in programs of wheat improvement because it carries genes controlling bread-making quality as well as those for disease resistances and other agronomic characters. The current D genome has been extensively modified since its original introduction into hexaploid wheat. In order to study the effects and other evolutionary considerations of the removal of the D genome from hexaploid wheat, Kaltsikes et al. (1969) developed three derived tetraploid wheat cultivars from their hexaploid parents: 'TetraPrelude' from 'Prelude', 'TetraRescue' from 'Rescue' and 'TetraThatcher' from 'Thatcher'. These three pairs consist of the hexaploid wheat parent (genomic formula AABBDD) and its extracted tetraploid counterpart (genomic formula AABB). Thus, in each pair the two lines share the same A and B genomes, but differ by the presence or absence of the D genome. These genetic materials have been used extensively in genetic and cytogenetic studies to document the effects of the removal of the D genome on several traits (Kaltsikes et al. 1969, 1970). By virtue of their genomic composition, this material provides an opportunity for the study of the effects involving the presence of either the entire present-day D genome, or a small segment of it, on any traits controlled by genes residing on its chromosomes. The material can also be used in a study of DNA polymorphism within the D genome and the isolation of genome-specific probes.

The purpose of the study presented here was to utilize DAMD-PCR to detect polymorphisms specific to the presence of the D genome in the hexaploid/tetraploid system and to develop novel molecular markers for cultivated wheat.

Materials and methods

Plant materials and genomic DNA extraction

The plant material used in this study included the 3 spring-type common wheats (AABBDD), 'Prelude', 'Rescue' and 'Thatcher', and their derived tetraploid varieties (AABB), 'TetraPrelude', 'TetraRescue' and 'TetraThatcher', as described by Kaltsikes et al. (1969, 1970). *Triticum durum* var. 'Stewart 63' was used in the extraction of the AABB component for the above 3 tetraploid wheats (Kaltsikes et al. 1969). Other plant material used included 4 hexaploid wheat cultivars, 'Chinese Spring', 'Holdfast', 'Marika', and 'Pavon 76', 1 tetraploid wheat cultivar, 'Cocorit 75', and the various *Triticum* species *T. monococcum* (A^m), *T. timopheevii* (AG), *T. dicoccoides* (AB), *T. urartu* (A^u), *T. tauschii* (D) and *T. durum* (AB) from USDA-Sears Collection (University of Missouri, Columbia). Two hexaploid triticale (AABBRR) (x-*Triticosecale* Wittmack) cultivars 'Drira^{++}' (Merker 1973) and 'Rosner^{++}' (Roupakias and Kaltsikes 1977) were also included in the analysis. Leaf material was harvested from 1- to 3-month-old plants grown in a greenhouse at the University of Missouri-Columbia, lyophilized and ground to a fine powder. Total genomic DNA was isolated following the method of Saghai-Maroof et al. (1984).

DNA amplification

Primers were synthesized based on the following minisatellite core sequences: (1) FVIIex8 (ATGCACACACACAGG) (Murray et al. 1988); (2) HBV3 (GGTGAAGCACAGGTG) (Nakamura et al. 1987); and (3) Rice HVR(−) (CCCTCCTCCTCCTTG) (Winberg et al. 1993). Another primer (4) FVIIex8-c (TACGTGTGTGTGTCC) was synthesized based on the complementary strand of the core sequence of FVIIex8 (Murray et al. 1988). PCR reactions were performed in a Perkin Elmer Cetus Model 480 DNA Thermal Cycler. The amplification was programmed as: 1 min at 95°C for denaturation, followed by 1 min at 60°C for annealing and 1.5 min at 72°C for extension, for 35 cycles. Each reaction was carried out in the volume of 20 µl containing 80 ng of genomic DNA, 200 µ*M* of each dNTP, 40 pmole of a single primer, 1 unit of *Taq* DNA polymerase (Gibco BRL), 20 m*M* TRIS-HCl, 50 m*M* KCl, and 2.5 m*M* $MgCl_2$. Annealing temperatures of 50°C, 55°C and 60°C were also tested in order to optimize the PCR conditions. Other parameters, such as the quantity (1 unit or 0.5 unit) and quality (different supplier, Boehringer) of *Taq* DNA polymerase were also tested. The PCR products were electrophoresed in 2% (w/v) agarose, and 1 × TBE at 100 V for 3–4 h. Amplified DNA fragments were visualized by staining with ethidium bromide.

Southern hybridization

Twenty-three DAMD-PCR-derived fragments were re-amplified using the band-stab method (Bjourson and Cooper 1992) and the PCR conditions described above. The re-amplified fragments were separated on 1.5% (w/v) low-melting-point agarose and purified using β-Agarase I (New England BioLabs) digestion following the manufacturer's protocol. Genomic DNA from each of the varieties and species was digested with *Dra*I, *Hin*dIII or *Hin*fI (Promega) under the conditions recommended by the manufacturers. The digestions

of 10 μg for each diploid, 15 μg for each tetraploid and 20 μg for each hexaploid species were fractionated on 0.8% (w/v) agarose gels and transferred onto Hybond-N^+ charged nylon membrane (Amersham Int) following the manufacturer's recommended procedures. DNA fragments were labelled with α-[^{32}P]dCTP using the random primer method (Feinberg and Vogelstein 1983). Prehybridization, hybridization and autoradiography were as described by Zhou and Gustafson (1995).

PCR procedure and screening, cloning and sequencing

The DAMD-PCR-generated fragments revealing polymorphic banding patterns were selected for further screening by Southern hybridization and subsequent cloning. The DNA products from the re-amplification were blunt-ended with T4 DNA polymerase and T4 DNA Polynucleotide Kinase as described by Wang et al. (1994) prior to electrophoresis. Gel-purified PCR products were ligated into the pBluescript II KS(−) vector (Stratagene Cloning System). The cloned fragments were sequenced from both directions with T3 and T7 sequencing primers using a DyeDeoxy™ Terminator Cycle Sequencing Kit (Perkin-Elmer Corp) using double-stranded template DNA. Sequence homology searching against the DNA databases was carried out using BLAST (Altschul et al. 1990).

Results and discussion

Detection of polymorphism in hexaploid/tetraploid wheat with DAMD-PCR

Four minisatellite core sequences were used as primers in PCR reactions utilizing the template DNA from the three pairs of hexaploid wheat varieties and their extracted tetraploid derivatives. DAMD-PCR yielded variable polymorphism levels among the hexaploid and tetraploid wheat cultivars. In addition, different primers produced different electrophoretic profiles (Fig. 1). The primer FVIIex8 revealed bands that were present only in 'Thatcher' and 'TetraThatcher' and absent in the other two pairs of wheats. With primer FVIIex8-c, the hexaploid varieties, 'Rescue' and 'Thatcher', and their derived tetraploids showed an extra intense band that was absent from 'Prelude' and 'TetraPrelude' (Fig. 1). Polymorphic bands were also observed among the hexaploid-tetraploid pairs with the other two primers (Fig. 1). Of the four primers used, primer FVIIex8-c revealed the most polymorphisms. No within-cultivar variation was detected using any of the primers when three to five individual plants from the same cultivar were analyzed (data not shown).

The DAMD-PCR technique detected polymorphisms within each of the hexaploid/tetraploid pairs (Fig. 1). Six intense bands that were present in all 3 hexaploid wheats, but were absent from their tetraploid counterparts, were observed independent of which primer was used. In each case, the amplified hexaploid-specific fragments were of the same molecular weight. According to Kaltsikes et al. (1969), each pair of the hexaploid/tetraploid wheats shared a common AABB genome. Therefore, the presence of extra DAMD-PCR product bands from the hexaploid wheats suggest that these amplified fragments originate from the D genome.

Reproducibility of DAMD-PCR was tested extensively by changing different parameters, such as quantity and quality of *Taq* DNA polymerase from different suppliers with the manufacturer's suggested buffers. Consistent amplification profiles were obtained throughout the study. Different annealing temperatures were used in the PCR amplifications for all four primers, and the results indicated that somewhat sharper bands with less background could be obtained at 60°C (data not shown).

The directed amplification of minisatellite-region DNA produced RAPD-like results. However, the high reaction stringencies in this PCR application greatly limited the amplification of artifacts which commonly occur with RAPDs. The results confirmed that DAMD-PCR with a single minisatellite core sequence primer was highly reproducible, as has been previously reported (Heath et al. 1993; Somers et al. 1995).

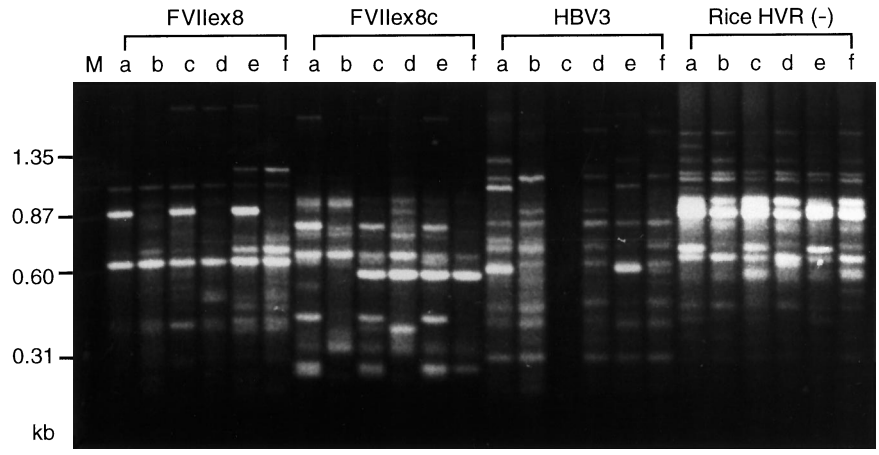

Fig. 1 Results of the directed amplification of minisatellite-region DNA in hexaploid and tetraploid extracted wheat. Molecular marker is identified in *lane M*. *Lane a* 'Prelude', *b* 'TetraPrelude', *c* 'Rescue', *d* 'TetraRescue', *e* 'Thatcher', *f* 'TetraThatcher'. No amplification occurred in *lane c* with the primer HBV3

DNA cloning and Southern hybridization of DAMD-PCR fragments

The DAMD-PCR-generated DNA fragments were screened for their potential as molecular markers in detecting restriction fragment length polymorphism (RFLP) among wheat varieties. Twenty-three PCR-amplified fragments, derived from utilizing different primers and genotypes, were used as probes to hybridize with genomic DNA of wild and cultivated wheat of different ploidy levels. The DAMD-PCR fragments detected various levels of polymorphism among the wheats analyzed. Some probes gave putative single-locus RFLP-like patterns, while the others yielded multi-locus, highly polymorphic RFLP-like patterns. The probes derived from DAMD-PCR fragments that were monomorphic usually produced similar hybridization patterns, and when used as probes on Southern blots of DAMD-PCR products, they were also found to cross-hybridize to each other between different genotypes. Of the 23 isolated DAMD-PCR fragments, 8 yielding different hybridization patterns were reamplified for further analysis (Table 1).

The cloned PCR fragments yielded similar hybridization patterns, but a lower non-specific background was observed as compared to that produced by probes prepared from the uncloned DAMD-PCR fragments. Clones pBC27.1, pBC27.3, pBC27.4 and pBC27.6, showed 1–5 bands and detected little polymorphism among the different cultivars. However, all of these clones exhibited polymorphism among different species and/or species with different ploidy levels. At least 1 polymorphic band could be found between the hexaploid wheats and their tetraploid derivatives. One example is shown by clone pBC27.4 which hybridized to 1 band in diploid wheat *T. tauschii* (Fig. 2, lane 5). The same-sized band was also present in the hexaploid wheats, which contain the D genome (Fig. 2, lanes 7, 8, 10, and 12). It was also interesting to note that pBC27.4, which was derived from hexaploid wheat

Table 1 Characteristics of the cloned DAMD-PCR fragments from wheat

Clone	Insert size (kb)	G + C content (%)[a]	Primer	DNA template
pBC27.1	0.87	–	FVIIex8	Prelude
pBC27.2	0.805	52.8	FVIIex8-c	Prelude
pBC27.3	0.304	52.3	FVIIex8-c	Prelude
pBC27.4	0.578	45.9	FVIIex8-c	Rescue
pBC27.5	0.43	51.9	FVIIex8-c	Rescue
pBC27.6	0.39	42.0	FVIIex8-c	TetraRescue
pBC27.7	0.6	–	HBV3	Thatcher
pBC27.8	0.672	50.0	Rice HVR(−)	Prelude

[a] Sequences of clones pBC27.1 and 27.7 were not determined

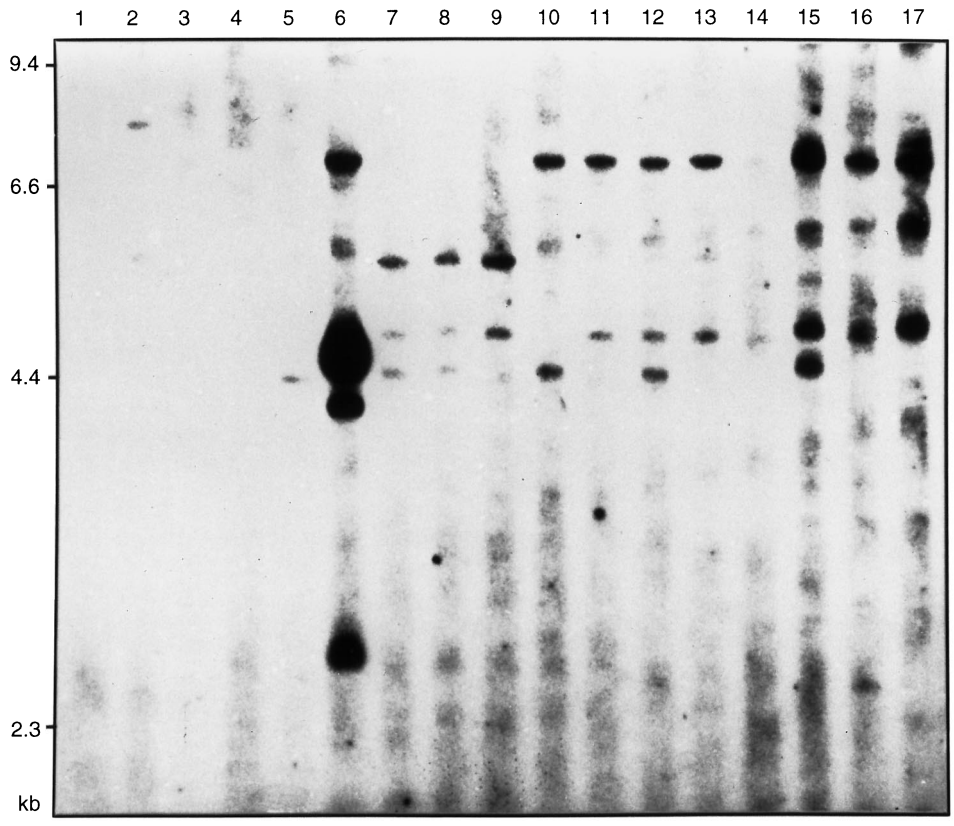

Fig. 2 Hybridization patterns detected by the probe pBC27.4 in *Dra*I digests of the genomic DNA from 17 genotypes of wheat and triticale. *Lanes 1–17 T. timopheevii*, *T. dicoccoides*, *T. urartu*, *T. speltoides*, *T. tauschii*, *T. durum*, 'Chinese Spring', 'Prelude', 'TetraPrelude', 'Rescue', 'TetraRescue', 'Thatcher', 'TetraThatcher', 'Pavon 76', 'Holdfast', 'Drira^{++}' 'Rosner^{++}'. One to two faint hybridization bands were visible on the original autorads on *lanes 1*, *3* and *4*. Washing stringency was 1 × SSC/0.1% SDS, 65°C

'Rescue' using primer FVIIex8-c (Table 1), hybridized strongly to 3 bands in tetraploid wheat (Fig. 2, lane 6). The sequence(s) homologous to pBC27.4 appeared to exist in much larger numbers in tetraploid wheat than in any other species. This might be due to the amplification of pBC27.4 in tetraploid wheat. However, the exact genome organization of pBC27.4 has not been characterized.

Other cloned DAMD-PCR fragments detected multiple, highly polymorphic loci and revealed cultivar-specific DNA fingerprint-like patterns. pBC27.5 hybridized to a single band of the genomic DNA from *T. tauschii* but to multiple fragments in all other wild species and cultivars (Fig. 3). High levels of polymorphism were detected between different wild species and among different cultivars. One to four polymorphic bands could be found between each pair of the hexaploid/tetraploid wheats (Fig. 3).

Among the cloned DAMD-PCR fragments, pBC27.2 revealed the most polymorphism between different wild species or cultivars. From 19 to 31 discrete fragments, ranging in size from 1 kb to 23 kb, were detected on the *Hin*dIII-digested genomic DNA of different genotypes of wheat and triticale (Fig. 4). Significant differences were observed not only between the hexaploid/tetraploid wheats, but also among the various cultivars, irrespective of ploidy level. High levels of variation between 2 triticales, 'Drira^{++}' and 'Rosner^{++}', were also detected (Fig. 4, lanes 16 and 17). Even greater

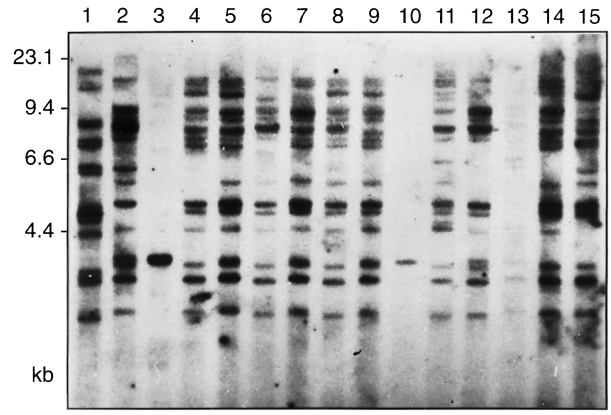

Fig. 3 Hybridization patterns detected by the probe pBC27.5 in *Dra*I digests of the genomic DNA from 14 genotypes of wheat and triticale. *Lanes* 1–15 *T. monococcum*, *T. durum*, *T. tauschii*, 'TetraPrelude', 'Prelude', 'TetraRescue', 'Rescue', 'TetraThatcher', 'Thatcher', *T. tauschii*, *T. dicoccoides*, 'Cocorit 75', 'Chinese Spring', 'Drira^{++}', 'Rosner^{++}'. Washing stringency was 1 × SSC/0.1% SDS, 65°C

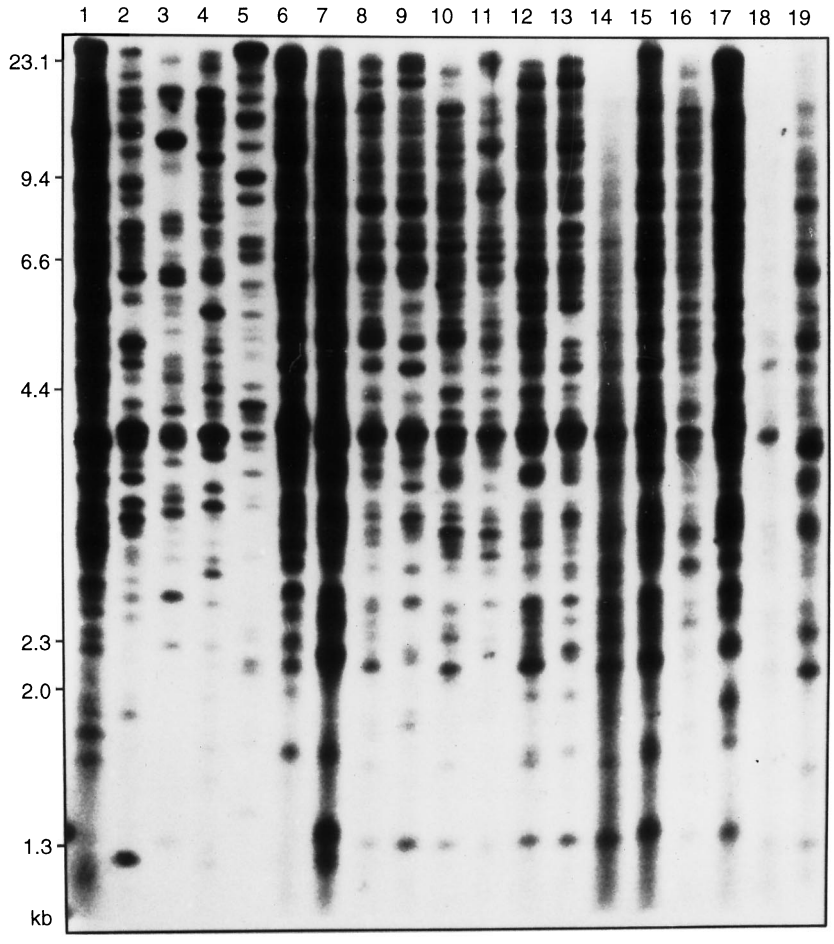

Fig. 4 Hybridization patterns detected by the probe pBC27.2 in *Hin*dIII digests of the genomic DNA from 18 genotypes of wheat and triticale. *Lanes 1–19 T. timopheevii*, *T. dicoccoides*, *T. urartu*, *T. speltoides*, *T. tauschii*, *T. durum*, 'Chinese Spring', 'Prelude', 'TetraPrelude', 'Rescue', 'TetraRescue', 'Thatcher', 'TetraThatcher', 'Pavon 76', 'Holdfast', 'Drira^{++}', 'Rosner^{++}', 'Marika', 'Chinese Spring'. The sample in *lane 19* was the same as in *lane 7* but only half amount of DNA was used in the restriction. DNA in *lanes 14* and *18* was degraded. Washing stringency was 0.1 × SSC/0.1% SDS, 65°C

Table 2 Sequences with high homology to pBC27.3

DNA sequences	Nucleotide sequence and region	Homology
Wheat pBC27.3	153 GAGGGACGTCACCGAGCTGAATGTGTGTAGATCGTGGAGGTGCCGTGCGTTCGATACT 96	79%
Wis 2-1A*	1512 GGGAGACGTCGTCGAGCTGTACGTGTGTTGAACGCGGAGGTGCCGTCCGTTCGGCACT 1569	
Wheat pBC27.3	188 AAACTCTCCCTCGGCCTCAGCTGGATCAAG 159	76%
Wis 2-1A*	1478 AACCTCTCCCTCTCTCCTTGCTGGATCAAG 1507	
Wheat pBC27.3	82 CGCGAAGACGTTCGACTACATCAACCGCGTTACTAAATGCTTACGCTTT 34	79%
BARE-1†	12236 CGTGAGGACGTTCCACTACATCAACCGCGTGTATTAACGCTTCTGCTGT 12284	
Wheat pBC27.3	79 GAAGACGTTCGACTACATCAACCGCGTTACTAAA 46	85%
BARE-1†	1981 GAGGACGTTCCACTACATCAACCGCGTTTATTAA 2014	

* *T. aestivum* Wis 2-1A retrotransposon-like element (Lucas et al. 1992)
† H. vulgare DNA for BARE-1 copia-like retroelement (Mannien and Schulman 1993)

polymorphism levels were found among the different wild wheat species (Fig. 4, lanes 1–5). With other enzymes, such as *Dra*I and *Hin*fI, pBC27.2 also detected multiple, highly polymorphic RFLPs among all wild wheat species and cultivars studied. Each of the wheat species and cultivars showed an individual-specific DNA fingerprinting profile.

Like pBC27.2, clones pBC27.7 and pBC27.8 also detected multiple, highly polymorphic RFLPs on Southern blots and revealed individual-specific hybridization patterns not only for each of the wild species but also for the wheat cultivars and the 2 triticales (data not shown). However, the polymorphism detected by pBC27.8 was relatively low among the wheat cultivars as compared to pBC27.2 or pBC27.7. Clone pBC27.8 detected 11 bands, on average, but few were polymorphic among the different cultivars within a species. Nevertheless, the results clearly demonstrated that DAMD-PCR could be an efficient tool for the isolation of informative molecular markers from wheat genomes. Out of 8 cloned DAMD-PCR fragments, 4 have potential use for DNA fingerprinting wheat.

All clones derived from hexaploid-specific DAMD-PCR fragments also hybridized to at least 1 band of genomic DNA from the tetraploid (AABB), or diploid wheats (AA, or BB) using a high stringency ($0.1 \times$ SSC, 0.1% SDS, 65°C) wash (Figs. 2, 3). This suggested that the DAMD-PCR fragments which appeared to be hexaploid-specific were not D genome-specific sequences. We noted that all hexaploid-specific DAMD-PCR fragments also hybridized to all of the extracted tetraploid cultivars, and to the other diploid species. This suggested that sequences homologous or homoeologous to those hexaploid-specific DAMD-PCR fragments were present in the AA or BB genomes but were not amplified by PCR. However, DAMD-PCR was quite capable of revealing D genome-specific polymorphism (Fig. 1), and the hexaploid-specific PCR fragments probably came from the D genome of hexaploid wheat. During the evolutionary process, the homologous or homoeologous sequences which exist in the other genomes may have lost the DAMD primer site(s), and therefore only the sequences present in the D genome were amplified by PCR.

Characterization of cloned DAMD-PCR fragments

The length of the inserts and their nucleotide composition of 6 cloned DAMD-PCR fragments are given in Table 1 (complete sequencing data are available on request). One of the clones, pBC27.2 was found to have two additional short repeat sequences which were distinct from the primer FVIIex8-c. Another clone, pBC27.4, contained a short microsatellite $(GA)_8$ sequence. However, no minisatellite tandem repeat array was found within any of the 6 DAMD-PCR clones. Similar results have been reported in fish (Heath et al. 1993) and in wheat and rice (Somers et al. 1995, Zhou et al. 1997). It is believed that DAMD-PCR amplifies the regions containing minisatellite sequences (Heath et al. 1993), but the amplified fragments will not always contain tandemly repeated arrays. Most likely, a single-copy DNA region adjacent to (flanking) a minisatellite was amplified; this would occur if a local inversion or other rearrangement was present between two inverted repeat core sequences (Heath 1993).

The DNA nucleotide sequence database was searched for sequences similar to the cloned DAMD-PCR fragments. Three clones, pBC27.2, pBC27.5 and

Table 3 Summary of the data analysis of wheat DNA fingerprints

	Probe/enzyme combination		
	pBC27.2/*Dra*I	pBC27.2/*Hin*dIII	pBC27.7/*Dra*I
No. of bands per accession			
Average N ± Standard deviation (SD)	23.0 ± 2.8	31.1 ± 4.0	20.0 ± 4.8
Range in N	15–27	25–37	15–27
Proportion of matching bands (R)*			
Average R ± SD	0.48 ± 0.12	0.48 ± 0.11	0.52 ± 0.15
Range in R	0.27–0.88	0.26–0.78	0.29–0.86
Probability of two accessions matching (*P*)			
$P = R^N$	4.66×10^{-8}	3.89×10^{-12}	2.09×10^{-6}

* Proportion of matching bands was calculated based on Rogers and Tanimoto's coefficient (Sneath and Sokal, 1973)

pBC27.7, showed no significant homology to any known sequences. However, clones pBC27.3, pBC27.4 and pBC27.6 had various degrees of similarity with a number of known sequences. Clone pBC27.3 showed 79% and 76% homology with two segments of the *T. aestivum* Wis 2-1A retrotransposon-like element (Lucas et al. 1992) (Table 2). Clone pBC27.3 also showed 79% and 85% homology with two regions in the BARE-1 *copia*-like retroelement of *Hordeum vulgare* (Manninen and Schulman 1993) (Table 2). This suggested that pBC27.3 may be part of a retrotransposon-like element in wheat var 'Prelude'. Whereas, pBC27.4 showed an average 68% of homology with a number of known sequences from animals and plants, most of the similarity was due to the short microsatellite motif in pBC27.4 (data not shown). Clone pBC27.6 showed 86% of homology (283 bp overlapped) with a wheat glutathione-S-transferase (*gst*) gene (Mauch et al. 1991). The significant similarity between these two sequences suggested that pBC27.6 may be part of the *gst* gene. Three clones that showed no homology with other known sequences yielded multiple-banding patterns on Southern blots (Figs. 3 and 4). These DAMD-PCR-generated fragments might be associated with dispersed, repeated sequences in the wheat genome. The remaining 3 clones may represent single-copy or coding DNA owing to the low number of hybridization bands observed on Southern blots (Fig. 2).

Analysis of wheat DNA fingerprints

The DNA fingerprints were analyzed from the hybridization patterns of restricted genomic DNA with the DAMD-PCR clones, pBC27.2 with *Hin*dIII (Fig. 4), pBC27.2 with *Dra*I and pBC27.7 with *Dra*I (pictures not shown). Since clone pBC27.5 detected only 1 band in *T. tauschii* (Fig. 3) and clone pBC27.8 revealed relatively low levels of polymorphism between different wheat cultivars, they were not analyzed. pBC27.2 detected an average of 31 and 23 fragments on the autorads with the restriction enzymes of *Hin*dIII and *Dra*I, respectively (Table 3). Clone pBC27.7 revealed 20 bands with *Dra*I (Table 3). An average similarity of 49.3% was observed among the genotypes studied with these 2 probes and two enzyme combinations (Table 3). On the basis of these data, the probability of two individuals having the same fingerprints and all bands are independently inherited is $4.66 \times 10^{-8} \times 3.89 \times 10^{-12} \times 2.09 \times 10^{-6} = 3.79 \times 10^{-25}$. The results indicated that using enzymes *Hin*dIII and *Dra*I, theoretically up to 10^{25} wheat genotypes, similar to those analyzed in the present study, could be distinguished using probes pBC27.2 and pBC27.7. However, the above genotype number will be drastically decreased when the cultivars analyzed would be more closely related. Beckmann and Soller (1986) estimated that at least 20–30 single-copy RFLP markers may be required to differentiate large numbers of maize cultivars. Liu et al. (1992) reported that a moderately repeated wheat DNA probe which detects an average 10.4 bands per individual could identify 56 bread wheat cultivars with at least three enzymes. In the present study, pBC27.2, or pBC27.7, detected at least 15 fragments in each individual (Table 3) and yielded extremely variable hybridization patterns among the genotypes analyzed. Our results clearly demonstrated that pBC27.2 and pBC27.7 can be used for DNA fingerprinting wheat germplasm.

Conclusions

We have demonstrated that DAMD-PCR can be a reliable technique for detecting high levels of DNA polymorphism in hexaploid and tetraploid wheat cultivars and related diploid species. Without the massive requirement of screening a genomic library, DAMD-PCR is a simple and efficient tool for isolating informative molecular markers from wheat. The results clearly demonstrate that the cloned DAMD-PCR fragments pBC27.2 and pBC27.7 can be used in DNA

fingerprinting for wheat germplasm identification. Analyzing the wheat DNA fingerprints would enable one to study the genetic relationship between different species and cultivars. Even though the numbers of species, cultivars and accessions used in the study were limited, the fingerprinting probes detected extensive variability between individuals and were shown to be more informative than available single-copy RFLP markers. DNA fingerprinting of more wheat cultivars with DAMD-PCR clones is currently in progress. This technique can yield species-specific profiles as well as genome-specific profiles and can reliably detect polymorphism and differentiate among wheat germplasm.

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