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Direct amplification of minisatellite-region DNA with VNTR core sequences in the genus *Oryza*

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Abstract A polymerase chain reaction (PCR) application, involving the directed amplification of minisatellite-region DNA (DAMD) with several minisatellite core sequences as primers, was used to detect genetic variation in 17 species of the genus *Oryza* and several rice cultivars (*O. sativa* L.). The electrophoretic analysis of DAMD-PCR products showed high levels of variation between different species and little variation between different cultivars of *O. sativa*. Polymorphisms were also found between accessions within a species, and between individual plants within an accession of several wild species. The DAMD-PCR yielded genome-specific banding patterns for the species studied. Several DAMD-PCR-generated DNA fragments were cloned and characterized. One clone was capable of detecting multiple fragments and revealed individual-specific hybridization banding patterns using genomic DNA from wild species as well as rice cultivars. A second clone detected only a single polymorphic locus, while a third clone expressed a strong genome specificity by Southern analysis. The results demonstrated that DAMD-PCR is potentially useful for species and genome identification in *Oryza*. The DAMD-PCR technique also allows for the isolation of informative molecular probes to be utilized in DNA fingerprinting and genome identification in rice.

Key words *Oryza* · Rice · PCR · DNA fingerprinting · Minisatellite

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Introduction

The polymerase chain reaction (PCR) is well established as an efficient tool for the amplification of specific sequences of genomic DNA. With arbitrary primers, the random amplification of polymorphic DNA (RAPD) has been used in a wide range of plant and animal species for genetic mapping and gene tagging (Martin et al. 1991; Rafalski et al. 1991), for parentage determination (Welsh et al. 1991), and for species identification (Arnold et al. 1991; Chapco et al. 1992). The advantage of using RAPDs in genetic analysis is cost-efficiency (Ragot and Hoisington 1993). The procedure requires little DNA (Welsh et al. 1991) and allows the use of simple procedures for the isolation of genomic DNA (Williams et al. 1990). However, one drawback of using short (10-base), random primers in RAPDs is the low stringency necessary for successful amplification. This can and does lead to

non-reproducible results, which severely limit the application of RAPDs in plant and animal genome analyses.

Minisatellites, also called hypervariable repeats (HVR) and/or variable number of tandem repeats (VNTR), are tandem repeat DNA sequences which generally consist of 10–60-bp motifs. Regions containing minisatellites usually show high levels of restriction fragment length polymorphism (RFLP). Extreme variation in the tandem repeat copy number of minisatellite loci is considered to be the source of the observed polymorphisms in humans (Jeffreys et al. 1985). To-date, a number of minisatellite sequences have been identified in humans (Jeffreys et al. 1985) and in a variety of animal and plant species (Jeffreys and Morton 1987; Broun and Tanksley 1993; Winberg et al. 1993; Tourmente et al. 1994). Most of the minisatellites share a common motif known as the core sequence (Wong et al. 1986; Nakamura et al. 1988). Studies have shown that minisatellite loci are inherited in a Mendelian fashion and are dispersed throughout the genome in many species, including mouse (*Mus musculus* L.) (Julier et al. 1990), bovine (*Bos taurus* L.) (Georges et al. 1991), tomato (*Lycopersicon esculentum* Mill.) (Broun and Tanksley 1993), and rice (*Oryza sativa* L.) (Gustafson, unpublished data). This makes minisatellites attractive candidates as DNA fingerprinting probes.

Recently, Heath et al. (1993) reported a new technique, called direct amplification of minisatellite-region DNA (DAMD), which uses PCR to direct the amplification to regions rich in minisatellite repeats. By using the core sequence of minisatellites as a single primer, this PCR application is capable of producing RAPD-like results for the identification of specific regions in a genome. It has been speculated that if a portion of a minisatellite array is involved in an inversion this would make PCR possible using a single minisatellite core sequence as a primer. If inversions have taken place, then the repeats would most likely have a piece of single-copy flanking DNA inserted between them. Therefore, DAMD could also amplify sequences formerly adjacent to hypervariable minisatellite loci (Heath et al. 1993). Furthermore, since minisatellite core sequences are longer than RAPD primers, DAMD-PCR can also be effectively carried out at relatively high stringencies, thus yielding highly reproducible results (Heath et al. 1993).

The purpose of the current study was to utilize DAMD-PCR in order to detect polymorphisms among species and cultivars within the genus *Oryza*. A few DAMD-generated DNA fragments were isolated and cloned. The DNA sequences were characterized, and subsequent Southern analysis of wild and cultivated rice was carried out. The potential use of DAMD-PCR for rice species identification and genetic analysis will be discussed.

Materials and methods

Plant materials and DNA extraction

The plant material used in this study included 20 accessions representing 17 species of the genus *Oryza* from the International Rice Research Institute (IRRI, Manila, Philippines) and 19 varieties of cultivated rice from the China National Rice Research Institute (CNRRI, Hangzhou, P.R. China) (Table 1). A species formerly classified as *Oryza* (Tateoka 1963), *Rhynchoryza subulata* Baillon, was also analyzed. Leaf material was harvested from one to four plants of each accession grown in a greenhouse, freeze-dried, and ground to a fine powder with a Tekmar sample mill (Tekmar Co., Ohio, USA). Total genomic DNA was extracted following the method of Saghai-Maroof et al. (1984).

Primers and polymerase chain reaction

Primers were synthesized based on the following minisatellite core sequences: 33.6 (GGAGGTGGGCA) (Jeffreys et al. 1985), HBV5 (GGTGTAGAGAGGGGT) (Nakamura et al. 1987), and rice HVR(−) (CCTCCTCCCTCCT) (Winberg et al. 1993). The PCR was performed in a Perkin-Elmer thermal cycler (Model 480, Perkin-Elmer Corp., Conn., USA). Each reaction contained 40 pmole of one of the primers, 250 μM of each dNTP, 1.0 unit of *Taq* DNA polymerase (Boehringer Mannheim, Indiana, USA), 50 mM KCl, 10 mM Tris (pH 8.3), 2.5 mM $MgCl_2$, and 100 ng of template genomic DNA in a total volume of 20 μl. Reactions were covered with mineral oil and run for one cycle of 95°C for 2 min, 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min, and one cycle of 72°C for 5 min. The reaction products were then fractionated on 2.0% (w/v) agarose gels for 3–4 h at 100 V in 1 × TBE and visualized with ethidium bromide staining.

PCR fragment selection and hybridization

Individual DAMD-PCR DNA fragments which were abundant and/or polymorphic between the tested genotypes were re-amplified using the band-stab method (Bjourson and Cooper 1992) under the same conditions described above. This method preserves the PCR fragment in a small gel plug at −20°C. The resulting re-amplification reactions were electrophoresed on 1.5% (w/v) low-melting-point agarose, and the fragments were purified using β-Agarase I following the procedure recommended by the supplier (New England Biolabs, Massachusetts, USA). The isolated fragments were labeled with β-^{32}P-dCTP by the random primer method (Feinberg and Vogelstein 1983) for Southern analysis. Genomic DNA (10 μg) from each genotype (Table 1) was digested with *Dra*I or *Hin*dIII under conditions recommended by the supplier (Promega, Wisconsin, USA). Electrophoresis, Southern blotting, hybridization, and autoradiography were carried out as described previously (Zhou and Gustafson 1995).

DNA cloning and sequencing

The DAMD-PCR fragments that appeared to detect highly variable loci among the tested genotypes by Southern analysis were selected for cloning and characterization. The DAMD-PCR bands corresponding to these fragments were re-amplified from the gel plugs (Bjourson and Cooper 1992) under the same conditions described above. The re-amplified DNA fragments were blunt-ended with T4 DNA polymerase, following the procedure of Wang et al. (1994), and

Table 1 List of accessions from the genus *Oryza* and rice cultivars (*O. sativa*) included in study

Sample	IRRI accession	Species or cultivar name or type	Genome	Origin[a]
1	101395	*O. alta* Swallen	CCDD	South America
2	100122	*O. barthii* A. Chev.	AA	Gambia
3	101231	*O. brachyantha* A. Chev. et Roehr.	FF	West Africa
4	101422	*O. eichingeri* A. Peter	CC	Uganda
5	102201	*O. glaberrima* Steud	AA	West Africa
6	101405	*O. grandiglumis* Prod.	CCDD	Brazil
7	100962	*O. latifolia* Desv.	CCDD	Guatemala
8	101378	*O. longistaminata* A. Chev et Roehr.	AA	Mali
9	100957	*O. malampuzhaensis* Krish. et Chand	BBCC	India
10	101147	*O. meridionalis* Ng	AA	Australia
11	101089	*O. minuta* J. S. Presl	BBCC	Philippines
12	101508	*O. nivara* Sharma et Shastry	AA	India
13	100896	*O. officinalis* Wall.	CC	Thailand
14	101409	*O. punctata* Kotschy	BB, BBCC	Ghana
15	100821	*O. ridleyi* Hook f.	Tetraploid	Thailand
16	103831	*O. sativa* f. spontanea Roschev.	AA	Bangladesh
17	100913	*Rhynchoryza subulata* Baillon	?	South America
18	100137	*O. glaberrima*	AA	West Africa
19	100963	*O. latifolia*	CCDD	Guatemala
20	101973	*O. nivara*	AA	?
21	PI 271672	Taichung Native 1 (TN1), Japonica		Taiwan
22	PI 312627	IR8 (IR 8–288–3), Indica		Philippines
23	–	Ai-Zai-Zhan, Indica		China
24	–	Tai Hei, Japonica		China
25	–	Dular, Indica		India
26	–	Ai-Ma-Bai-Gu, Indica		China
27	–	Yuan-Feng-Zhao, Indica		China
28	–	Zhen-Zu 19 (Nuo), Indica		China
29	–	IR29, Indica		Philippines
30	–	China 91, Japonica		China
31	–	Suwon 283, Indica		Korea
32	–	Pecos, Indica		USA
33	–	La-Ai 64, Indica		China
34	–	Qiu-Guan, Japonica		China
35	–	Zhen-Zu 19, Indica		China
36	–	Dourado Precoce, Indica		Brazil
37	–	Bellemont, Indica		USA
38	–	Ketan NangKa, Javanica		?
39	–	Lun-Hui 422, Japonica		?
40	–	Guan-Lu-Ai 4, Indica		China
41	–	T-986, Japonica		China
42	–	Xue-He-Ai-Zhao, Indica		China
43	–	02428, Japonica		China
44	–	Zhe-Hu 114, Japonica		China
45	–	CPSLO 17, Javanica		USA
46	–	3264, Japonica		China
47	–	CPSLO 19, Javanica		USA
48	–	IR58, Indica		Philippines
49	–	Ai-Liao-Nan-Te, Indica		China
50	–	Dee-Gee-Woo-Gen, Indica		Taiwan
51	–	Yuan-Feng-Zhao, Indica		China
52	–	Nan-Te-Hao, Indica		China
53	–	Zhao-Sha-Keng, Japonica		China
54	–	Nanjing 11, Indica		China
55	–	Gui-Hua-Huang, Indica		China

[a] ?, information not available

cloned into the pBluescript II KS(−) vector (Stratagene Cloning System, Calif., USA). Cloned inserts were used in Southern hybridization as described above. Double-stranded DNA was prepared from clones and subsequently sequenced in both directions with T3 and T7 primers using a DyeDeoxy Terminator Cycle Sequencing Kit (Perkin-Elmer Corporation). Sequence comparison with the DNA database was performed using BLAST (Altschul et al. 1990).

Results and discussion

Polymorphism among species in the genus *Oryza* and cultivated rice with DAMD-PCR

There were from two to nine clearly distinguishable bands amplified by DAMD-PCR in the different *Oryza*

species; several bands were observed to be genome-specific. Significant differences were revealed among the 17 *Oryza* species with the rice HVR(−) primer (Fig. 1 A). Highly polymorphic banding patterns were also observed with the primers 33.6 and HBV5. Since the PCR reactions were run at high stringency, DAMD-PCR yielded highly reproducible results with all the primers used in all genotypes tested.

The DAMD-PCR profiles also revealed variation between accessions within a species and among individual plants of the same accession (Fig. 1 B). In addition, within an accession different primers yielded different degrees of variation. *Oryza nivara* Sharma et Shastry (Fig. 1 B; lanes 13–18) showed an extreme example of such within-species polymorphism where more than one polymorphism was observed between the two accessions (101508 and 101973). At least one polymorphic fragment was found among the individual plants of *O. nivara* (Fig. 1 B). Wang et al. (1992) reported that 23% of the rice accessions they analyzed were found to have within-species variation based on an analysis using 15 RFLP markers. Due to a limited sampling size, we were unable to determine the exact percentage of polymorphic accessions with DAMD-PCR. However, our observations suggested that DAMD-PCR, using minisatellite core sequences as primers, was sensitive in detecting genetic variation between *Oryza* species and between accessions of a single species.

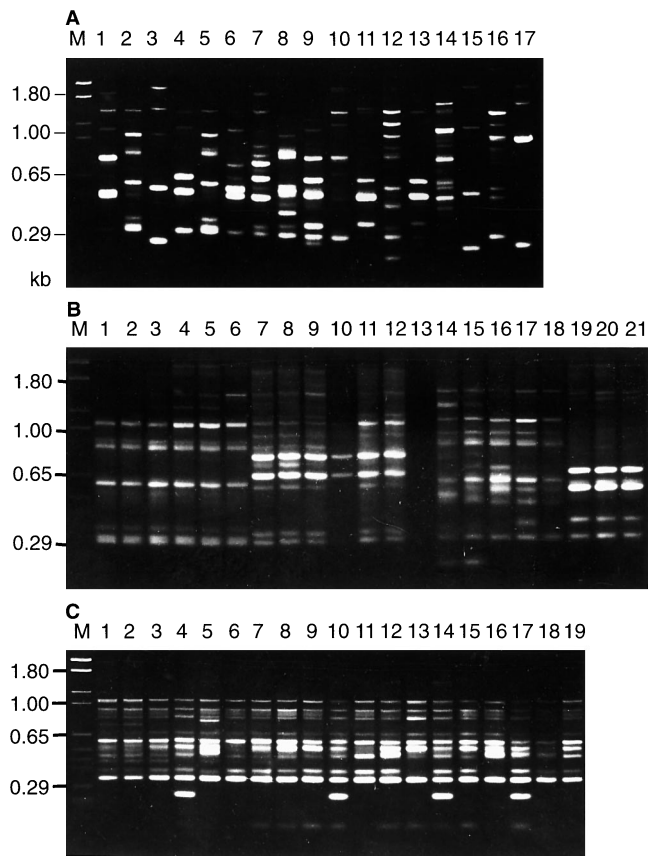

Fig. 1A–C Results of the directed amplification of minisatellite-region DNA. **A** Amplification with primer rice HVR(−) in *Oryza* species, *lanes 1–17* correspond to the samples 1–17 (Table 1). **B** Amplification with primer 33.6 in individual plants of different *Oryza* species: *lanes 1–3 O. glaberrima* (102201); *4–6 O. glaberrima* (100137); *7–9 O. latifolia* (100962); *10–12 O. latifolia* (100963); *13–15 O. nivara* (101508); *16–18 O. nivara* (101973); *19–21 O. officinalis* (100896). No amplification occurred in *lane 13*. **C** Amplification with primer 33.6 in *O. sativa* cultivars, *lanes 1–19* correspond to the samples 21–39 (Table 1). The molecular marker is identified in *lane M*

Species classified as having the same genome constitution had more bands in common than they shared with other species. Species with the AA genome (*O. barthii* A. Chev., *O. glaberrima* Steud., *O. longistaminata* A. Chev. et Roehr., *O. meridionalis*, and *O. sativa* f. spontanea Roschev.; Fig. 1 A; lanes 2, 5, 8, 10, and 16, respectively) shared two similar-sized DAMD-PCR fragments (0.31 and 1.5 kbp). Species containing the CCDD genomes (*O. alta* Swallen, *O. grandiglumis* Prod., and *O. latifolia*; Fig. 1 A; lanes 1, 6, and 7, respectively), shared two bands in common (0.51 and 0.78 kbp). Although it is not known if the common fragments amplified were from the same loci in the different species, our results were consistent with other morphological, cytogenetic, and molecular studies on *Oryza* (Hu 1970; Second 1982; Wang et al. 1992).

Limited polymorphism was revealed by DAMD-PCR among 19 rice cultivars using the primer 33.6 (Fig. 1 C). Similar results were also found in the PCR profiles using the two other primers (data not shown). It appears that DAMD-PCR alone may be somewhat limited in its ability to detect genetic variation among cultivated rice varieties (within the species *O. sativa*). This would most likely be due to the more narrow genetic base among rice cultivars coming from a single breeding program as compared to rice species, and to the restricted number of loci amplified by DAMD-PCR.

Southern hybridization with cloned DAMD-PCR fragments

When DAMD-PCR-amplified fragments were hybridized to genomic DNA from different wild species and cultivated rice varieties, the hybridization patterns showed: (1) multi-locus, highly polymorphic bands, (2) single-locus, polymorphic banding patterns, and (3) strong genome specificity. Two cloned DAMD-PCR fragments, clones pOl 5.1 and pOm 6.1, were particularly interesting in revealing polymorphisms among the accessions of wild rice species and cultivars of *O. sativa* (Figs. 2, 3, 4).

Clone pOl 5.1 was derived from *O. latifolia* with the primer HBV5, and detected multiple fragments both in the genomic DNA of wild rice species and cultivars of *O. sativa* (Figs. 2, 3). Clone pOl 5.1 was also derived

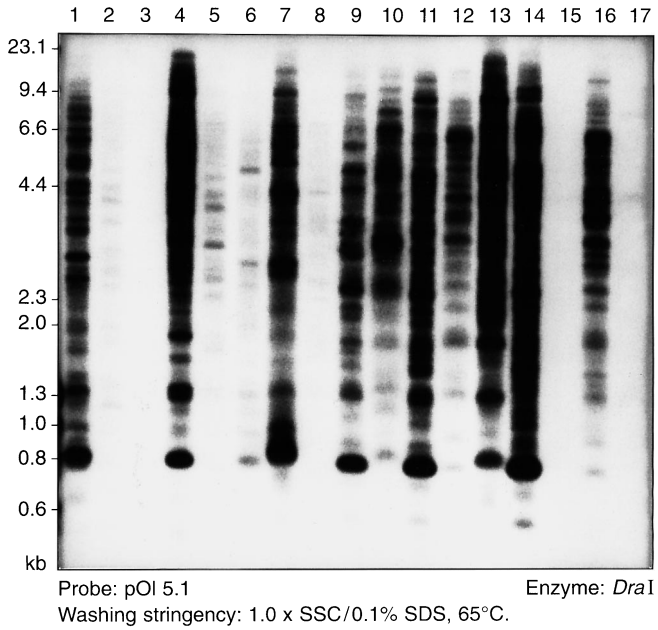

Fig. 2 Hybridization patterns detected by the probe pOl 5.1 in *Dra*I digests of genomic DNA from *Oryza* species. *Lanes 1–17* correspond to the samples 1–17 (Table 1). One-to-three faint hybridization bands were visible on the original autoradiogram in *lanes 3*, *15*, and *17*. Washing stringency was 1 × SSC/0.1% SDS, 65°C

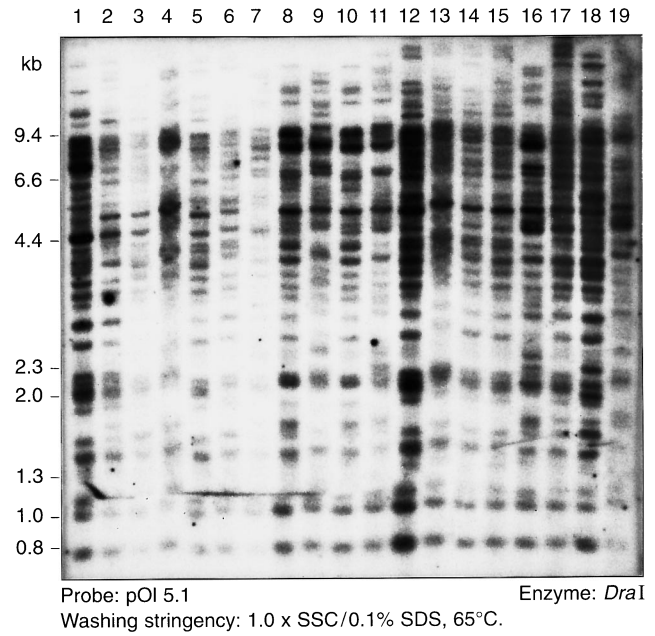

Fig. 3 Hybridization patterns detected by the probe pOl 5.1 in *Dra*I digests of genomic DNA from *O.sativa* cultivars. *Lane 1* TN1, *lane 2* IR 8, *lanes 3–19* correspond to the samples 40–55 (Table 1). Washing stringency was 1 × SSC/0.1% SDS, 65°C

from *O. latifolia* and exhibited variable homology to the other wild species. This probe strongly hybridized to AA, BBCC, CC, CCDD genomes but not to the FF genome. The cultivated rice varieties exhibited approximately 30 detectable fragments at the 1 × SSC, 65°C stringency level (Fig. 3). The restriction patterns differed among all cultivars studied, and at least one polymorphic fragment could be found even among closely related cultivars. Although the electrophoresis profiles from DAMD-PCR products alone are limited in detecting DNA polymorphisms between rice cultivars, our results demonstrated that the DAMD-PCR-generated clone pOl 5.1 was an informative molecular marker and has the potential for DNA fingerprinting in both *Oryza* species (Fig. 2) and cultivars (Fig. 3).

Clone pOm 6.1 was derived from *O. meridionalis* with the primer 33.6. A single locus with a high level of polymorphism (six alleles) was detected among the 19 rice cultivars surveyed (Fig. 4). It is interesting to note that clone pOm 6.1 revealed null alleles in both Indica and Japonica cultivars (Fig. 4; lanes 7, 9, 11, 16, and 19). Under low-stringency conditions (1 × SSC, 65°C), clone pOm 6.1 was found to hybridize readily with 2–3 fragments in all cultivars tested (data not shown), while at high-stringency levels (0.1 × SSC, 65°C), it did not hybridize at all, or else only very faintly, to some of the cultivars (Fig. 4; lane 17).

It is not surprising that the locus detected by pOm 6.1 was absent from some cultivars, since it was derived from the wild rice species *O. meridionalis*. Similar results for RFLPs have been reported (McCouch et al. 1988) where probes derived from an Indica library show null alleles in Japonica.

One of the clone ligation reactions, involving *O. brachyantha* A. Chev. et Roehr. and using primer 33.6, from six transformants screened, yielded clones pOb 6.1.1 and pOb 6.1.2 which showed different hybridization patterns on genomic DNA of wild rice species (Fig. 5 A, B). Clone pOb 6.1.1 hybridized to a single band in *O. brachyantha* and *O. ridleyi* Hook f. (Fig. 5 A), and pOb 6.1.2 detected one to two major bands in all species examined (Fig. 5 B). Minisatellites are found to be dispersed throughout the genomes of many species (Julier et al. 1990; Georges et al. 1991; Broun and Tanksley 1993; Gustafson, unpublished data). We suggest that similar-sized amplicons from different loci comigrated to the same position during electrophoresis and were subsequently cloned. The sequences of clones pOb 6.1.1 and pOb 6.1.2 were not determined. Multiple ligation products occurred in a concurrent study with

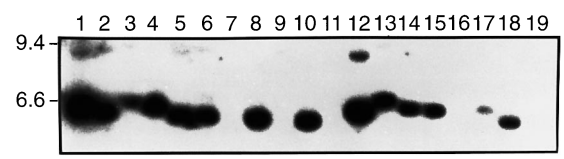

Fig. 4 Restriction patterns detected by the probe pOm 6.4 in *Hin*dIII digests of genomic DNA from *O. sativa* cultivars. *Lanes 1–19* correspond to the samples 21–39 (Table 1). A null allele was detected in *lanes 7*, *9*, *11*, *16*, and *19*. Washing stringency was 0.1 × SSC/0.1% SDS, 65°C

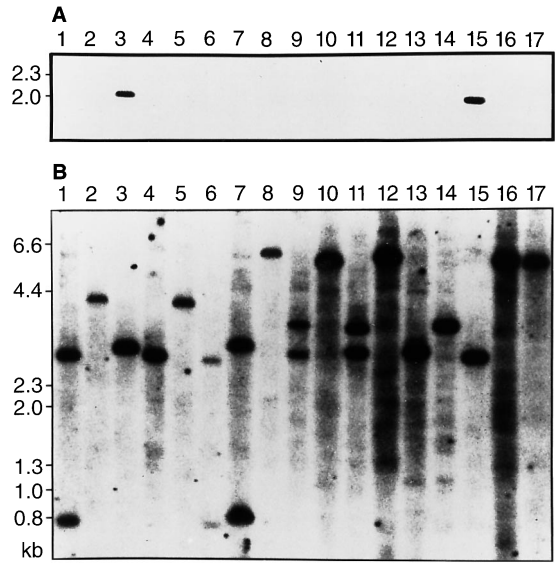

Fig. 5A, B Hybridization patterns detected on the same blot by the probes pOb 6.1.1 **A** and pOb 6.1.2 **B** in *Dra*I digests of genomic DNA from *Oryza* species. *Lanes 1–17* correspond to the samples *1–17* (Table 1). Washing stringency was 1 × SSC/0.1% SDS, 65°C in both hybridizations

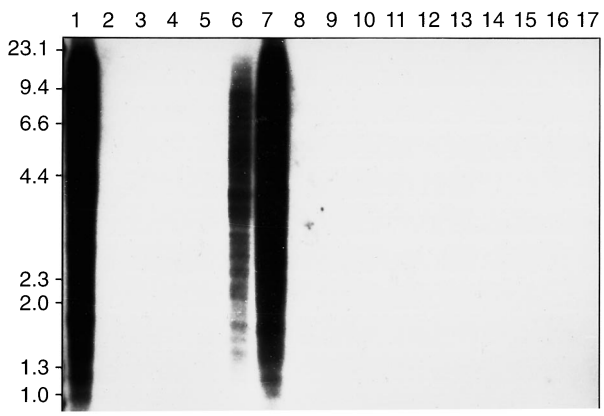

Fig. 6 Hybridization patterns detected by the probe pOa 6.4 in *Dra*I digests of genomic DNA from *Oryza* species. *Lanes 1–17* correspond to the samples 1–17 (Table 1). Species of the CCDD genomes are: in *lane 1 O. alta*, *lane 6 O. grandiglumis*, and *lane 7 O. latifolia*. Washing stringency was 0.1 × SSC/0.1% SDS, 65°C (discrete bands in *lanes 1* and *7* were clearly visible on the original autoradiogram)

wheat (*Triticum aestivum* L. em Thell.) where sequencing demonstrated that there were two different amplicons (Somers et al. 1996).

To isolate genome-specific DNA sequences, several DAMD-PCR-generated fragments were isolated and used as probes to hybridize with the genomic DNA from different rice species. One of the DAMD-PCR-generated fragments derived from *O. alta* (CCDD genome) showed no cross-hybridization with any species containing other genomes (Fig. 6). The fragment was cloned and designated pOa 6.4 for further characterization.

Hybridization with pOa 6.4 detected multiple fragments in the species containing the CCDD genomes (*O. alta*, *O. grandiglumis*, and *O. latifolia*). These three species showed genome-specific banding patterns (data not shown). In order to test whether pOa 6.4 would cross-hybridize to less-homologous DNA sequences in species not containing the CCDD genomes, low-stringency washing (1 × SSC and 65°C) and prolonged autoradiography exposure time were employed. At low stringency, the pOa 6.4 probe hybridized readily only to DNA of the species containing the CCDD genomes, and showed smears on the lanes of *O. alta*, *O. grandiglumis*, and *O. latifolia* DNA with a 4-h exposure. A 7-day exposure (the normal exposure for this experiment was 4 h), detected one to two very faint bands in some species [*O. meridionalis* Ng (AA), *O. nivara* Sharma et Shastry (AA), *O. officinalis* Wall. (CC), and *O. sativa* f. spontanea Roschev. (AA), data not shown]. Thus, under normal conditions, pOa 6.4 did not cross-hybridize with any other wild rice species except CCDD tetraploids. Our results suggested that pOa 6.4 is a genome-specific DNA sequence having originated from either the C or D genome of *O. alta*. We might speculate that pOa 6.4 was derived from the DD genome since BBCC and CC species showed no homology. A DD-genome species is not available for the definitive test. The hybridization of pOa 6.4 to fragments with a wide range in size (from 0.8 kb to >9.6 kb) suggested that it is indeed a repetitive sequence.

Nucleotide sequences of DAMD-PCR fragments

The nucleotide sequences of clones pOl 5.1 and pOm 6.1 are 760 bp and 345 bp, respectively. As anticipated, the minisatellite core sequences (priming sequences)

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1    GGTGTAGAGAGGGGTGAATAGGTATTTCTTAAAATTTAAAAAATTCATTT   50
51   GCTGCGGATTAGTAGCACCCGGAATCTCCGGGCTTGGGACACCGAAGTCC  100
101  TCGGGCTAGTTAGGAAAAAATGCCCTTCAAAGCGGAGACAAACCACTAGC  150
151  AAAAAATCTACTTGAACTTGACAAACAAGTTACATAAGCAATATATATAG  200
201  TCACCAACTAGATCAAACAAGCATACAAAGCAGATCGGGCAAGATAAGTA  250
251  TTAATAACGATAAATGCACAAGAGAGAGAGCGGAGACAAAATTTGGTTTC  300
301  CCGAAGTCAGATTCTCCACCAAAGATCCTACGTCTTCGTTGAGAAAGCTC  350
351  CTAAGGAACCGTTTCTCTTTCATCCACTTTCCTCACCCAGATWTCAACCA  400
401  CGAGAAGTCTWGGTTTTTGGAGGACTACTAGATTAGTAAGTCTCCAAGCG  450
451  ACCTAAGGGTCCAAGCTTGGACGGACGAGCCTCGATCCACCGGTGAAGCT  500
501  TTGTCTWCCACGCAACCTCCTAGAAGATAGCTGGGTTTCCWSTWAATTAC  550
551  CTCTTTTCTTGTGGAGGTGTGGGTACCTTCACAAATTTCCCGCGGYTCAC  600
601  CACACTCTTGGGAGCTAACTGGCAACGCTTTTCCGTCTAGGAGGCTCAAC  650
651  CTCCAAGAGTAACAAACACAATCGAAGATTTGCTTGCTATGAACCCAAGT  700
701  ACTCAGATTTGAGTGAAGCTCACTAGCTCTCAACTTCTCTTCCCAACCCC  750
751  TCTCTACACC                                          760
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Fig. 7 Nucleotide sequence of rice clone pOl 5.1. The DAMD PCR primer sequence is *underlined*. Several short repetitive sequences are shown *double underlined* or *dot underlined* (nucleotide codes *S*: G or C; *W*: A or T; *Y*: T or C)

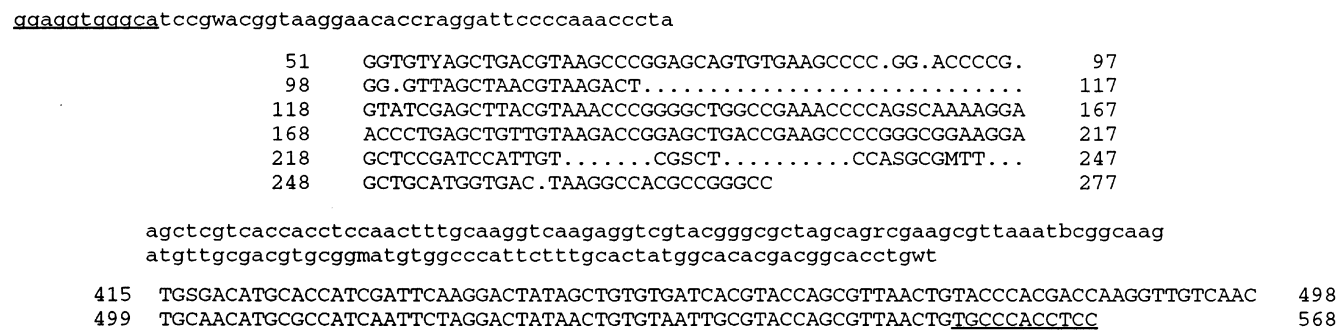

Fig. 8 Nucleotide sequence of the rice clone pOa 6.4 (nucleotide codes *B*: G, T, or C; *M*: A or C; *R*: G or A; *S*: G or C; *W*: A or T; *Y*: T or C). The sequences flanking the repetitive region are in *lower case*. *Gaps* (.) are introduced to facilitate sequence alignment. The DAMD PCR primer sequence is *underlined*

were present in opposite orientation at either end of the clones. Clone pOl 5.1 contained 45.5% G + C (guanine and cytosine), and clone pOm 6.1 contained 58.8% G + C. Neither clone appeared to contain a tandem repetitive sequence like those found in typical minisatellite or VNTR sequences. The presence of several types of short repeat structures in clone pOl 5.1 (Fig. 7) suggested that it was derived from a region rich in repetitive sequences. The presence of such repeat structures within pOl 5.1 may account for its hybridization to multiple genomic DNA fragments in contrast to pOm 6.1 in rice (Fig. 4).

Clone pOl 5.1 had a low degree of homology with three known sequences detected using BLAST (Altschul et al. 1990). Clone pOm 6.1 was shown to have a significant similarity ($P > 0.001$) with a *Spinacia oleracea* L. mRNA for the photosystem-II 22-kDa protein (Wedel et al. 1992). A 71% homology was found in the bp sequence region 245 to 336. This suggested that pOm 6.1 may be associated with a functional gene in the rice genome. Since pOm 6.1 itself does not contain repetitive sequences and hybridizes to only one high-molecular-weight locus (Fig. 4), we speculate that the polymorphism in allele size detected at this locus may result from minisatellite variation(s) flanking pOm 6.1. This would also indicate that DAMD-PCR-generated fragments are derived from regions rich in minisatellite sequences.

Sequence analysis revealed that clone pOa 6.4 comprised 57.7% G + C (guanine and cytosine), which was much higher than the average 44% G + C-content of the cultivated rice genome (Wu and Wu 1992). The 33.6 minisatellite core sequence was present in opposite orientations at either end of the sequence and terminated the amplification region (Fig. 8). There were two distinct types of tandem repetitive sequences found within pOa 6.4. The first consisted of two repeats of 83 and 71 bp that showed 82% homology over the 71%-bp length. This sequence data is strong evidence that DAMD-PCR was able to amplify a putative minisatellite region which included tandem repeats and core-sequence components.

The second type of tandem repeats found in clone pOa 6.4 were imperfect and varied from 20 to 50 bp due to several insertions and deletions. The G + C content of this region was 60.9%, which is similar to characterized minisatellites. We could not identify a core-sequence component within these tandem repeats.

In this study, DAMD-PCR produced one DNA fragment with a potential for DNA fingerprinting in rice cultivars, one highly polymorphic single locus RFLP marker, and one repetitive, genome-specific, probe. Such probes will be useful in breeding programs to address questions relevant to rice genetic relatedness, cultivar identification, or marker-mediated backcross breeding utilizing wild species. At present, the number of RFLP probes that can be obtained via DAMD-PCR appears to be limited. Even so, DAMD-PCR will complement traditional genomic-library screening to isolate molecular markers.

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