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A repetitive sequence specific to *Oryza* species with BB genome and abundant in *Oryza punctata Kotschy ex Steud*

Yueh-Yun CHENG, Shao-An FANG, Yao-Cheng LIN, and Mei-Chu CHUNG*

Institute of Plant and Microbial Biology, Academia Sinica, Taipei 115, Taiwan, Republic of China

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ABSTRACT. Molecular markers are capable of discriminating specific constituents of genome and monitoring genomic introgression of interspecific hybrids. In this study, we isolate and characterize a BB genome specific RAPD, *Opun*210, from *Oryza punctata Kotschy ex Steud* (W1593), an African native wild rice. We demonstrate the *Opun*210 as a highly species-specific marker. The *Opun*210 sequence is 789 base pairs in length and estimated at 5.3×10^4 copies in *O. puntata* (W1593) haploid genome, which contains the most repeats of *Opun*210 among *Oryza* species. The results of DNA sequence alignments among *Opun*210 and a few hits in the GenBank found that a relatively high similarity was in position ~500 nucleotides regions at the 5′ ends, but a low similarity was in the rest of the nucleotides at the 3′ ends. SCAR-PCR profiles indicates that this fragment was specific to BB genome. Furthermore, the *Opun*210 sequence at position 430~480 nucleotides putatively encodes a peptide with 88% identity to a *Ty3-gypsy* retrotransposon protein or a peptide with 94% identity to a hypothetical protein. The results of Southern hybridization and fluorescent *in situ* hybridization (FISH) indicated that the repetitive *Opun*210 sequences dispersed throughout the entire genome of *O. punctata*. The origin and divergence of the *Opun*210 sequence in genus *Oryza* is discussed based on the investigations in this study.

Keywords: Fluorescent *in situ* hybridization (FISH); *Oryza punctata*; Repetitive sequence; Species-specific RAPD.

INTRODUCTION

Cultivated rice is one of the most important staple food crops in the world. In addition to two cultivated species, *O. sativa* and *O. glaberrima*, the genus *Oryza* is comprised of more than twenty wild species (Aggarwal, 1997; Ge et al., 1999; Vaughan et al., 2003). *Oryza* species are classified genetically into ten genome types, i.e. the AA, BB, CC, BBCC, CCDD, EE, FF, GG, JJHH and JJKK according to the chromosomal pairing behavior at meiosis of interspecies hybrids, genomic DNA hybridization, and DNA sequence analysis of nuclear and chloroplast genes (Moringa, 1964; Oka, 1988; Vaughan, 1994; Khush, 1997; Ge et al., 1999), thus the phylogenetic relationships of the genus *Oryza* was described (Ge et al., 1999).

Wild rice species are important genetic resources and have been broadly introduced into rice breeding programs for a long time (Chang et al., 1975; Sitch et al., 1989; Brar and Khush, 1997; Khush, 1997; Nakagahra et al., 1997; Xiao et al., 1998). Various molecular markers have been proven efficient for discriminating specific genomes in hybrids and monitoring genome introgression in some of the breeding programs mentioned above.

Eukaryotic genomes contain abundant repetitive DNA sequences. Most repetitive sequences spread throughout genomes; however, a few repetitive sequences cluster at unique chromosomal positions, which are useful landmarks for chromosome identification. Because most repetitive DNA sequences do not encode proteins, mutations on such sequences usually will not make significant changes in the phenotype. Therefore, repetitive DNA sequences could rapidly accumulate a great diversity in comparing with the unique coding DNA sequences during evolution. However, they are also constantly homogenized via a molecular process called "concerted evolution", so that few variants can be fixed (for review see, Elder and Turner, 1995). Such a rapid divergence and homogenization process results in some of the repetitive sequences to become highly specific to a species (Dover 1982; Grellet et al., 1986; Ganal et al., 1988; De Kochko et al., 1991), or even specific to a chromosome (Willard and Waye, 1987; Wang et al., 1995). Some repetitive sequences play important roles in chromosomal functions, such as centromeric repeats (Ananiev et al., 1998; Dong et al., 1998; Page et al., 2001) or telomeric repeats

*Corresponding author: E-mail: bomchung@gate.sinica.edu.tw; Tel: 886-2-27892701; Fax: 886-2-27827954.

(Richards and Ausubel, 1988). Therefore, the organization of repetitive DNA sequences and the correlation among different repetitive sequences and unique sequences in the genome are very important aspects of eukaryotic genome characterization.

More than 20 repetitive DNA sequences have been isolated and characterized from various *Oryza* species. Some of the repetitive sequences were highly genome-specific (for review see: Yan et al., 2002), and some of them were physically localized to individual chromosomes by using (fluorescent) *in situ* hybridization (Wu et al., 1991; Wang et al., 1995; Ohmido and Fukui, 1997; Uozu et al., 1997). However, none sequence with specificity to BB genome has been previously described. In this study, we firstly report a BB genome specific RAPD (random amplified polymorphic DNA), which was amplified from *O. punctata*.

The RAPD method allows investigating genomic variation without prior knowledge of DNA sequences (Williams et al., 1990). Most RAPD bands are known to be generated from repetitive DNA sequences (Williams et al., 1990; Devos and Gale, 1992; Echt et al., 1992); however, a few RAPDs were derived from low-copy sequences in the *Hordeum* species (Marillia and Scoles, 1996) and in the *Triticeae* species (Svitashev et al., 1998). Several interspersed, species-specific repetitive elements isolated from RAPD products were useful for phylogenetic relationship studies and for interspecific hybrids identification; some of them even can be used as FISH markers for chromosome identification (Skinner 1992; Ko et al., 2002).

Here we report the isolation and characterization of the first BB genome specific sequence, *Opun*210, which was a RAPD generated from *O. punctata* (W1593). *Oryza punctata,* a wild species of rice native in Africa, together with *O. grandiglumis*, *O. latifolia*, *O. minuta*, and *O. officinalis* belong to *Oryza* ser. *Latifoliae*. Two morphological types exist in *O. punctata*; the diploid species (*2n=24*) with BB genome, while the allotetraploid species (*2n=4x=48*) with BBCC genome. Our results reveal that *Opun*210 sequence disperses throughout *O. puntata* (W1593, BB) genome and presents more frequently in this genome than in other *Oryza* species. *Opun*210 can be use as a specific marker for characterizing BB genome.

MATERIALS AND METHODS

Plant material

The accessions used in this study are shown in Figures 1 and 4, and Table 2. The seeds of wild species of rice were kindly provided by the late Professor H. I. Oka, National Institute of Genetics, Japan (Wild Rice Database, http://www.pgcdna.co.jp/cgi-bin/wrdb/content.cgi). Plants have been propagated for many years at the experimental field of the Institute of Plant and Microbial Biology, Academia Sinica, Nankang, Taipei, Taiwan.

Isolation and cloning of genome-specific DNA

Total genomic DNA extracted from young leaves of plants following the protocol described by McCouch et al. (1988). Protocol for RAPD analysis follows the description in our previous report (Wu et al., 2002). The primers were obtained from the University of British Columbia, Vancouver, BC, Canada (UBC Kit 1-4). Each 25 μl reaction mixture contained 25 ng of template DNA, 1× PCR buffer (10 mM Tris-HCl, pH8.8, 1.5 mM $MgCl_2$ 50 mM KCl, and 0.1% Triton X-100), 200 μM dNTPs, 0.2 μM of a given pair of primers, and one unit of HotstarTaq DNA polymerase (Qiagen). The amplification was carried out in a thermal cycler (GeneAmp PCR System 9600, Perkin-Elmer, Norwalk, USA), programmed for an initial denaturing for 15 min at 95°C, followed by 45 cycles of 5 sec at 94°C, 20 sec at 36°C, 90 sec at 72°C, and a final extension for 10 min at 72°C. Reaction products were separated by electrophoresis through 1% Nusieve GTG plus 1% SeaKem LE agarose gels (Cambrex Bio Sciences Rockland, Inc. Rockland, USA) in 0.5X Tris-borate-EDTA buffer (1X TBE: 89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA), and visualized by ethidium bromide staining under UV illumination. The sizes of bands were estimated by referring to 100-bp ladders (New England Biolabs) in each gel.

Putatively genome-specific RAPD bands were excised from the gel after electrophoresis; DNA fragments were eluted and purified by a QIAquick Gel Extraction kit (Qiagene), then cloned into pCRTMII vector using a TA cloning kit (Invitrogen, Carlsbad, California, USA) following suppliers' instructions. The positive colonies (white) were verified by PCR to identify cloned fragments. Inserts were sequenced by using an Autoread sequencing kit and an autosequencer (Amersham Pharmacia Biotech).

Southern Hybridization

Genomic DNA samples were completely digested with various restriction enzymes (New England Biolabs), then separated by electrophoresis at 40 kV (1 kV/cm) for overnight on a 1% agarose gel in 0.5X TBE buffer, and then blotted to Hybond-N^+ nylon membranes (Amersham Pharmacia Biotech) by Southern transfer. Probe preparation, membrane hybridization, and signal detection were performed following the instructions of ECL™ direct nucleic acid labeling and detection system (Amersham Pharmacia Biotech).

Copy number estimation

To estimate the copy numbers of cloned DNA sequences within each genomes, total genomic DNA of each accession and a series of diluted recombinant DNA were applied to a Hybond-N^+ nylon membranes through a slot-blot template (Bio-Dot Slot Format, Bio-Rad Laboratories, Philadelphia, PA) and performed Southern hybridization as described above. The intensities of hybridization signals on X-ray film were quantified with a densitometer (Molecular Dynamics) and ImageQuant™

software (Amersham Pharmacia Biotech). The copy number of the repetitive units estimation depended on the relative intensity compared between the signal derived from the genomic DNA and that from the series dilution of plasmid DNA as previously described by Rivin et al. (1986).

Chromosome preparation

Healthy root tips were harvested from germinating rice seeds, pretreated in 2 mM 8-hydroxyquinoline at 20°C for 2 h to accumulate prometaphase cells, rinsed with distilled water, then fixed in fresh prepared Farmer's Fluid (95% ethanol : glacial acetic acid = 3:1) at room temperature for overnight. Chromosome preparations were carried out following the protocol described in Wu et al. (1991). Root tips were macerated with 6% cellulose (Onoauka R-10, Yakukt Honsha, Japan) and 6% pectinase (Sigma Chemical Co., St. Louis, Mo.) in 75 mM KCl, (pH=4.0) at 37°C for 70 min. After rinsing with water, tissues were squashed onto slides in the same fixative. Slides were air-dried and stored at -80°C until used for FISH. Slides were dehydrated through an ethanol series (70%, 95%, and 100%, five minutes each) prior to be used in FISH.

Fluorescence *in situ* hybridization

The FISH procedure was performed following the protocol described previously (Kao et al., 2006). Digoxigenin-11-dUTP-labeled probes were detected using a rhodamine-conjugated anti digoxigenin antibody (Roche Diagnostics GmbH, Penzberg Germany). Chromosomes were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI). All images were captured by using a CCD camera (Cool SNAPfx, Photometrics, Tucson, AZ), which was driven by Image-Pro Plus software (version 4.5.1, Media Cybernetics, Yorktown, VA, USA), attached to a Zeiss axioplan epifluorscent microscope (Axioplan, Carl Zeiss AG, Germany). Final image adjustments were done with Adobe Photoshop 6.0 (Adobe Systems Incorporated, San Jose, CA, USA).

RESULTS

Identification and isolation of genome specific DNA sequence in rice

A total of 131 decameric random primers were screened for RAPD with highly polymorphic and well-resolved genome-specific bands. Abundant polymorphism was detected with all primers used in this study. Forty-three among these primers could generate 243 products, which showed distinctive and satisfactory amplification profiles. The average number of discrete bands generated per primer was 5.65, ranging from a single band (from W1577 by UBC 189) to 16 bands (from W1564 by UBC 101). The amplified products were approximately 300 to 2800 base pairs (bp) in size. Reproducible amplification profiles were further evaluated for the specificity of RAPD. Polymorphism is defined as the presence/absence of a particu-

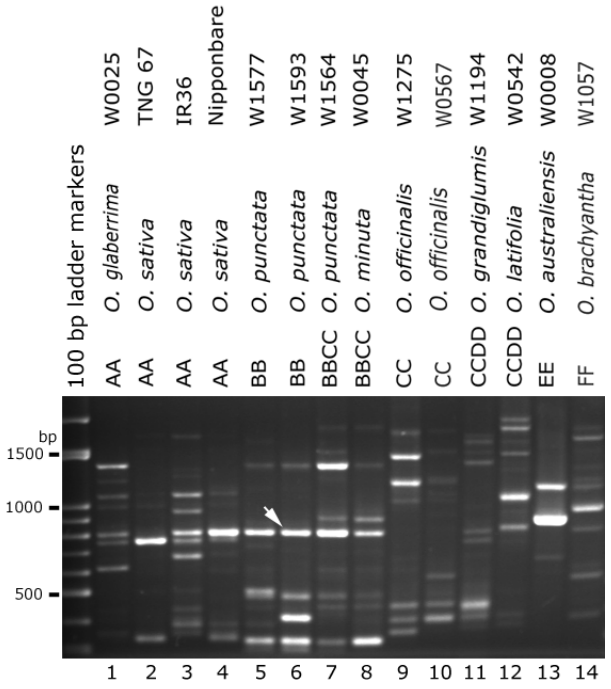

Figure 1. RAPD profiles showing inter-specific/genomic, intra-genomic/specific polymorphism detected among 14 *Oryza* species with primer UBC 210. DNA fragments ca. 800 bp amplified from *O. punctata* (W1593; arrow) were eluted and cloned for further characterization.

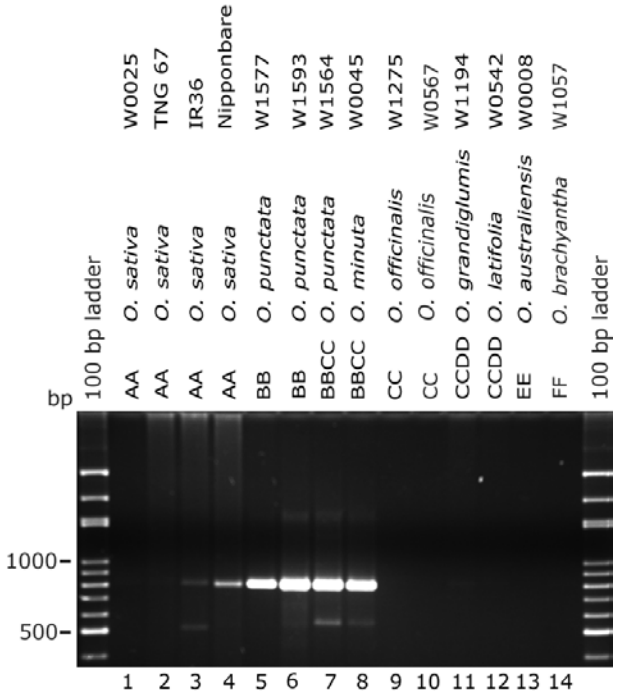

Figure 2. SCAR-PCR profiles showing fragments ca. 800 bp specifically amplified from accessions with BB genome. SCAR primers were designed according to the sequences of the cloned *Opun*210 sequence and UBC 210 primer. Products amplified from cultivars Nipponbare and IR36 were far less than those from accessions with BB genomes.

lar band generated from different accessions by the same primer.

In this study, polymorphism presents among genome types and species, even among the different accession numbers in same species. As shown in Figure 1, these RAPD profiles amplified from 14 accessions with primer UBC 210 (5′-GCACCGAGAG) presented several distinguishable patterns of intragenomic/intergenomic variations. RAPD profiles (Figure 1) were distinguishable between *O. glaberrima* (AA, lane 1) and *O. sativa* (AA; lanes 2-4), between *O. punctata* and *O. minuta* (BBCC; lanes 7 and 8), and between *O. grandiglumis* and *O. latifolia* (CCDD; lanes 11 and 12). Polymorphism even presented among different cultivars of *O. sativa* (lanes 2-4). RAPD profiles of *indica* type cultivars IR36 (lane 3) were different from those from *japonica* type cultivars TNG 67 (lane 2) and Nipponbare (lane 4). Although the same primer was used, the RAPD profiles (Figure 1) generated from diploid *O. punctata* (BB genome), both W1977 (lane 5) and W1593 (lane 6), and tetraploid *O. punctata* (BBCC; lane 7) were similar but not identical. Polymorphic profiles were also generated from two *O. officinalis* accessions, W1275 (lane 9) and W0567 (lane 10) in this case. The remaining accessions, *O. australiensis* (EE; lane 13) and *O. brachyantha* (FF; lane 14) showed unique amplification profiles with primer UBC 210.

One distinct band ca. 800 bp commonly presented in those profiles (Figure 1) of *Oryza* species with BB (lanes 5 and 6) and BBCC genomes (lanes 8 and 9) and two cultivars of *O. sativa* (AA genome), *indica* cv. IR 36 (lane 3) and *japonica* cv. Nipponbare (lane 4). The DNA fragments amplified from *O. punctata* (W1593; lane 6) were chosen to be eluded from agrose gel and cloned for further characterization.

One clone, designated as *Opun*210 was further characterized in this study. *Opun* denotes its origin species *O. punctata* and 210 refers to the number of primer UBC 210 in UBC kit. The nucleotide sequence of *Opun*210, 789 base pairs in length, appears in the GenBank nucleotide sequence databases under the accession number DQ104697.

This sequence has been compared with 3,243,863 sequences in a databank including GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS, environmental samples or phase 0, 1 or 2 HTGS sequences) by the similarity searching method with the Basic Local Alignment Search Tool (BLAST) program from the Web at http://www.ncbi.nlm.nih.gov/BLAST/. The results of this similarity search with EXPECT threshold value better than 10 obtained 77 high-scoring segment pairs (HSP) without gapping. The expectation values (E-values) reported by similarity programs indicate nearly exact matches between query sequences with each of the database sequences. The results of alignments between the *Opun*210 sequence and the top 50 matches showed about 90% similarity in approx. 500 nucleotides regions at the 5′ ends, but of relative low similarity to the rest at 3′ ends.

Furthermore, the results of the similarity search for coding proteins by BLASTX showed that the *Opun*210 sequence at position 430~480 nucleotides putatively encoded a peptide with 88% identity to a *Ty3-gypsy* subclass retrotransposon protein annotated by *O. sativa*, *japonica* cultivar-group (gi|62734207|gb|AAX96316.1|) or a peptide with 94% identity to a hypothetical protein LOC_Os11g22630.

We designed a pair of Sequence Characterized Amplified Region (SCAR) primers based on the sequence data obtained above. Each SCAR primer contained the original ten-bases of the UBC 210 primer at the 5′ end and the subsequent 14 internal bases from the end. The synthesized 24-mers were: SCAR 210a (5′-GCACCGAGAGAAGGAAGGAAGGGG) and SCAR 210b (GCACCGAGAGTATATTGAACTGGT-3′). SCAR-PCR amplification of the rice genomic DNA of each species was performed in a standard PCR. The SCAR-PCR profiles are shown in Figure 2. A distinct band ca. 800 bp was amplified from several species with either BB or BBCC genomes, including *O. punctata* (W1577, W1593, and W1564; Figure 2, lanes 5-7) and *O. minuta* (W0045; Figure 2, lane 8), respectively. The bands amplified from cultivars IR36 and Nipponbare (*O. sativa*; Figure 2, lanes 3-4) were relative faint. No products were amplified from the remaining accessions by this SCAR 210a/b primer pair (Figure 2). These results confirmed that the *Opun*210 sequence was specific to the BB genome.

The abundance of *Opun*210 repeats in the rice genome

The nucleotide sequence of *Opun*210 was also BLAST searched against new genomic survey sequence (GSS) databases with BLAST identity length > 120. The results, as shown in Table 1, revealed that this fragment appeared repeatedly, although at low hit percentages, in those rice species with AA genome. The copy numbers of the *Opun*210 sequence in *Oryza* genomes were estimated by comparing the relative intensities of the signals of quantitative slot-blot hybridization between standard (Figure 3, left) and each genome (Figure 3, right). A dilution series of cloned *Opun*210 DNA was referred as a copy number standard in these estimations (Figure 3, left). The haploid genome size of each species referred to the data published in Plant DNA C-values Database (Bennett and Leitch 2001, release 3.1, http://www.rbgkew.org.uk/cval/homepage.html).

Table 1. The frequencies of *Opun*210 sequence found in GSS database of rice genomes with BLAST identity length > 120.

	O. sativa (*japonica*)	*O. nivara*	*O. rufipongon*
Library name	OSJNBa	OSJNBb	OR_Bba	OR_Cba
Reads NO.	73362	54097	106130	71006
Hits number in database	43	17	88	74
Hits %	0.0586	0.0314	0.0829	0.1042

Oryza punctata (W1593), from which *Opun*210 was cloned, was found to have the most repeats of *Opun*210 among all accessions (Table 2). There were estimated 5.3×10^4 repeats of *Opun*210 in the *O. punctata* (W1593) haploid genome, approximately 7.6% of its haploid DNA contents. For the convenience of comparison, the copy number of *Opun210* sequences in each genome is presented as a relative percentage of that found in *O. punctata* (W1593), which is referred as 100%. The relative copy numbers (%) are ranged from 4% in IR36 (AA) to 19% in *O. minuta* (W0045, BBCC) among the *Oryza* species. In comparing with *O. puctata* (W1593), less *Opun210* repeats were found in another *O. puctata* accession (W1577) and in tetraploid *O. punctata* (W1564, BBCC), 14% and 11% in respective genome. In *O. officinalis* (W1275, CC) and Nipponbara, the relative copy numbers were 12% and 13%, respectively. In *O. glaberrima* (AA) and *O. gradiglumis* (W1194, CCDD), the relative copy numbers are below 10%. No *Opun210* homologous sequences were detected in the *O. latifolia* (W0542, CCDD) genome or in the *O. australiensis* (W0008, EE) genome.

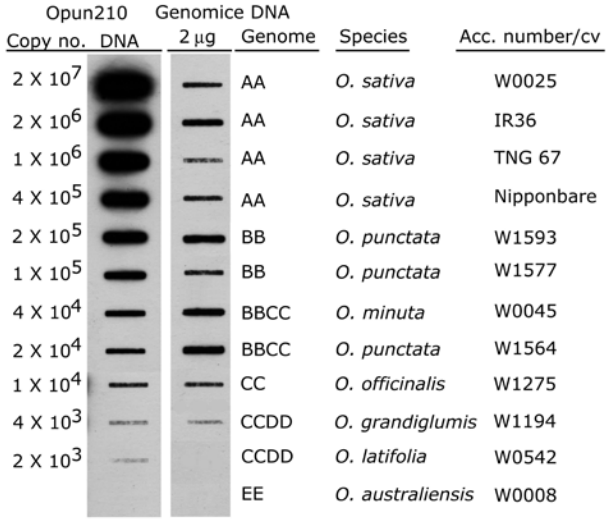

Figure 3. Quantitative slot-blot hybridization for estimation of the copy numbers of *Opun*210 sequences in rice genomes. A diluted series of plasmid *Opun*210 DNA (left) and 2 µg genomic DNA of each accession (right) were blotted on nylon membrane and hybridized to *Opun*210.

Distribution of clone *Opun*210 sequence in *O. punctata* genome

The results of Southern hybridization showed multiple distinguishable bands with a smear background in all lanes of different restriction enzyme digestions (Figure 4). Those bands were not in ladder pattern, thus *Opun*210 sequences were not considered as the typical pattern of tandem repeats. These results also indicated that the *Opun*210 sequences moderately repeated and dispersed throughout the entire *O. punctata* genome.

In situ hybridization results also demonstrated that *Opun*210 sequences dispersed over all chromosomes of the *O. puntata* (W1593). Although several chromosomes obviously have more *Opun*210 sequences than the rest, the localizations of *Opun*210 sequences were evenly distributed on individual chromosome. No banding patterns showing conspicuous signals as repeats clustering sites were observed on individual chromosome (Figure 5). FISH results indicated that *Opun*210 sequence was not a chromosome specific repetitive sequence and was unsuitable for chromosome identification.

Table 2. The copy numbers of *Opun*210 sequence found in rice genomes.

Species	Genome designation	Genome size (pg/1C)[a]	Copy number/haploid (relative copy number, %)
O. sativa, *Japonica*, cv. TNG 67	AA	0.43[b]	3.2×10^3 (6)
O. sativa, *Indica*, cv. IR36	AA	0.48	1.9×10^3 (4)
O. sativa, *Japonica*, cv. Nipponbare	AA	0.43	6.2×10^3 (12)
O. glaberrima (W0025)	AA	0.43	7.5×10^3 (6)
O. punctata (W1577)	BB	0.55	7.4×10^3 (14)
O. punctata (W1593)	BB	0.55	5.3×10^4 (100)
O. punctata (W1564)	BBCC	1.13[c]	6.0×10^3 (11)
O. minuta (W0045)	BBCC	1.18	1.0×10^4 (19)
O. officinalis (W1275)	CC	0.73	7.4×10^3 (13)
O. gradiglumis (W1194)	CCDD	1.00	3.4×10^3 (6)
O. latifolia (W0542)	CCDD	1.15	Not detectable
O. australiensis (W0008)	EE	0.98	Not detectable

[a]Genome sizes as listed in Plant DNA C-values Database (http://www.rbgkew.org.uk/cval/homepage.html).
[b]Estimated according to the genome size of *O. sativa*, *Japonica*, cv. Nipponbare.
[c]Genome sizes as previously reported by Salles e al. (2001).

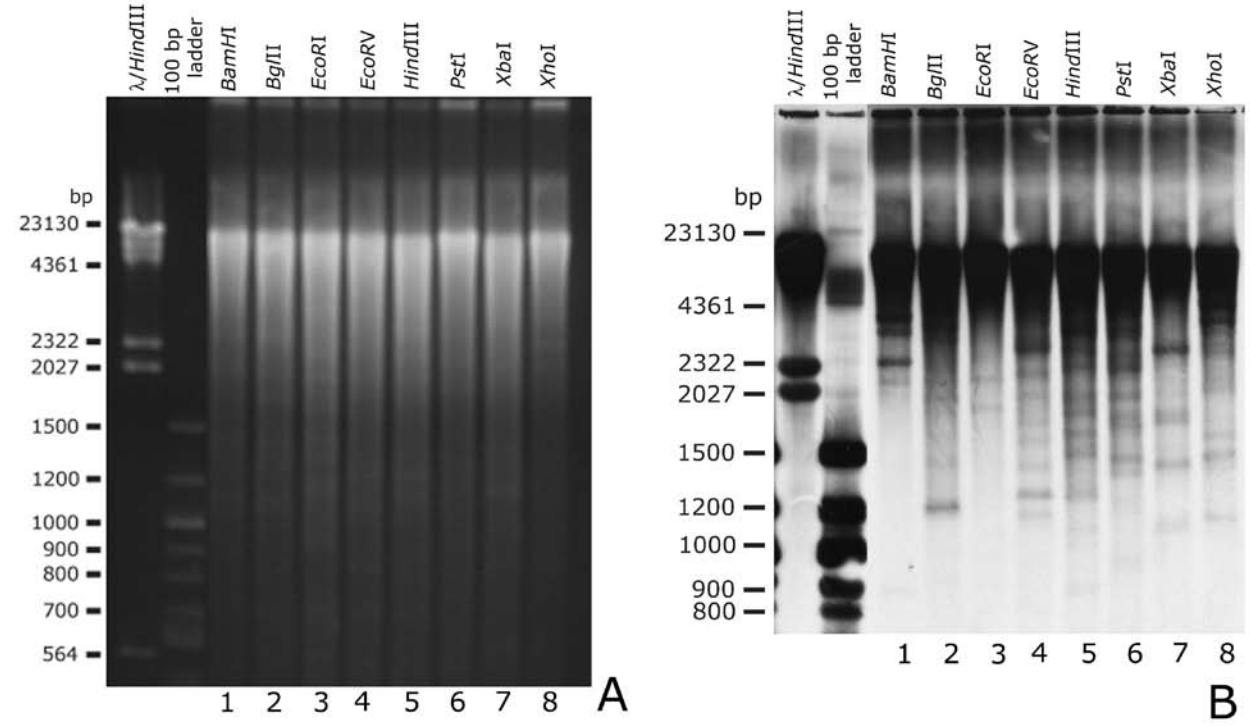

Figure 4. Electrophoretic fractionation (A) and Southern hybridization analysis (B) of the organization of *Opun*210 sequence in *O. puntata* (W1593) genome.

DISCUSSION

In our studies, except for the *Opun*210, most of the putative species-specific RAPDs finally showed no obvious genomic specificity by Southern hybridization (data not shown). Our results suggested that RAPDs, although polymorphic in size, often presented high sequence similarity, as it has been reported previously (Williams et al., 1993). RAPDs with similar sequences were thought to be amplified from segments flanked by same priming sites, but separated in different distances. Such RAPDs were considered as the consequent events of insertion/deletion within these regions during evolution. However, co-migration RAPDs in the electrophoresis gel may contain unrelated DNA sequences (Thorman and Osborn, 1992). Nevertheless, RAPDs are efficient and inexpensive molecular markers, and have been proven successfully in various taxonomic and phylogenetic studies (Kazan et al., 1993; Wilkie et al., 1993). In this study, we have proven the RAPD method as an efficient approach for isolation a genome/species-specific repetitive sequence. Our results indicated that *Opun*210, which was amplified from *O. punctata* (W1593) with UBC 210 primer, was a whole genome dispersed repetitive sequence and was specific to the BB genome.

In this study, we want to evaluate the efficiency of RAPD method in differentiation genome/species-specific RAPDs and investigation the organization of rice genomes. Although there were very few primers could differentiate among three accessions of *O. punctata*, including

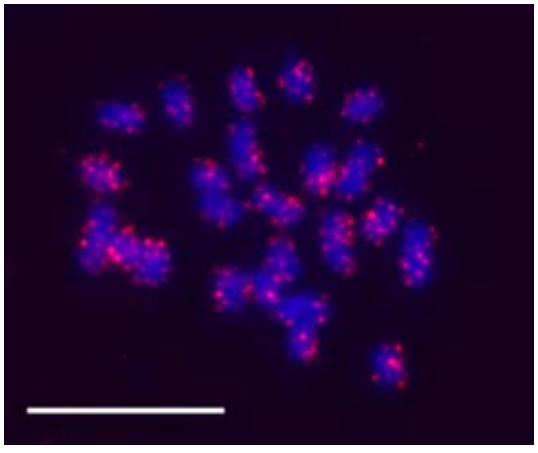

Figure 5. The distribution of the *Opun*210 sequences on *O. puntata* (W1593) mitotic chromosomes. The *Opun*210 sequence was mapped to mitotic nuclei by FISH with a digoxigenin labeled probe and immunologically detected by rhodamine-conjugated anti digoxigenin antibody (red). Chromosomes were counterstained with DAPI (blue). Scale bar = 10 μm

W1577, W1593, and W1564, UBC 210 primer could generate reproducible and distinguishable RAPD profiles from each accession (Figure 1, lanes 5-7). This primer also could discriminate two *O. officinalis* accessions W1275 and W0567 (Figure 1, lanes 9-10). These results suggest that those segments flanked by UBC 210 primer are various in copy number and size among genomes, therefore, *Opun*210 can represent as a genome specific marker. Our

results suggest that RAPDs can provide not only detailed analysis for verification at species level, but also an efficient approach to isolate genome/species-specific markers. Although the *Opun*210 sequences showed no specific distribution on individual chromosome, it still could be used as an *O. punctata* specific RAPD marker. Such markers will be useful for monitoring genome introgression in interspecies hybridization breeding programs involving this accession.

As shown in Figure 1 and Table 2, *Opun*210 is commonly found in species with BB genome, especially in *O. punctata* (W1593, BB). In *O. officinalis* (W1275, CC), although the band corresponding to *Opun*210 was absent from lanes 9 and 10 in Figure 1, amount of *Opun*210 sequences could be detected by quantitative slot-blot hybridization (Table 2). This implies that those distinct bands mentioned above were amplified from fragments with similar sequences but in polymorphic sizes. *Oryza officinalis* (W1275, CC) and *O. minuta* (W0045, BBCC) both geographically distribute in Asia. This may suggest a close relationship between them based on the amount of *Opun*210 repeats found in their genomes. According to the phylogenetic relationships of the genus *Oryza*, species with AA, BB, and CC genome types were grouped in the same clade (Ge et al., 1999). The accessions with AA genome, except a *japonica* type cultivar Nipponbare, have less *Opun*210 repeats than those accessions with BB or CC genomes. Nipponbare has as many *Opun*210 repeats as *O. punctata* (W1577, BB; W1564, BBCC) and *O. officinalis* (W1275, CC). It indicated that AA genome was eventually less close to BB genome than CC genome did, while Nipponbara may have progenitors with BB or CC genome. *Opun*210 repeats were less, or even absent in species geographically distributed in central/south America or Australia, such as *O. gradiglumis* (W1194, CCDD), *O. latifolia* (W0542, CCDD), and *O. australiensis* (W0008, EE) (Table 2). These species were grouped in different clands from that contained AA, BB, and CC genomes (Ge et al., 1999). Therefore, those bands, either conspicuous or faint, present in profiles of CCDD (Figure 1, lanes 11 and 12), EE (Figure 1, lane 13), and FF (Figure 1, lane 14) genomes were amplified from unrelated sequences with the same priming sites.

About half of the rice genome is composed of repetitive sequences (Kurata et al., 1994). The polymorphic distributions of retrotansposons have been found different among rice varieties. Retrotransposons are considered to play important roles in rice genome diversity (Wang et al., 1997; 1999). Based on the full genome draft sequences, *in silico* survey of different kinds of repetitive sequences revealed that there are approximately 38 Mb of long repetitive sequences and 150 Mb of short repetitive DNA in the rice genome (Goff et al., 2002). A large fraction of the moderately repeated sequences comprises transposon- and other mobile DNA-related sequences (Mao et al., 2000). Sequence analysis revealed that *Opun*210 might originate from one of the retrotransposons, which commonly existed in ancestral *Oryza* species. Mutations occurring at the 3' end flanking sequences and consequentially rapid amplification with other relative elements might drive them to become abundant and specific to *O. punctata* (W1593) during evolution. Such putative possibilities can be reflected in the results of Southern hybridization. Southern hybridization analysis showed that repetitive *Opun*210 sequences presented as several distinct bands or as smeared patterns in restricted digestions (Figure 4). The distinct bands, larger than *Opun*210 in size, may suggest that those fragments contained sequences complementary to the probe were present in several discrete configurations, presumably next to other repetitive sequences. Smeared hybridization signals were most probably due to the probe sequence being hybridized to dispersed repeats or being adjacent to single or low-copy sequences as previously reported (Evans et al., 1983; Saul and Potrykus, 1984; Rivin et al., 1986).

A comparative genomics program entitled the 'Oryza Map Alignment Project' (OMAP) has been embarked (Wing et al., 2005). The OMAP aims to construct and align BAC/STC -based physical maps of ten wild rice species and one cultivated rice to *O. sativa* ssp. *japonica* c.v. Nipponbare genome sequence finished by IRGSP. The results of our study suggest that genome/species specific repetitive DNA sequences are various in types and amounts among the *Oryza* species and may play important roles in the organization and speciation of rice genomes. Therefore, such kinds of repetitive sequences may consequentially determine the efficiency of alignments.

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LITERATURE CITED

Aggarwal, R.K., D.S. Brar, and G.S. Khush. 1997. Two new genomes in the *Oryza* complex identified on the basis of molecular divergence analysis using total genomic DNA hybridization. Mol. Gen. Genet. **254:** 1-12.

Ananiev, E.V., R.L. Philips, and H.W. Ring. 1998. Chromosome-specific molecular organization of maize (*Zea mays* L.) centromeric regions. Proc. Natl. Acad. Sci. USA **95:** 13073-13078.

Brar, D.S. and G.S. Khush. 1997. Alien introgression in rice. Plant Mol. Biol. **35:** 35-47.

Chang, T.T., S.H. Qu, M.D. Pathak, K.C. Ling, and H.E. Kauffman. 1975. The search for disease and insect resistance in rice germplasm. *In* O.H. Frankel and J.G. Hawkes (eds.), Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, Cambridge, England, pp. 183-200.

De Kochko, A, M.C. Kiefer, F. Cordesse, A.S. Reddy, and M. Delseny. 1991. Distribution and organization of a tandemly repeated 352-bp sequence in the *Oryza* family. Theor. Appl. Genet. **82:** 57-64.

Devos, K.M. and M.D. Gale. 1992. The use of random amplified polymorphic DNA markers in wheat. Theor. Appl. Genet. **84:** 567-572.

Dong, F., J. Miller, S.A. Jackson, G.L. Wang, P.C. Ronald, and J. Jiang. 1998. Rice (*Oryza sativa*) centromeric regions consist of complex DNA. Proc. Natl. Amer. Sci. USA **95:** 8135-8140.

Dover G.A. 1982. Molecular drive: a cohesive mode of species evolution. Nature **299:** 111-117.

Echt, C.S., L.A. Erdahl, and T.J. McCoy. 1992. Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. Genome **35:** 84-87.

Elder, J.F. Jr. and B.J. Turner. 1995. Concerted evolution of repetitive DNA sequences in eukaryotes. Q. Rev. Biol. **70:** 297-320.

Evans, I.J., A.M. James, and S.R. Barnes. 1983. Organization and evolution of repeated DNA sequences in closely related plant genomes. J. Mol. Biol. **170:** 803-826

Ganal, M.W., N.L.V. Lapitan, and S.D. Tanksley. 1988. A molecular and cytogenetic survey of major repeated DNA sequences in tomato (*Lycopersicon esculentum*). Mol. Gen. Genet. **213:** 262-268.

Ge, S.T. Sang, B.R. Lu, and D.Y. Hong. 1999. Phylogeny of rice genomes with emphasis on origins of allotetraploid species. Proc. Natl. Amer. Sci. USA **96:** 14400-14405.

Goff, S.A., D. Ricke, T.-H., Lan, G. Presting, R. Wang, M. Dunn, J. Glazebrook, A. Sessions, P. Oeller, H. Varma et al. 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science **296:** 92-100.

Grellet, F., D. Delcasso, F. Panabieres, and M. Delseny. 1986. Organization and evolution of a higher plant alphoid-like satellite DNA sequence. J. Mol. Biol. **187:** 495-507

Kao, F.I., Y.Y. Cheng, Chow TY, H.H. Chen, S.M. Liu, C.H. Cheng, and M.C. Chung. 2006. An integrated Map of *Oryza sativa* L. Chromosome 5. Theor. Appl. Genet. **112:** 891-902.

Kazan, K., J.M. Manners, and D.F. Cameron. 1993. Genetic relationships and variation in the *Stylosanthes guianensis* species complex assessed by amplified polymorphic DNA. Genome **36:** 43-49.

Khush, G.S. 1997. Origin, dispersal, cultivation and variation of rice. Plant Mol. Biol. **35:** 25-34.

Ko, J.M., G.S. Do, D.Y. Suh, B.B. Seo, D.C. Shin, and H.P. Moon. 2002. Identification and chromosomal organization of two rye genome-specific RAPD products useful as introgression markers in wheat. Genome **45:** 157-164.

Kurata N, Y. Nagamura, and K. Yamamoto. 1994. A 300 kilobase interval genetic map of rice including 880 expressed sequences. Nature Genetics **8:** 365-372.

Mao, L., T.C. Wood, Y. Yu, M.A. Budiman, J. Tomkins et al., 2000. Rice transposable elements: a survey of 73,000 sequence-tagged-connectors. Genome Res. **10:** 982-990.

Marillia, E.F. and G. Scoles. 1996. The use of RAPD markers in *Hordeum* phylogeny. Genome **39:** 646-654.

McCouch, S., G. Kochert, Z. Yu, Z. Wang, G.S. Khush, W. Coffman, and S. Tanksley. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. **76:** 815-829

Moringa, T. 1964. Cytogenetical investigations on *Oryza* species. *In* IRRI (ed.), Rice Genetics and Cytogenetics, Elsevier, Amsterdam, pp. 91-110.

Nakagahra, M., K. Okuno, and D. Vaughan. 1997. Rice genetic resources: history, conservation, investigative characterization and use in Japan. Plant Mol. Biol. **35:** 69-77.

Ohmido, N. and K. Fukui. 1997. Visual verification of close disposition between a rice A genome-specific DNA sequence (TrsA) and the telomere sequence. Plant Mol. Biol. **35:** 963-968.

Oka, H.I. 1988. Origin of Cultivated Rice. Elsevier, Amsterdam, 255 pp.

Page, B.T., M.K, Wanous, and J.A. Birchler. 2001. Characterization of a maize chromosome 4 centromeric sequence: Evidence for an evolutionary relationship with the B chromosome centromere. Genetics **159:** 291-302.

Richards, E.J. and F.M. Ausubel. 1988. Isolation of a higher Eukaryotic telomere from *Arabidopsis thaliana*. Cell **53:** 127-136.

Rivin, C.J., C.A. Cullis, and V. Walbot. 1986. Evaluating quantitative variation in the genome of *Zea mays*. Genetics **113:** 1009-1019.

Saul, M.W. and I. Potrykus. 1984. Species-specific repetitive DNA used to identify interspecific somatic hybrids. Plant Cell Rep. **3:** 65-67.

Sitch, L.A., R.D. Dalmacio, and G.O. Romero.1989. Crossability of wild *Oryza* species and their potential use for improvement of cultivated rice. Rice Genet. Newslett. **6:** 58-60.

Skinner, D.Z. 1992. A PCR-bssed method of identifying species-specific repeated DNAs. Biotechniques **13:** 210-214.

Svitashev, S., T. Bryngelsson, X. Li, and R.R. Wang. 1998. Genome-specific repetitive DNA and RAPD markers for genome identification in *Elymus* and *Hordelymus*. Genome **41:** 120-128.

Thormann, C.E. and T.C. Osborn. 1992. Use of RAPD and RFLP markers for germplasm evaluation. *In* Applications of RAPD Technology to Plant Breeding: Proceedings of the Joint Plant Breeding Symposia Series, Minneapolis, Minn., *In* D. Hoisington and A. McNab (eds.), Crop Science Society of America. American Society for Horticultural Science. American Genetic Society, Minneapolis, Minn., pp. 9-11.

Uozu, S., H. Ikehashi, N. Ohmido, H. Ohtsubo, E. Ohtsubo, and K. Fukui. 1997. Repetitive sequences: cause for variation in genome size and chromosome morphology in the genus *Oryza*. Plant Mol. Biol. **35:** 791-799.

Vaughan, D.A. 1994. The wild relatives of rice: a genetic resources guide book. International Rice Research Institute, Los Banos, The Philippines.

Vaughan, D.A., H. Morishimay, and K. Kadowaki. 2003. Diversity in the *Oryza* genus. Curr. Opin. Plant Biol. **6:** 139-146.

Wang, S., N. Liu, K. Peng, and Q. Zhang. 1999. The distribution and copy number of *copia*-like retrotransposons in rice (*Oryza sativa* L.) and their implications in the organization and evolution of the rice genome. Proc. Natl. Acad. Sci. USA **96**: 6824-6828.

Wang, Z.X., N. Kurata, S. Saji, Y. Katayose, and Y. Minobe. 1995. A chromosome 5-specific repetitive DNA sequence in rice (*Oryza sativa* L.). Theor. Appl. Genet. **90:** 907-913.

Wang, S, Q. Zhang, P.J. Maughan, and M.A. Saghai Maroof. 1997. *Copia*-like retrotransposons in rice: sequence heterogeneity, species distribution and chromosomal locations. Plant Mol. Biol. **33:** 1051-1058.

Wilkie, S.E., P.G. Isaac, and R.J. Slater. 1993. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. Theor. Appl. Genet. **86:** 497-504.

Willard, H.F. and J.S. Waye. 1987. Hierarchical order in chromosome-specific human alpha satellite DNA. Trends Genet. **3:** 192-198.

Williams, J.G.K., M.K. Hanafey, J.A. Rafalski, and S.V. Tingey. 1993. Genetic analysis using random amplified polymorphic DNA markers. Methods Enzymol. **218:** 704-740.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:** 6531-6535.

Wing, R.D., J. S.S. Ammiraju, M. Luo, H. Kim, Y. Yu, D. Kudrna, J. L. Goicoechea, W. Wang, W. Nelson, K. Rao, D. Brar, D. J. Mackill, B. Han, C. Soderlund, L. Stein, P. San-Miguel, and S. Jackson. 2005. The Oryza Map Alignment Project: the golden path to unlocking the genetic potential of wild rice species. Plant Mol. Biol. **59:** 53-62.

Wu, H.K., M.C. Chung, T.Y. Wu, C.N. Ning, and R. Wu. 1991. Localization of specific repetitive DNA sequences in individual rice chromosomes. Chromosoma **100:** 330-338.

Wu, J.Y., H.K. Wu, and M.C. Chung. 2002. Co-dominant RAPD markers closely linked with two morphological genes in rice (*Oryza sativa* L.). Bot. Bull. Acad. Sin. **43:** 171-180.

Xiao, J., J. Li, S. Grandillo, S.N. Ahn, L. Yuan, S.D. Tanksley, and S.R. McCouch. 1998. Identification of trait-improving quantitative trait loci alleles from wild rice relative, *Oryza rufipogon*. Genetics **150:** 899-909.

Yan, H.H., G.Q. Liu, Z.K. Cheng, X.B. Li, G.Z. Liu, S.K. Min, and L.H. Zhu. 2002. A genome-specific repetitive DNA sequence from *Oryza eichigeri*: Characterization, localization, and introgression to *O. sativa*. Theor. Appl. Genet. **104:** 177-183.

野生水稻物種中 B 型基因組特有的重複序列之研究

鄭月雲　方紹安　林耀正　鍾美珠

中央研究院 植物暨微生物學研究所

利用分子標記可以辨認特定的基因組及監測種間雜交後代之基因組滲入程度。本文報導由野生稻 *Oryza punctata Kotschy ex Steud* (W1593) 分離得的逢機引子擴增 DNA 片段 (RAPD)，命名為 *Opun*210，此為稻屬物種中帶有 B 型基因組者所特有的重複序列。以逢機引子 UBC 210 對稻屬各物種進行之聚合酶增幅反應，增幅所得產物中有一個 B 型基因組專一性的條帶出現，稱之為 *Opun*210。本研究選殖並分析由非洲野生水稻 *O. punctata* (W1593) 基因組 DNA 擴增所得的 *Opun*210 片段；*Opun*210 是第一個被報導的稻屬B型基因組物種專一性的重複序列，具有高度的物種專一性，甚至可以有效的分辨同一物種之不同族群。*Opun*210 片段長度為 789 bp，在 *O. puntata* (W1593) 單套基因組內約有 5.3×10^4 個重複。將*Opun*210 序列與基因資料庫中的已知序列比對，可找到少數的同源序列，且在 5′ 端約有 500 bp 長度的核苷序列的同源性較高， 而在 3′ 端核苷序列的同源性較低。SCAR-PCR 的結果確定 *Opun*210 序列為帶有 B 型基因組之稻屬物種特有。 此外，在 *Opun*210 序列中，位在第 430~480 的核苷序列與一個 Ty3-gypsy 型的反轉錄跳躍子之蛋白的預測編碼區有 88% 相同，或與一個未知蛋白的編碼有 94% 相同。 南方墨漬分析及螢光原位雜合反應的結果顯示，高度重複的 *Opun*210 序列散布在 *O. puntata* 全基因組內。根據所得到的資料，本文討論 *Opun*210 序列的起源與其在稻屬各物種間的歧異性。

關鍵詞：螢光原位雜合反應；野生稻 *Oryza punctata*；重複序列；物種專一性；逢機引子多型性標誌。