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RESEARCH ARTICLE

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Comparative analysis of genomes in *Oryza sativa*, *O. officinalis* and *O. meyeriana* with C_0t-*1* DNA and genomic DNA of cultivated rice

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Abstract Fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) were applied to somatic chromosome preparations of *Oryza sativa*, *O. officinalis* and *O. meyeriana* with labeled probes of C_0t-*1* DNA and genomic DNA from cultivated rice. The coverage percentage (%) and size (Mb) of C_0t-*1* DNA in *O. sativa*, *O. officinalis and O. meyeriana* were 47.1 ± 0.16, 38.61 ± 0.13, 44.38 ± 0.13 and 212.33 ± 1.21, 269.42 ± 0.89, 532.56 ± 1.68, respectively. The coverage percentage and size of probe signals with genomic DNA from *O. sativa* in *O. officinalis* and *O. meyeriana* were 91.0%, 93.6% and 634 Mb, 1 123 Mb respectively, in which there were 365 and 591 Mb in *O. officinalis* and *O. meyeriana* which came from *O. sativa* genomic DNA not from repetitive sequences of *O. sativa*, and the uncovered genome size in *O. officinalis* and *O. meyeriana* was 64 and 78 Mb, respectively. In addition, karyotype analysis was conducted based on the signal bands of C_0t-*1* DNA in *O. sativa*, *O. officinalis* and *O. meyeriana*. The results showed that highly and moderately repetitive sequences in *Oryza* genus were conserved as the functional genes during the evolution process. The repetitive sequence reduplication might be one of the important causes of genome enlargement in *O. officinalis* and *O. meyeriana*; the *O. officinalis* genome enlarged more slowly compared with *O. meyeriana*. Based on the above results, it is concluded that *O. officinalis* and *O. meyeriana* formed by reduplication, rearrangement and gene selective loss during the evolution process.

Keywords C_0t-*1* DNA, comparative genomic hybridization (CGH, karyotype), *O. officinalis*, *O. meyeriana*

1 Introduction

Rice is one of the most important crop species and a biomodel in plant genome studies. Rice upgrades have the same or similar genetic sources (Chang, 1984), resulting in genetic backgrounds strait of cultivated rice, gene loss, and a diversity decline of species (Tanksley and McCouch, 1997). Wild rice species are important germplasm pools that contain various resistance genes obtained from natural selection under complex geographical and ecological environments. The genetic diversity of wild rice is richer than cultivated rice (Sun et al., 2000; Dong 2003), and many excellent genes have been discovered, including cytoplasmic male sterility and high resistance to diseases, insects, cold and heat climate (Khush et al., 1990; Xiao et al., 1996). Using these genes, people could increase their products of cultivated rice, encouraging more research applications on wild rice (He, 1998; Lu, 1998; He and Shu, 2003). The genomes of *O. officinalis* and *O. meyeriana* are CC and GG, which differ from *O. sativa* (AA). Previous studies showed that wild rice with non-AA genome possessed richer molecular polymorphism, which was helpful for the utilization of wild rice germplasm in rice breeding (Jena and Khush, 1990; Wu et al., 1998). Owing to the differentiation between AA and non-AA genomes, reproductive inability often appeared in interspecific hybrids of genus *Oryza*, and germplasm transference and intercommunion among different species were impeded. Molecular biology played an important role in studying and utilizing genes, analyzing genetic content, checking foreign DNA fragment, and locating and cloning important functional

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genes of wild rice (Zhang et al., 1998; Qin et al., 2002; He and Shu, 2003).

The repetitive DNA sequence is an important characteristic of eukaryotic genomes that is dispersed in the whole genome (Flavell et al., 1974; Bennetzen et al., 2005). They usually account for more than 50% in genomes of higher plants such as rice, maize, wheat, rye, and lemon; the content percentages of their repetitive sequences are 50%, 78%, 83%, 92%, and 95%, respectively (McCouch and Tanskley, 1991; Bennetzen et al., 2005;). A majority of these sequences are non-coding, making it difficult to know their roles in the evolution process among different genomes. Used as molecular markers, the repetitive DNA sequences were important in comparing genomes, analyzing the structure and function of genomes, and studying the evolution of chromosomes and genomes. For example, microsatellite DNA was used for studies that include genetic linkage map construction and variety identification and improvement (Yin and Hartemink, 2005).

In this study, the highly and moderately repetitive sequences of Guang Lu Ai 4 of *O. sativa*, i.e. C_0t-*1* DNA, was used as a probe for in situ hybridization on mitotic metaphase chromosomes of *O. sativa*, *O. officinalis* and *O. meyeriana*, and the signal distribution of the repetitive sequences was analyzed in these three genomes. The genomic DNA of the cultivated rice was also used for a probe into genomic in situ hybridization (GISH) in the three species, namely comparative genomic hybridization (CGH). The results revealed the genomic structure and distribution of different DNA sequences in the genomes. Furthermore, the evolutional relationship among *O. sativa*, *O. officinalis*, and *O. meyeriana* was studied by comparing the homologous sequences among different species in genus *Oryza*. These results would be helpful for using wild rice species in the development of cultivated rice.

2 Materials and methods

2.1 Plant material and chromosome preparation

Guang Lu Ai 4 of *O. sativa* was supplied by Professor Zeng Zuokui, Hubei Academy of Agriculture Sciences. The line 1 589 of *O. officinalis* was supplied by Guang Dong National Wild Rice Garden, *O. meyeriana* was supplied by the Genetic Institute of Wuhan University, while chromosome preparations were performed according to the method of Yan et al. (1998) and Ren et al. (1997).

2.2 Preparation of C_0t-*1* DNA

Total genomic DNA of Guang Lu Ai 4 of *O. sativa* was extracted using the CTAB method according to Doyle and Doyle (1990). C_0t-*1* DNA preparation was based on the work of Zwick et al. (1997). Genomic DNA was sterilized under 0.14 Mpa for 3–15 min and broken into fragments of 800–1 500 bp. The DNA was reannealed at 65°C for the required time calculated according to the formula C_0t-*1* = mol/L × *T*s, after which S1 nuclease (2 U/μg DNA, Promega, USA) was added. The tube was placed in a water bath at 37°C for 1 h. The DNA was extracted twice by equal volumes of phenol-chloroform, precipitated with isopropanol and washed with prechilled 70% ethanol, and dried and resuspended in TE buffer. The C_0t-*1* DNA was quantified and stored at –20°C before use.

2.3 Probe labeling

C_0t-*1* DNA of *O. sativa* was labeled with biotin-11-dUTP using a nick translation kit (Roche, Germany) according to the manufacturer's instructions. The length of the C_0t-*1* DNA used as the probe for FISH was estimated by gel electrophoresis to be between 300 and 500 bp. The labeled probe was separated from unincorporated nucleotides by passage through a Sepharose CL-6B (Sigma, USA) column. Incorporation of biotin-11-dUTP was evaluated by means of dot blots through alkaline phosphatase conjugate (Roche, Germany) detection.

2.4 In situ hybridization and detection

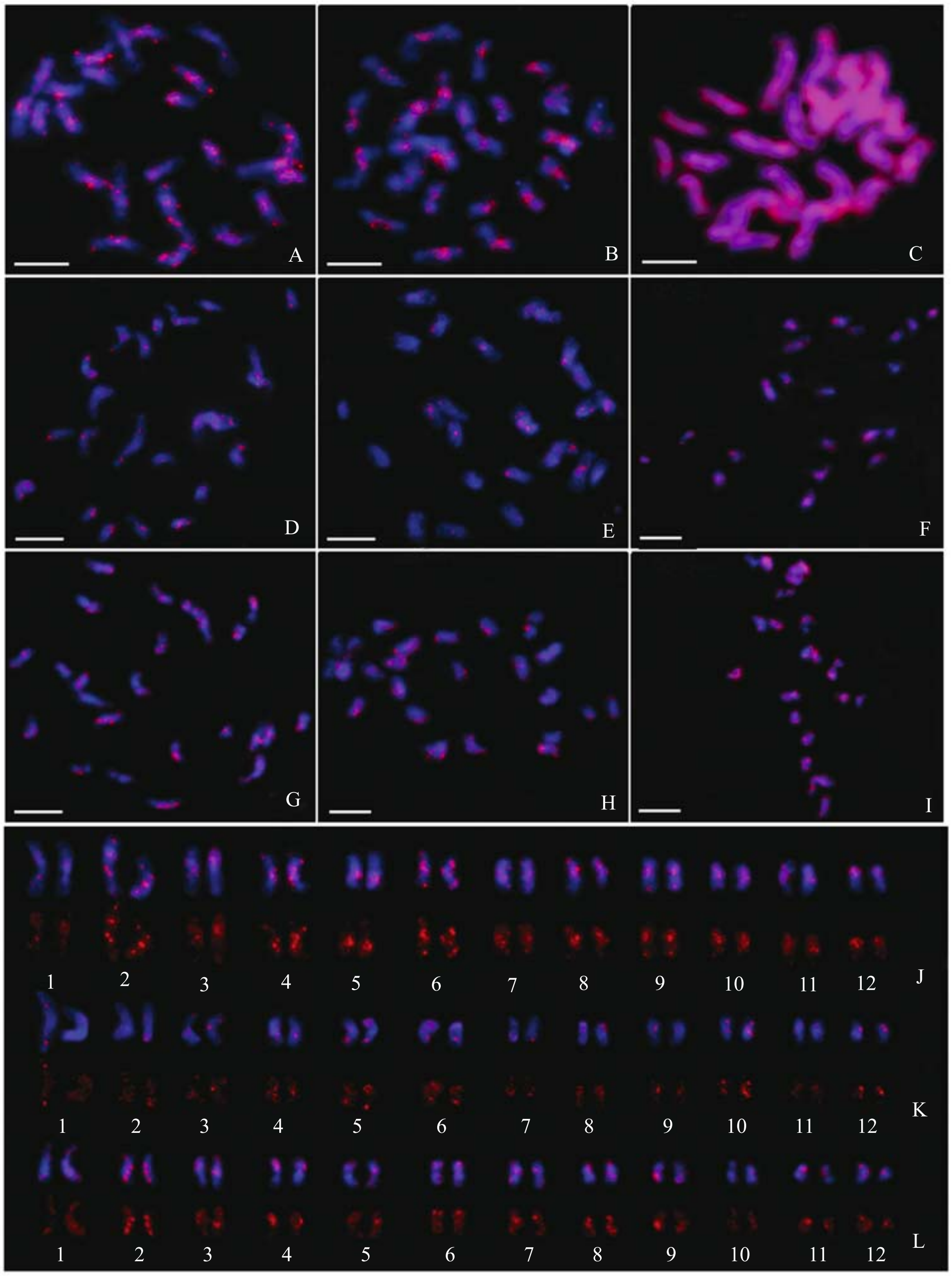
Fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) were carried out following the method of Jiang et al. (1995) and Wei et al. (2001) with some modifications. The chromosome preparations were dried at 60°C for 1 h, pretreated with RNase A (Promega, USA) at 37°C for 1 h, and followed by a 5 min wash in 2 × SSC at 37°C twice. Chromosomal DNA was denatured by immersing the slides in 70% deionized formamide (Sigma, USA) in 2 × SSC at 70°C for 3.5–5 min, dehydrated in an ice-cold ethanol series (70%, 95% and 100%), and air-dried.

The hybridization mixture consisted of 50% deionized formamide, 8% (w/v) sodium dextran sulphate (Amresco, USA), 0.5% (w/v) SDS, 0.5 μg of salmon sperm DNA (Sigma, USA) in 2× SSC, and 80 ng of labeled probes for each slide. Denatured in boiled water for 10 min, the mixture was immediately chilled in ice for 10 min. The 50 μL of hybridization mixture was applied per slide and covered with a plastic slip. The slides were placed in a humidity chamber and denatured at 90°C for 10 min, then incubated at 37°C overnight. To detect the fluorescent signals, the slides were washed first in SSC, then in PBS at room temperature. Detection of a biotin-labeled probe was achieved with streptavidin-Cy3 (Rockland, USA). The preparations were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). The chromosomes were observed with an Olympus BX61 fluorescence microscope and photographed with a Cool-1300QS CCD controlled by a Manager Expo 2.1.1 imaging system. Karyotype analysis was carried out using the chromosome analyzing system FISHView EXPO 2.0 software. The relative chromosome lengths were measured by SPOT advanced software.

3 Results

3.1 Fluorescence in situ hybridization of C_0t-*1* DNA

C_0t-*1* DNA was used as a probe to in situ hybridize somatic chromosome preparations of *O. sativa*, *O. officinalis* and *O. meyeriana*. Figure 1(A) and (B) are FISH images of *O. sativa s* probed with its own C_0t-*1* DNA. Figure 1(D), (E) and (G), (H) are FISH images of *O. officinalis* and *O. meyeriana* probed with C_0t-*1* DNA from *O. sativa*, respectively. The results showed that C_0t-*1* DNA signals distributed mainly in the areas of centromeres, subcentromeres and telomeres, and much fewer signals in the middle regions of chromosome arms. The signals were detected in all chromosomes of the three species, but the coverage of signals was different on chromosomes of the species (Fig. 1(A), (B), (D), (E), (G) and (H)). The coverage area of hybridization signals of C_0t-*1*



Notes: A and B stand for FISH images of *O. sativa* probed with its own C_0t-*1* DNA; C for GISH image of *O. sativa* probed with its own genomic DNA; D and E for FISH images of *O. officinalis* probed with C_0t-*1* DNA from *O. sativa*; F for GISH image of *O. officinalis* probed with genomic DNA from *O. sativa*; G and H for FISH images of *O. meyeriana* probed with C_0t-*1* DNA from *O. sativa*; I for GISH image of *O. meyeriana* probed with genomic DNA from *O. sativa*; and J, K and L for Karyotypes of *O. sativa*, *O. officinalis* and *O. meyeriana* reconstructed based on C_0t-*1* DNA signal bands, respectively (Bar = 10 μm).

Fig. 1 Comparative analysis of genomes in *Oryza sativa*, *O. officinalis* and *O. meyeriana* with C_0t-*1* DNA and genomic DNA

DNA was calculated using SPOT advanced software. The percentage of *O. sativa* C_0t-1 DNA on chromosomes of *O. sativa*, *O. officinalis* and *O. meyeriana* genomes is shown in Table 1. The coverage percentage (%) of C_0t-1 DNA in *O. sativa*, *O. officinalis* and *O. meyeriana* was 47.1 ± 0.16, 38.61 ± 0.13 and 44.38 ± 0.13, respectively. It was established that the genome size of *O. sativa*, *O. officinalis* and *O. meyeriana* was 450, 697 and 1 201 Mb (Uozu et al., 1997). Thus, the content of C_0t-1 DNA in *O. sativa*, *O. officinalis* and *O. meyeriana* was 212.33 ± 1.21, 269.42 ± 0.89 and 532.56 ± 1.78 Mb, respectively (Table 1).

Table 1 Distribution data of C_0t-1 DNA and genomic DNA hybridization signals in *O. sativa*, *O. officinalis* and *O. meyeriana*

Species	Signal coverage percentage of C_0t-1 DNA/%	Signal coverage percentage of genomic DNA/%	C_0t-1 DNA content /Mb	Genomic DNA content /Mb
O. sativa	47.17 ± 0.16[a)]	100 ± 0.15	212.33 ± 1.21	450.00
O. officinalis	38.61 ± 0.13	91.03 ± 0.12	269.42 ± 0.89	634.67 ± 1.07
O. meyeriana	44.38 ± 0.11	93.56 ± 0.10	532.56 ± 1.78	1123.56 ± 1.12

Notes: [a)] means standard deviation.

3.2 Comparative genomic hybridization analysis

Genomic DNA was used as a probe for CGH in *O. sativa*, *O. officinalis*, and *O. meyeriana*. In Fig. 1, C, F and I show the GISH images of *O. sativa*, *O. officinalis*, and *O. meyeriana* probed by the genomic DNA from *O. sativa*, respectively. The hybridization signals covered whole chromosomes of *O. sativa* (Fig. 1(C)). Strong fluorescent signals were detected on chromosomes of *O. officinalis* and *O. meyeriana* (Fig. 1(F) and (I)), signal coverage percentage of genomic DNA was 91.03% ± 0.12 and 93.56% ± 0.10 using SPOT advanced software, and the coverage size were 634.67 ± 1.07 and 1123.56 ± 1.12 Mb, respectively (Table 1).

3.3 Karyotype analysis based on FISH images

The relative lengths of *O. sativa*, *O. officinalis*, and *O. meyeriana* chromosomes were measured by SPOT advanced software, and the karyotypes were reconstructed using FISHView EXPO 2.0 software (Fig. 1(J), (K) and (L)). Figure 1(K) and (L) are karyotype images of *O. officinalis* and *O. meyeriana*. In each image, the upper part is a combined image, while the lower part represents red hybridization signals of C_0t-1 DNA.

Karyotype analysis of *O. sativa* was performed according to the standard karyotype of the International Rice Institute, and the conventional method was also adopted in *O. officinalis* and *O. meyeriana*. According to FISH images, repetitive sequences had specific signal bands on chromosomes. Since the homologous chromosomes exhibited similar signal bands, karyotype analysis was conducted based on the C_0t-1 DNA specific band possible in the three species. The results of karyotype analysis of *O. sativa*, *O. officinalis* and *O. meyeriana* are summarized in Table 2. C_0t-1 DNA signals mainly distributed on chromosomes 2, 4, 8 and 10 of *O. sativa*, with the fewest on chromosomes 1, 3 and 12 (Fig. 1(J)). In *O. officinalis*, C_0t-1 DNA signals mainly distributed on chromosomes 5, 6 and 10, with only a few on chromosomes 1, 2, 3, 7, 9 and 11 (Fig. 1(K)). Relatively uniform signals were detected on the chromosomes in *O. meyeriana* except chromosomes 10, 11 and 12 (Fig. 1(L)).

4 Discussion

The repetitive DNA sequences are usually more than 50% in genomes of higher plants (Bennett and Leitch, 2005), and they can be classified as low, moderately and highly repetitive sequences. C_0t-1 DNA contains mainly highly and moderately repetitive DNA sequences (Zwick et al., 1997). Past research on plant genome principally concentrated on tandem-repeated DNA sequences: satellite repeat sequences, centromere-specific repeated DNA sequences, telotrisomics repeated DNA sequences and retrotransposons (Wang et al., 2000; Quiroz, 2002; Yu et al., 2003; Bennett and Leitch, 2005). The analysis of repetitive sequences using FISH mostly focused on low repetitive sequences (Cheng et al., 2001; Zhao et al., 2005). The analysis of moderately and highly repetitive sequences mostly focused on animals and humans, and just a few studies were reported by Zwick et al. (1997). There has been no report yet about the quantitative signal analysis of highly and moderately repetitive sequences between different species using FISH. Comparison of physical locations of resistance genes *Pi-5(t)* and *Gm-6* between *O. sativa* and *O. officinalis* using BAC-FISH was reported in the previous study. It had shown chromosomes of *O. officinalis* displaying many non-specific hybridization signals probing with a labeled BAC clone obtained from cultivated rice. The study also indicated a relatively high homology of the repetitive DNA sequences between the genomes of cultivated rice and *O. officinalis* (Qin et al., 2001). Thus, the cultivated rice C_0t-1 DNA was used as a probe to make a comparative analysis between *O. sativa*, *O. officinalis* and *O. meyeriana* in this study. Figure 1 shows many highly and moderately repetitive sequences of cultivated rice in the genome of *O. sativa*, *O. officinalis* and *O. meyeriana*, with coverage percentage (%) and content (Mb) of 47.1 ± 0.16, 38.61 ± 0.13, 44.38 ± 0.13 and 212.33 ± 1.21, 269.42 ± 0.89, 532.56 ± 1.68, respectively (Table 1). These results indicated that highly and moderately repetitive sequences in genus *Oryza* were conserved as the functional genes during evolution.

The genome size of *O. officinalis* was 697 Mb, which was 1.5 times larger than that of *O. sativa* (450 Mb), and that of *O. meyeriana* was 1 201 Mb, approximately 2.7 times as large as that of *O. sativa* (Uozu et al., 1997). In this study, there were about 269 and 532 Mb C_0t-1 DNA in the genome of *O. officinalis* and *O. meyeriana* (Table 1), approximately 1.3 and 2.5 times as large as that of *O. sativa* (212 Mb). The multiple ratio of *O. sativa* C_0t-1 DNA in the genome of *O. officinalis* and *O. meyeriana* was close to that of genomic DNA in the

Table 2 Karyotype analysis of *O. sativa*, *O. officinalis* and *O. meyeriana*

No.	*O. sativa*			*O. officinalis*			*O. meyeriana*		
	AR±SD a)	RL±SD b)	PC c)	AR±SD	RL±SD	PC	AR±SD	RL±SD	PC
1	1.22±0.13	12.53±0.43	m	1.33±0.23	12.57±0.40	m	1.25±0.27	13.01±0.47	m
2	1.13±0.48	11.92±0.29	m	1.47±0.36	10.66±0.11	m	1.49±0.49	12.01±0.14	m
3	1.80±0.32	10.21±0.32	sm	2.25±0.67	10.49±0.53	sm	1.46±0.16	10.26±0.24	m
4	1.25±0.17	8.86±0.55	m	1.23±0.24	9.07±0.21	m	1.15±0.31	9.33±0.55	m
5	1.40±0.27	8.50±0.48	m	2.21±0.37	8.83±0.32	sm	1.22±0.51	9.02±0.19	m
6	1.10±0.47	7.73±0.31	m	1.31±0.45	7.91±0.28	m	1.13±0.27	8.73±0.22	m
7	2.81±0.43	7.57±0.27	sm	1.90±0.49	7.41±0.50	sm	1.33±0.24	7.31±0.36	m
8	1.73±0.44	6.94±0.31	sm	1.20±0.49	7.39±0.11	m	1.24±0.41	7.01±0.11	m
9	1.26±0.29	6.68±0.48	m	1.32±0.25	7.31±0.57	m	1.77±0.17	6.55±0.46	sm
10	1.28±0.31	6.51±0.37	m	1.27±0.46	6.23±0.44	m	1.90±0.33	6.34±0.53	sm
11	1.82±0.38	6.36±0.17	sm	1.21±0.39	6.14±0.39	m	1.11±0.49	5.46±0.17	m
12	1.15±0.33	6.24±0.54	m	2.22±0.21	6.06±0.48	sm	1.32±0.14	5.07±0.20	m

Notes: a) stands for arm ratio±SD, b) for relative length±SD, and c) for position of centromere.

genome of *O. officinalis* and *O. meyeriana*. This suggested that repetitive sequence reduplication might be one of the important causes of genome enlargement in the evolutional process. C_0t-*1* DNA content in *O. meyeriana* genome (532 Mb) was very large (Table 1), and C_0t-*1* DNA distributed among most chromosomes in *O. meyeriana* (Fig. 1(L)). This indicates that the genome of *O. meyeriana* originated from a primal genome and was formed by sudden DNA reduplication. Compared with *O. meyeriana*, the genome in *O. officinalis* enlarged more slowly and only 269 Mb C_0t-*1* DNA distributed on the chromosome, which was 57 Mb more than *O. sativa* (212 Mb). In addition, C_0t-*1* DNA mainly distributed on chromosomes 5, 6 and 10 (Fig. 1(K)), which indicated that the highly and moderately repetitive sequence reduplication took place only in some regions of the chromosomes.

The results of CGH analysis of *O. sativa*, *O. officinalis* and *O. meyeriana* indicated that the coverage percentage of hybridized signals probing with labeled genomic DNA from *O. sativa* on chromosomes of *O. officinalis* and *O. meyeriana* was approximately 91.0% and 93.6%, respectively (Table 1), suggesting a high homology of genomes among these species. In addition, the homologous genomes of the corresponding C_0t-*1* DNA, and C_0t-*1* DNA content in the two species were 269 and 532 Mb, while genome sizes of *O. officinalis* and *O. meyeriana* were about 634 and 1 123 Mb, respectively. There were 365 and 591 Mb of DNA sequences not belonging to the highly and moderately repetitive sequences, but to the low and simple repetitive sequences from *O. sativa*. These sequences may be genes or non-coding sequences; for the genes, low and simple repetitive sequences had been proven to be conserved in synteny and colinearity in different plant species and genomes (Feuillet and Keller, 1999; Choi et al., 2004), which was consistent with the results. The results also revealed the feasibility of the new method for comparative genome quantitative analysis by FISH. In addition, some regions of chromosomes of *O. officinalis* and *O. meyeriana* genome were not covered by *O. sativa* genomic DNA probes. These genome contents, which were 64 and 78 Mb, respectively, include specific low, highly and moderately repetitive sequences. These may have originated from a primal genome and formed by DNA reduplication and gene mutation accumulation, with DNA sequences lost in the process. Previous studies also showed that the DNA reduplication, rearrangement and gene selective loss are common in plant genomes (Ku et al., 2000; Multani et al., 2003; Yin and Hartemink, 2005; Yogeeswaran et al., 2005). In this study, the differentiations in genomes between *O. meyeriana* and *O. sativa* were more than those of *O. officinalis* and *O. sativa* (Table 1). This may be one of the reasons causing variations between wild rice and cultivated rice, and can explain why a crossbreeding between *O. officinalis* and *O. sativa* is easier than that between *O. meyeriana* and *O. sativa* (He and Shu, 2003).

The chromosomes of *Oryza* are small in size, similar in configuration, and inconspicuous in centromere, making it difficult to identify homologues and distinguish different chromosomes in generating a karyotype by morphological means. The BAC-FISH was once used to overcome the difficulties in our previous study (Cheng et al., 2001). Comparison of BAC-FISH analysis on the chromosome 4 between *O. officinalis* and cultivated rice was conducted based on their homology on comparative RFLP-maps, but not the lengths of chromosomes (Cheng et al., 2001). Compared with conventional karyotype analysis, the technique reported in this study was based on the genome constitution and was faster and more exactly matched the homologous chromosomes and discriminated between different chromosomes. At present, comparative karyotype analysis of other chromosomes between *O. officinalis* and *O. meyeriana* is being carried out. The results showed that specific signal bands of labeled C_0t-*1* DNA from *O. sativa* displayed on all the chromosomes of *O. sativa*, *O. officinalis* and *O. meyeriana* (Fig. 1(J), (K) and (L)) and the homologous chromosomes exhibited similar signal bands. This karyotype analysis based on C_0t-*1* DNA signal bands differed from the conventional karyotype analysis. It was established beyond doubt that the technology combining the band patterns of C_0t-*1* DNA with BAC-FISH analysis should increase the veracity of chromosome recognition in *Oryza* plants. Furthermore, this study was based upon comparative genomics, and could enhance the efficiency of studying the evolution of chromosomes, genomes and repetitive sequences, and consequently accelerate the establishment of the large genetic system for *Oryza* plants, even for the genera of the grass family.

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