Front. Agric. China 2011, 5(2): 173–180
DOI 10.1007/s11703-011-1092-4

RESEARCH ARTICLE

Comparative karyotypic analysis of A and C genomes in the genus *Oryza* with C_0t-1 DNA and RFLP

Junbo ZHOU[1], Weizhen LAN (✉)[2], Rui QIN (✉)[3]

[1] *College of Life Sciences, Huzhou Teachers College, Huzhou 313000, China*
[2] *The Third Middle School of Wuyi, Jinhua 321200, China*
[3] *Key Laboratory of State Ethnic Affairs Commission for Biological Technology, College of Life Sciences, South-Central University for Nationalities, Wuhan 430074, China*

Abstract **Fluorescence *in situ* hybridization (FISH) was applied to somatic chromosomes preparations of *Oryza sativa* L. (AA), *O. glaberrima* (AA), and *O. officinalis* Wall. (CC) with a labeled probe of C_0t-1 DNA. Genomic *in situ* hybridization to its own chromosomes (self-GISH) was conducted in a control experiment. The homologous chromosomes showed similar signal bands probed by C_0t-1 DNA, while karyotypic analysis of chromosomes between A genome in the two cultivated species and C genome in *O. officinalis* were conducted based on the band patterns. The ideograms with C_0t-1 DNA signal bands were also built. The nonuniform distribution of hybridization signals of C_0t-1 DNA from *O. sativa* and that on its own chromosome of *O. officinalis* were observed. However, the similarity and correspondence between C_0t-1 DNA signal patterns and genomic DNA signal patterns indicated that the self-GISH signals actually resulted from the hybridization of genomic repetitive sequences to the chromosomes. The restriction fragment length polymorphism (RFLP) marker, R2676, from the chromosome 8 of *O. sativa* and *O. officinalis*, was used as a probe to somatic hybrid on chromosomes for comparative karyotypic analysis between *O. glaberrima* and *O. officinalis*. The results showed that R2676 was located on the short arm of chromosome 7 in *O. officinalis* and chromosome 4 in *O. glaberrima*. The percentage distances from the centromere to hybridization sites were 91.56±5.62 and 86.20±3.17. Our results revealed that the relative length of *O. officinalis* chromosome 8 does not follow conventional chromosome length in descending order of number. C_0t-1 DNA of A genome signals were detected in the end of the short arm of *O. officinalis* chromosome 8, indicating that the highly and moderately repetitive DNA sequences in this region were considerably similar between C and A genomes. However, the fluorescence intensity on the chromosomes of C_0t-1 DNA of A genome was less than that of its own C genome from *O. officinalis*, which would be one of the causes for the fact that highly and moderately repetitive DNA sequences were amplified in *O. officinalis*. No homology signal of C_0t-1 DNA from *O. sativa* was detected in the end of the long arm of *O. glaberrima*, indicating that repetitive DNA sequences of A genome in two cultivated rice were lost in the evolutional history. In this paper, using comparative karyotypic analysis of RFLP combined C_0t-1 DNA signal bands, the evolutionary mechanism of genome in genus *Oryza* was also discussed.**

Keywords C_0t-1 DNA, RFLP, karyotype, *in situ* hybridization, *Oryza*

Introduction

The genus *Oryza* comprises more than 20 species including two cultivated species, *O. sativa* L. and *O. glaberrima* Steud (Vaughan, 1994). The number of rice chromosome was $2n$ = 24 or 48, and the genomes of species in *Oryza* are classified into 10 types: AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, and HHKK (Lu, 1999; Vaughan, 2003). Historically, advances in rice chromosome research are quite limited, as the small chromosome of *Oryza*, similar configuration, and unconspicuous centromere make it difficult to identify homologs and distinguish different chromosomes to generate a karyotype by morphological means (Shishido et al., 2001).

Received November 11, 2010; accepted January 18, 2011
Correspondence: [a]Weizhen LAN; [b]Rui QIN
E-mail: [a]lanwz@hotmail.com; [b]qinrui@hotmail.com

With the first *in situ* hybridization on rice chromosomes reported in 1987, using I_{125} labeled rRNAs, the genomes *in situ* hybridization (GISH) on rice chromosomes and chromosome painting developed very quickly for rice chromosome analysis. Repetitive DNA sequences are either organized in tandem arrays or dispersed throughout the whole genome (Harrison and Heslop-Harrison, 1995; Ohmido et al., 2000). The DNA sequences of a repeat and its copy number at each chromosome site can all be evolved rapidly, leading to its specificity to a certain genome species and even a chromosome (Wang et al., 1995; Matyásek et al., 1997; Uozu et al., 1997; Hall et al., 2004). In this respect, the genome specific repetitive DNA sequence is a proper molecular marker in analyzing the nuclear genome chromosome composition and derivatives (Cheng et al., 2001a; Cheng et al., 2002; Mishima et al., 2002; Yan et al., 2002; Jin et al., 2004; Jin et al., 2005). During the last decade, researches on specific repetitive DNA sequence, such as rDNA, DNA transposon, and centromere DNA showed that specific repetitive DNA site number is one of the most important evolutionary dynamics of genome (Zwick et al., 2000; Mishima et al., 2002; Hall et al., 2004; Jiang et al., 2004). It has been suggested that one of the main factors that determine the variation in genome size is the quantity of repetitive DNA sequences (Uozu et al., 1997; Jiang et al., 2004). Therefore, repetitive sequences are likely to play an important role in genome differentiation during speciation. (Jiang et al., 1996; Uozu et al., 1997; Jiang et al., 2004; Yan et al., 2005).

C_0t-*1* DNA contains mainly highly and moderately repetitive DNA sequences, including satellites, microsatellites, and those located in centromeres and telomeres, which determine the chromosome structure and characterization (Zwick et al., 1997). In our previous study, we used the C_0t-*1* DNA of Guang Lu Ai 4 of *O. sativa*, as a probe for *in situ* hybridization on mitotic metaphase chromosomes of *O. sativa*, *O. officinalis*, and *O. meyeriana* to analyze signals distribution of the repetitive sequences in these three genomes (Lan et al., 2007). The C genome C_0t-*1* DNA was also hybridized to the somatic chromosome preparations of *O. latifolia* (CCDD), *O. alta* (CCDD), and *O. punctata* (BBCC) to analyze the relationships among B, C, and D genomes by distribution of C_0t-*1* DNA signals on chromosomes of different genomes (Lan et al., 2006). However, there are not much reports about the comparative karyotypic analysis of A and C genomes with C_0t-*1* DNA signal bands in the genus *Oryza*. At present, many RFLP (Restriction Fragment Length Polymorphism) clones have been genetically mapped on rice chromosomes (http://rgp.dna.affrc.go.jp) (Causse et al., 1994; Kurata et al., 1994; Harushima et al., 1998).

The RFLP markers have been used for indicating or localizing genes directly on rice chromosome with fluorescence *in situ* hybridization (FISH) (Song and Gustafson, 1995; Ohmido et al., 1998; Cheng et al., 2001b; Qin et al., 2001). RFLP markers have not been applied extensively in comparative karyotype with FISH method for localizing nucleotide sequences directly to chromosomes or interphase nuclei. The RFLP marker, R2676, from the chromosome 8 of *O. sativa* as well as *O. officinalis* through comparative genetic mapping analysis (Tan et al., 2005). Thus, the same RFLP marker is used as a probe to somatic hybrid on chromosomes for comparative karyotypic analysis between *O. glaberrima* and *O. officinalis*. This method has been used as a powerful technique to study small plant chromosome comparative karyotype in our laboratory before (Qin et al., 2001).

In this report, we first demonstrated the comparative karyotypic analysis based on the comparative RFLP map between *O. sativa* and *O. officinalis* (Tan et al., 2005). Based on the C_0t-*1* DNA banding on their chromosomes, we further analyzed the *O. sativa*, *O. glaberrima*, and *O. officinalis karyotype*. Furthermore, we used total genomic DNA as a probe for hybridization on the chromosomes of their own to contrast with the results of the C_0t-*1* DNA distribution on their chromosomes. Then, in light of the C_0t-*1* DNA banding on homologous chromosomes in different species, we elucidated what kind of genetic accident occurred during these species genome evolution, and the role of repetitive DNA sequences to the genome size in the genus *Oryza* was also discussed.

Materials and methods

Materials and chromosome preparations

The rice variety "Guang Lu Ai 4" (*O. sativa* L.) was provided by Professor Zuokui Zeng at the Hubei Academy of Agriculture Sciences, China. *O. glaberrima* Steud was provided by academician Yonggen Lu at the Huanan Agricultural University, China. Accession 1589 of *O. officinalis* Wall. ex Watt was supplied by the National Wild Rice Garden in Guangdong Province, China, which possesses a number of valuable traits, such as resistance to pests and diseases and high protein content in grains. The RFLP marker R2676 (1.8 kb), hybridized exclusively to the long arm of the chromosome 8 of *O. glaberrima* and *O. officinalis*, was supplied by the Institute of Genetics, Wuhan University, China. Chromosome preparations were performed following the method by Ren et al. (1997).

Preparation of C_0t-*1* DNA

Total genomic DNA of *O. officinalis* was extracted by following the CTAB method according to Doyle (1990). The 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 the fragments of 800–1500 bp. DNA was reannealed at 65 write units for the required time calculated according to the formula C_0t-*1* = mol/L × Ts. Then, S_1 nuclease (2 U/μg DNA, Promega, USA) was added.

The tube was placed in a water bath at 37ºC for 1 h. The DNA was extracted twice by equal volumes of phenol–chloroform, precipitated with isopropanol, washed with prechilled 70% ethanol, and dried and resuspended in TE buffer. The *C_0t-1* DNA was quantified and stored at −20ºC before it was used.

Probe labeling

The *C_0t-1* DNA of *O. officinalis* 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 that was used as the probe for FISH was estimated by gel electrophoresis to be 300 bp to 500 bp. The labeled probe was separated from unincorporated nucleotides by passage through Sepharose CL–6B (Sigma, USA) column. Incorporation of biotin–11–dUTP was evaluated by means of dot blots following the alkaline phosphatase conjugate (Roche, Germany) detection.

In situ hybridization and image observation

Fluorescence *in situ* hybridization (FISH) was carried out following the method of Li et al. (2001) with some modifications (Li et al., 2001). The chromosome preparations were dried at 60ºC for 1 h, pretreated with RNase A (Promega, USA) at 37ºC for 1 h, and followed by washing twice for 5 min in 2 × SSC at 37ºC. Chromosomal DNA was denatured by immersing the slides in 70% deionized formamide (Sigma, USA) in 2 × SSC at 70ºC for 3.5–5.0 min, dehydrated in an ice–cold ethanol series (70%, 95% and 100%), and then air dried.

The hybridization mixture consisted of 50% deionized formamide, 8% (w/v) sodium dextran sulfate (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. After being denatured in boiled water for 10 min, the mixture was immediately chilled in ice for 10 min, 50 μL of the hybridization mixture was applied per slide and covered with a plastic coverslip. The slides were placed in a humidity chamber and denatured at 90ºC for 10 min and then incubated at 37ºC overnight. For detection of the fluorescent signals, the slides were washed first in SSC and then in PBS at room temperature. The detection of the biotin-labeled probe and digoxigenin-labeled probe was achieved with streptavidin-Cy3 (Rockland, USA) and anti-digoxigenin–FITC (Roche), respectively. The preparations were counterstained with 4', 6-diamidino-2-phenylindole (DAPI) or Propidium iodide (PI). The chromosomes were observed with an Olympus BX61 fluorescence microscope and photographed with Cool–1300QS CCD controlled by Manager Expo 2.1.1 imaging system. Karyotypic analysis was carried out using the chromosome analyzing system FISHView EXPO 2.0 software. The chromosome relative lengths were measured by SPOT advanced software.

Results

Fluorescence *in situ* hybridization (FISH) with *C_0t-1* DNA and GISH

O. sativaC_0t-1 DNA and their own total genomic DNA were used as probes for *in situ* hybridization on mitotic metaphase chromosomes of *O. sativa*, *O. glaberrima*, and *O. officinalis* (Figs. 1A–F) and FISH with its own *C_0t-1* DNA to mitotic metaphase chromosomes of *O. officinalis* (Figs. 1G and H). The hybridization stringency and the washing stringency were 65%–75% and 80%–90%, respectively (Li et al., 2001). Under such conditions, the hybridization signals were highly specific. Figures 1A, C, E, F, and H show the red hybridization signals of *C_0t-1* DNA probes on chromosomes of *O. sativa*, *O. glaberrima*, and *O. officinalis*, respectively, which indicates that *C_0t-1* DNA signals are distributed mainly in areas of centromeres, subcentromeres, and telomeres, with much fewer ones on the middle regions of chromosome arms. There were strong signals on the *O. sativa* and *O. glaberrima* chromosomes (Figs. 1A and C), but there were rather weak signals on the *O. officinalis* chromosomes by using the *O. sativa C_0t-1* DNA as probe (Fig. 1E). The *O. sativaC_0t-1* DNA signals banding were not distinct on the *O. officinalis* chromosomes (Fig. 1E), but the *O. officinalis C_0t-1* DNA signals banding were distinct, and the strong hybridization signals were distributed on the *O. officinalis* chromosomes (Figs. 1G and H). By contrast, total genomic DNAs were used as probes for GISH on the chromosomes of *O. sativa*, *O. glaberrima*, and *O. officinalis*, respectively, which indicated that strong red signals covered the whole chromosomes (Figs. 1 B, D, and F).

Chromosome karyotype analysis using *C_0t-1* DNA signals banding

The relative lengths of chromosomes *O. sativa*, *O. officinalis*, and *O. meyeriana* were measured by SPOT advanced software, and the karyotypes were reconstructed using FISHView EXPO 2.0 software (Fig. 1J, K and L). Figures 2A–D are karyotype idiograms of *O. sativa*, *O. glaberrima*, and *O. officinalis*, where the upper part of each is a combined idiogram, and the lower part shows the red hybridization signals of *C_0t-1* DNA. Karyotypic analysis of *O. sativa* was performed according to standard karyotype of International Rice Institute, and the conventional method was also adopted in *O. glaberrima* and *O. officinalis*. According to FISH images, the signals of *C_0t-1* DNA have specific band patterns on each chromosome, and the homologous chromosomes show the same banding patterns. Therefore, the ideograms of the species were constructed according to the relative length, arm ratio, and position of *C_0t-1* DNA signals of the chromosomes (Figs. 2A–D). The relative lengths of chromsomes were measured by SPOT advanced software. These data were summarized in Table 1 and graphically reported in

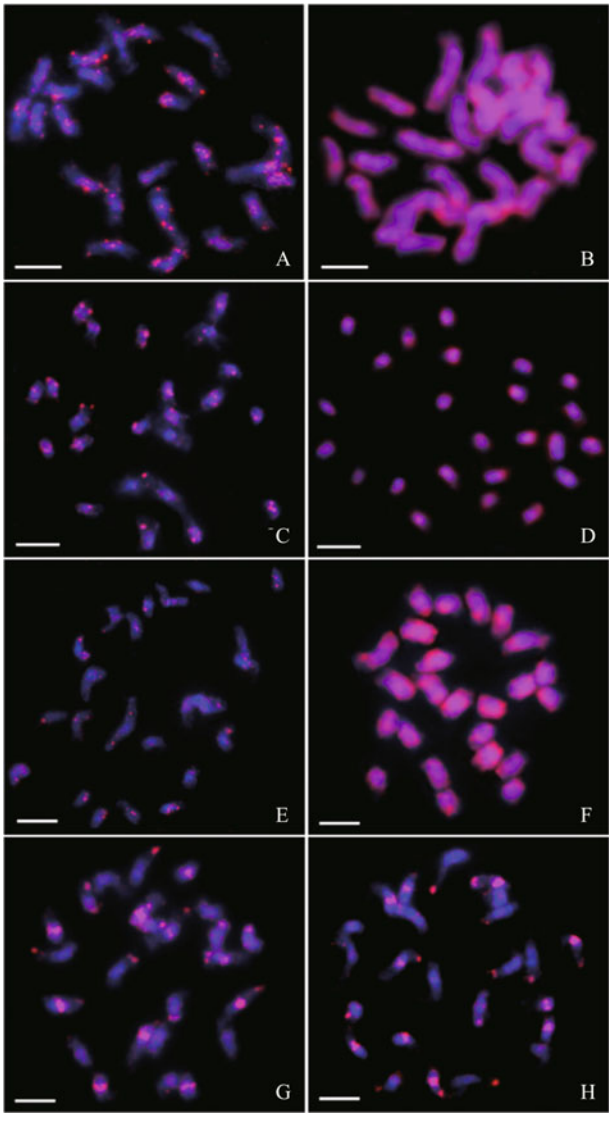

Figure 1 FISH images of prometaphase or metaphase chromosomes probed with C0t–1 DNA and their own total genomic DNA. Chromosomes stained with DAPI. Bars, 10 μm. FISH images A, C, and E show that the chromosomes are probed with *O. sativa* C_0t-*1* DNA of *O. sativa*, *O. glaberrima*, and *O. officinalis*, respectively. FISH images B, D, and F show that the chromosomes are probed with their own genomic DNA of *O. sativa*, *O. glaberrima*, and *O. officinalis*, respectively. G and H show that the chromosomes are probed with its own C_0t-*1* DNA of *O. officinalis*.

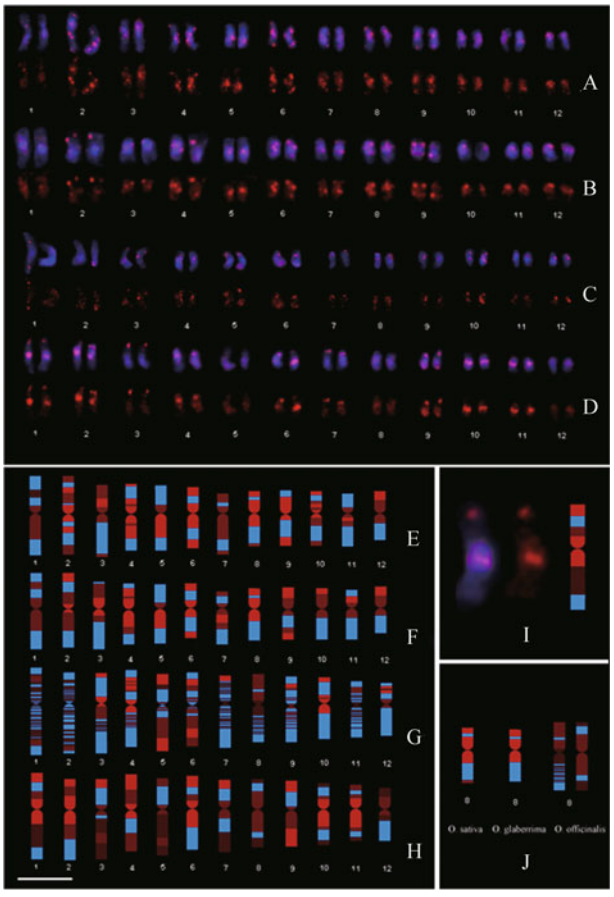

Figure 2 Karyotype reconstruction base on C_0t-*1* DNA banding and idiogram of chromosomal locations of C_0t-*1* DNA in *O. sativa*, *O. glaberrima*, and *O. officinalis*. Bars, 10μm. Karyotype idiograms A–C show that the chromosomes are probed with *O. sativa* C_0t-*1* DNA of *O. sativa*, *O. glaberrima*, and *O. officinalis*, respectively. D shows that the chromosomes are probed with its own C_0t-*1* DNA of *O. officinalis*. E, F, and G depict the distribution of the *O. sativa* C_0t-*1* DNA of *O. sativa*, *O. glaberrima*, and *O. officinalis*, respectively. H depicts the distribution of its own C_0t-*1* DNA of *O. officinalis*. I is C_0t-*1* DNA banding corresponding to the rearranged chromosome. J is the distribution of *O. sativa* C_0t-*1* DNA of chromosome 8 of *O. sativa*, *O. glaberrima*, and *O. officinalis* and its own C_0t-*1* DNA of chromosome 8 of *O. officinalis*, respectively.

Figs. 2E–H. Chromosomes were conventionally ordered in the idiograms according to Figs. 2A–D. The idiograms show that some chromosomes have the same C_0t-*1* DNA banding, i.e., chromosomes 1, 4, 5, 6, 8, and 12, of the two cultivated species, namely, *O. sativa* and *O. glaberrima* (Figs. 2E and F), whereas there are much more differentiations of distribution of *O. sativa*C_0t-*1* DNA on chromosomes between *O. officinalis* and cultivated rice (Figs. 2E, F and G). Besides, the distribution of *O. officinalis* C_0t-*1* DNA and *O. sativa* C_0t-*1* DNA on the *O. officinalis* differs from each other (Figs. 2G and H).

Comparative karyotype analysis by RFLP– FISH

The RFLP marker R2676 (1.8 kb) was found in chromosome 8 of cultivated rice, as well as in chromosomes of *O. officinalis* based on the comparative RFLP map (Tan et al., 2005). Figures 3A, B, E, and F show the results of FISH experiments using R2676 marker. Figures 3A and B show that the R2676 marker signals were detected in metaphase and interphase chromosomes of *O. glaberrima*, respectively.

Table 1 Karyotypic comparison of *O. sativa*, *O. glaberrima*, and *O. officinalis* based on the analysis of 10 metaphases

No.	Relative arm ratio pattern length±*SD*								
	O. sativa			*O. glaberrima*			*O. officinalis*		
1	12.94±0.41	1.25±0.12	m	12.26±0.19	1.21±0.32	m	12.42±0.40	1.35±0.22	m
2	12.14±0.19	1.23±0.45	m	11.15±0.22	1.12±0.48	m	10.64±0.14	1.50±0.36	m
3	10.02±0.32	1.85±0.42	sm	9.48±0.41	1.90±0.33	sm	10.66±0.33	2.25±0.47	sm
4	8.82±0.50	1.12±0.27	m	9.25±0.19	1.49±0.11	m	8.86±0.17	1.13±0.24	m
5	8.80±0.48	1.35±0.17	m	8.59±0.65	1.37±0.37	m	8.80±0.45	2.04±0.37	sm
6	7.89±0.21	1.13±0.44	m	8.37±0.44	1.20±0.30	m	7.64±0.21	1.34±0.45	m
7	7.61±0.37	2.61±0.45	sm	7.98±0.10	2.53±0.49	sm	7.43±0.50	1.21±0.49	m
8	6.92±0.61	1.71±0.34	sm	7.36±0.52	1.77±0.28	sm	7.38±0.61	1.31±0.49	m
9	6.73±0.18	1.36±0.22	m	6.91±0.24	1.40±0.46	m	7.34±0.27	1.26±0.25	m
10	6.44±0.47	1.42±0.39	m	6.53±0.39	1.39±0.17	m	6.23±0.24	1.27±0.46	m
11	6.26±0.47	1.83±0.18	sm	6.30±0.43	2.21±0.39	sm	6.12±0.34	1.23±0.39	m
12	6.04±0.24	1.18±0.33	m	5.97±0.11	1.24±0.25	m	6.03±0.38	2.25±0.21	sm

Note: Chromosomes are arranged according to their relative length, arm ratio, and position of C_0t-*1* DNA signals. *SD* is standard deviation, m is metacentric (arm length ratio = 1–1.5), and sm is submetacentric (arm length ratio > 1.5).

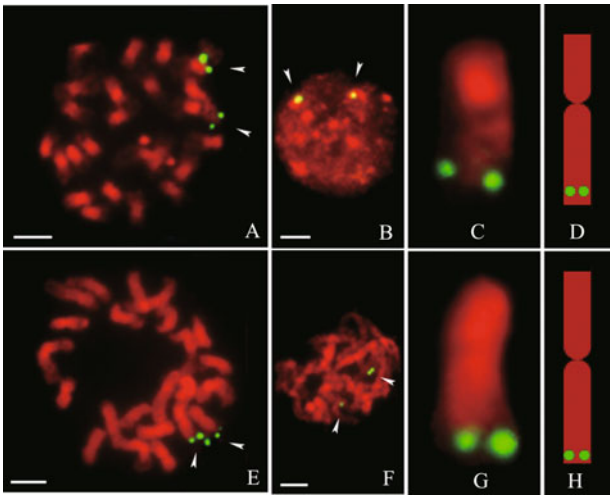

Figure 3 FISH images of physical location of an RFLP marker (R2676, 1.8 kb) on chromosomes of *O. glaberrima* and *O. officinalis*. Chromosomes are stained with PI. Bars, 10 μm. A and B show that the R2676 marker signals are detected in metaphase and interphase chromosomes of *O. glaberrima*, respectively. C and G are the enlarged idiograms of the signal-tagged chromosomes for R2676 marker of *O. glaberrima* and *O. officinalis*, respectively. D and H show that the signal locations are mapped on the chromosome 8 maps of *O. glaberrima* and *O. officinalis* by green dots. E and F show that the metaphase and prophase chromosomes are probed with R2676 marker of *O. officinalis*, respectively.

The metaphase and prophase chromosomes of *O. officinalis* were also probed with R2676 marker, respectively (Figs. 3E and F). Figures 3C and G show enlarged idiograms of the signal-tagged chromosomes. Clear double fluorescent signals were detected at an interstitial region of chromosome and at the end of chromosome of *O. glaberrima* and *O. officinalis*. It was clear that the two signals were localized at the region on the long arm of chromosome and using five metaphase spreads possessing a similar degree of condensation, and we measured and processed chromosomal lengths through chromosome analyzing system software (Table 2). The data of karyotypic analysis showed that the R2676 marker was located on chromosome 4 of *O. glaberrima* and chromosome 7 of *O. officinalis*, respectively. The distance of R2676 signals from the centromere of chromosome 4 of *O. glaberrima* and chromosome 7 of *O. officinalis* were 4.71±1.19 and 3.71±2.51; in addition, the marker was physically mapped at the 86.20% and 91.56% position from the centromere, respectively. The RFLP marker was physically mapped on the chromosome idiograms, as shown in Figs. 3D and H.

Discussion

The repetitive DNA sequences, as an important characteristic of eukaryotic genomes, disperse in the whole genome. They are usually more than 50% in genomes of higher plants (Flavell et al., 1974; Bennetzen et al., 2005), such as rice, maize, wheat, rye, and lemon. The content percentages of repetitive sequences are 50%, 78%, 83%, 92%, and 95%, respectively (Bennetzen et al., 2005). C_0t-*1* DNA contains mainly highly and moderately repetitive DNA sequences. In

Table 2 Karyotype comparison of chromosome 8 of *O. glaberrima* and *O. officinalis* based on 5 metaphase spreads possessing a similar degree of condensation

Species	Chromosome No.	Relative length±*SD*	Arm ratio	Section lengths	Pattern
O. glaberrima	4	9.21±0.18	1.46±2.33	4.71±1.19 (86.20±3.17%)	sm
O. officinalis	7	7.45±0.22	1.19±4.29	3.71±2.51 (91.56±5.62%)	m

Note: Chromosomes are identified by RFLP marker hybridization. SD is standard deviation, m is metacentric, and sm is submetacentric a distance of the signal from the centromere of chromosome.

this study, the fluorescence *in situ* hybridization was applied to the somatic chromosome preparations of *O. sativa*, *O. glaberrima* and *O. officinalis* with C_0t-*1* DNA as a probe. The results showed that the specific signal bands of C_0t-*1* DNA developed on the chromosomes of *O. sativa*, *O. glaberrima*, and *O. officinalis* (Figs. 1A, C, E, G, and H). The homologous chromosomes exhibited similar signal bands; therefore, karyotypic analysis was conducted based on C_0t-*1* DNA specific band possible in the three species. This karyotypic analysis based on C_0t-*1* DNA signal bands differed from the conventional karyotypic analysis. The chromosomes of *Oryza* were small and similar in configuration and inconspicuous centromere, so it was difficult to identify homologs and distinguish different chromosomes to generate a karyotype by morphological means. Figures 2D–F and H show the idiograms of chromosomal locations of C_0t-*1* DNA in *O. sativa*, *O. glaberrima*, and *O. officinalis*, and the results visually described the location of the repetitive sequences on chromosomes. There were more different distributions of repetitive sequences of each chromosome in a species, even in the two cultivated species of *O. sativa* and *O. glaberrima*, and there were very few chromosomes with similar C_0t-*1* DNA bandings, i.e., chromosomes 1, 4, 5, 6, 8, and 12. However, the similarity and correspondence between C_0t-*1* DNA signal patterns and genomic DNA signal patterns indicated that the self-GISH signals actually resulted from the hybridization of genomic repetitive sequences to the chromosomes. On the other hand, nonuniform distribution between C_0t-*1* DNA signal on their own chromosome of *O. officinalis* and *O. sativa* C_0t-*1* DNA on the chromosomes of *O. officinalis* was observed. There were much fewer *O. sativa* C_0t-*1* DNAs on the chromosomes of *O. officinalis* (Figs. 1E, G, and H). The genome size of *O. sativa* (450 Mb) differed from *O. officinalis* (697 Mb) (Uozu et al., 1997). In the evolutionary process, one of the important reasons for genome aggrandizement was the result of reduplication of highly and moderately repetitive DNA sequences (Flavell, 1986; Uozu et al., 1997; Kumar and Bennetzen, 1999). We speculated that repetitive sequences reduplication of the structure of AA genome (cultivated rice) was distinguished from CC genome (*O. officinalis*); in addition, the transposition and recombination of genome were also made differently between two species with the same genome, such as *O. sativa* and *O. glaberrima* (AA).

Figures 1B, D, and E show the GISH idiograms of *O. sativa*, *O. glaberrima*, and *O. officinalis* hybridized with total genomic DNA probes in their own mitotic metaphase. The hybridization stringency and the washing stringency were 65%–75% and 80%–90%, respectively. The posthybridization stringency is a very important factor to distinguish homology and similarity in the sequences in GISH. Li et al. (2001) reported that under low stringency (50%–60%), identity could not be distinguished in similarity genomes, and under 78%–86% stringency, the identity could be distinguished mostly (Li et al., 2001). The AA genome and CC genome existed in highly similar sequences; the results showed that, under the washing stringency at 80%–90%, the hybridization signals of C_0t-*1* DNA in target sequences were highly specific. By contrast, the GISH could enhance the reliability of C_0t-*1* DNA *in situ* hybridization, which indicates that the strong hybridization signals (red) covered the entire length of chromosomes of genome probes (Figs. 1B, D, and F) and also indicates clearly the locations of C_0t-*1* DNA on genome. Figures 1A, C, E, G, and H show that variable hybridization signals painted some specific regions of chromosomes.

RFLP marker-based genetic linkage maps have been constructed in numerous plant species. Much less effort has been devoted to physical mapping during the last decade although it can be applied to different species (Cheng et al., 2001c, 2001d). There are several methods for physical mapping used in plant species, for example, physical maps generated through a BAC contig assembly with large genome. However, it will be technically difficult to correctly achieve specific contigs in polyploid species or extensive sequence duplications. Secondly, the method to generate a physical map is to locate genetically mapped DNA markers to specific chromosomal segments using cytogenetic stocks (Werner et al., 1992; Riera-Lizarazu et al., 2000), but it is a daunting task to isolate a large number of cytogenetic stocks. Further cytogenetic stocks cannot be applied in all species. Another method for generation of a physical map is to directly visualize DNA sequences on chromosomes by fluorescence *in situ* hybridization (FISH) (Song and Gustafson, 1995; Jackson et al., 2000; Ohmido et al., 2000; Cheng et al., 2001c, 2001d; Zhao et al., 2002).

Comparative genetic maps of the cultivated rice (AA) with *O. officinalis* (CC) were constructed and used to name the two chromosomes with the strongest colinearity of the two species with the same numbers despite the configuration trait (Tan et al., 2005). We used the RFLP marker R2676 as the probe to hybridize it on the chromosomes of *O. officinalis* and named the chromosome with the detected signal number 8. The configuration trait of the chromosome 8 of *O. officinalis* is shown in Table 2. This chromosome 8 of *O. officinalis* was exactly the one we named number 7 by using tradition karyotype analysis (Fig. 2J and Fig. 3H). Our results revealed that the relative length of *O. officinalis* chromosome 8 does not follow conventional chromosome length in descending order of number. Similarly, chromosome 4 of *O. officinalis* named by using molecular marker RG214 was not the one named by using tradition karyotype analysis before (Qin et al., 2001). Figure 2J shows that C_0t-*1* DNA of A genome signals were detected in the end of the short arm of *O. officinalis* chromosome 8, indicating a relatively high homology in the repetitive DNA sequences in this region between A genome of cultivated rice and A genome of *O. officinalis*. However, the fluorescence intensity on the chromosomes of C_0t-*1* DNA of A genome was less than that of its own C genome from *O. officinalis*. As *O. officinalis*

genomes (697 Mb) were much larger than *O. glaberrima* genomes (420 Mb) and O. sativa genomes (449 Mb) (Uozu et al., 1997), which may be the result of reduplication of the highly and moderately repetitive DNA sequences in *O. officinalis* or the elimination of those sequences in *O. glaberrima* and *O. sativa* or species–specific sequences forming in the three species during long period of evolution, we concluded that the highly and moderately repetitive DNA sequences reduplication was one of the important causes due to the genome enlargement in *O. officinalis*. No homology signal of C_0t-*1* DNA from *O. sativa* was detected in the end of the long arm of *O. glaberrima* (Fig. 2J). *O. sativa* is grown worldwide but mostly in South and South-east Asia. Another cultivated A genome rice *O. glaberrima* is grown in western Africa. This suggested that the repetitive DNA sequences of A genome in two cultivated rice were lost for differentiations of growth environment in the evolutional history. Furthermore, our study was based upon comparative genomics and could enhance the efficiency of studying evolution of chromosomes, genomes, and repetitive sequences. It could be beneficial for making comparative analysis of single or repetitive sequences and consequently to accelerate the establishment of the large genetic system for *Oryza* plants, even for the genera of grass family.

Acknowledgements

This work was supported by the National Program of High Technology Development (No. 2004AA227120), the Scientific Research Foundation for the Returned Overseas Chinese Scholars, Ministry of Education of the People's Republic of China (No. BZY04003), China Postdoctoral Science Foundation (Grant No. 20040350574), and the Project of Science and Technology for Youth, Wuhan, China (No. 2004500607135).

References

Bennetzen J L, Ma J, Devos K M (2005). Mechanisms of recent genome size variation in flowering plants. Ann Bot, 95(1): 127–132

Causse M A, Fulton T M, Cho Y G, Ahn S N, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald P C, Harrington S E, Second G, McCouch S R, Tanksley S D (1994). Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics, 138(4): 1251–1274

Cheng Z K, Dong F G, Langdon T, Ouyang S, Buell C R, Gu M H, Blattner F R, Jiang J M (2002). Functional rice centromeres are marked by a satellite repeat and a centromere-specific retrotransposon. Plant Cell, 14(8): 1691–1704

Cheng Z K, Buell C R, Wing R A, Gu M H, Jiang J M (2001a). Toward a cytological characterization of the rice genome. Genome Res, 11(12): 2133–2141

Cheng Z K, Presting G G, Buell C R, Wing R A, Jiang J M (2001b). High-resolution pachytene chromosome mapping of bacterial artificial chromosomes anchored by genetic markers reveals the centromere location and the distribution of genetic recombination along chromosome 10 of rice. Genetics, 157(4): 1749–1757

Cheng Z K, Stupar R M, Gu M H, Jiang J M (2001c). A tandemly repeated DNA sequence is associated with both knob-like heterochromatin and a highly decondensed structure in the meiotic pachytene chromosomes of rice. Chromosoma, 110(1): 24–31

Cheng Z K, Yan H H, Yu H X, Tang S C, Jiang J M, Gu M H, Zhu L H (2001d). Development and applications of a complete set of rice telotrisomics. Genetics, 157(1): 361–368

Doyle J J (1990). Isolation of plant DNA from fresh tissue. Focus, 12: 13–15

Flavell R B (1986). Repetitive DNA and chromosome evolution in plants. Philos Trans R Soc Lond B Biol Sci, 312(1154): 227–242

Flavell R B, Bennett M D, Smith J B, Smith D B (1974). Genome size and the proportion of repeated nucleotide sequence DNA in plants. Biochem Genet, 12(4): 257–269

Hall A E, Keith K C, Hall S E, Copenhaver G P, Preuss D (2004). The rapidly evolving field of plant centromeres. Curr Opin Plant Biol, 7 (2): 108–114

Harrison G E, Heslop-Harrison J S (1995). Centromeric repetitive DNA sequences in the genus *Brassica*. Theor Appl Genet, 90(2): 157–165

Harushima Y, Yano M, Shomura A, Sato M, Shimano T (1998). A high–density rice gentic linkage map with 2275 markers using a single F2 population. Genetics, 144: 1883–1891

Jackson S A, Cheng Z K, Wang M L, Goodman H M, Jiang J M (2000). Comparative fluorescence *in situ* hybridization mapping of a 431-kb *Arabidopsis thaliana* bacterial artificial chromosome contig reveals the role of chromosomal duplications in the expansion of the Brassica rapa genome. Genetics, 156(2): 833–838

Jiang J M, Nasuda S, Dong F G, Scherrer C W, Woo S S, Wing R A, Gill B S, Ward D C (1996). A conserved repetitive DNA element located in the centromeres of cereal chromosomes. Proc Natl Acad Sci U S A, 93(24): 14210–14213

Jiang N, Feschotte C, Zhang X Y, Wessler S R (2004). Using rice to understand the origin and amplification of miniature inverted repeat transposable elements (MITEs). Curr Opin Plant Biol, 7(2): 115–119

Jin W W, Lamb J C, Vega J M, Dawe R K, Birchler J A, Jiang J M (2005). Molecular and functional dissection of the maize B chromosome centromere. Plant Cell, 17(5): 1412–1423

Jin W W, Melo J R, Nagaki K, Talbert P B, Henikoff S, Dawe R K, Jiang J M (2004). Maize centromeres: organization and functional adaptation in the genetic background of oat. Plant Cell, 16(3): 571–581

Kumar A, Bennetzen J L (1999). Plant retrotransposons. Anne Rev Genet, 33(1): 479–532

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio B A, Shomura A, Shimizu T, Lin S Y, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara Y, Hayasaka K, Miyao A, Monna L, Zhong H S, Tamura Y, Wang Z X, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994). A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet, 8(4): 365–372

Lan W Z, He G C, Wang C Y, Wu S J, Qin R (2007). Comparative analysis of genomes in *Oryza sativa, O. officinalis*, and *O. meyeriana* with C_0t-*1* DNA and genomic DNA of cultivated rice. Agric Sci China, 6(9): 1027–1034

Lan W Z, Qin R, Li G, He G C (2006). Comparative analysis of A, B, C and D genomes in the genus *Oryza* with C_0t-*1* DNA of C genome.

Chinese Sci Bull, 51(14): 1710–1720

Li C B, Zhang D M, Ge S, Lu B R, Hong D Y (2001). Differentiation and inter–genomic relationships among C, E and D genomes in the *Oryza officinalis* complex (Poaceae) as revealed by multicolor genomic in situ hybridization. Theor Appl Genet, 103(2–3): 197–203

Lu B R (1999). Taxonomy of the genus *Oryza* (Poaceae): historical perspective and current status. Int Rice Res Newslett, 243: 4–8

Matyásek R, Gazdová B, Fajkus J, Bezdek M (1997). NTRS, a new family of highly repetitive DNAs specific for the T1 chromosome of tobacco. Chromosoma, 106(6): 369–379

Mishima M, Ohmido N, Fukui K, Yahara T (2002). Trends in site-number change of rDNA loci during polyploid evolution in *Sanguisorba* (Rosaceae). Chromosoma, 110(8): 550–558

Ohmido N, Akiyama Y, Fukui K (1998). Physical mapping of unique nucleotide sequences on identified rice chromosomes. Plant Mol Biol, 38(6): 1043–1052

Ohmido N, Kijima K, Akiyama Y, de Jong J H, Fukui K (2000). Quantification of total genomic DNA and selected repetitive sequences reveals concurrent changes in different DNA families in indica and japonica rice. Mol Gen Genet, 263(3): 388–394

Qin R, Wei W H, Jin W W, He G C, Song Y C (2001). Physical location of rice Gm–6, Pi–5(t) genes in *O. officinallis* with BAC– FISH. Chinese Sci Bull, 46(8): 2427–2430

Ren N, Song Y C, Bi X Z, Ding Y, Liu L H, (1997). The physical location of genes *cdc2* and *prh1* in maize (*Zea mays* L.). Hereditas, 126(3): 211–217

Riera-Lizarazu O, Vales M I, Ananiev E V, Rines H W, Phillips R L (2000). Production and characterization of maize chromosome 9 radiation hybrids derived from an oat-maize addition line. Genetics, 156(1): 327–339

Shishido R, Ohmido N, Fukui K (2001). Chromosome painting as a tool for rice genetics and breeding. Methods Cell Sci, 23(1–3): 125–133

Song Y C, Gustafson J P (1995). The physical location of fourteen RFLP markers in rice (*Oryza sativa* L.). Theor Appl Genet, 90(1): 113–119

Tan G X, Jin H J, Li G, He R F, Zhu L L, He G C (2005). Production and characterization of a complete set of individual chromosome additions from *Oryza officinalis* to *Oryza sativa* using RFLP and GISH analyses. Theor Appl Genet, 111(8): 1585–1595

Uozu S, Ikehashi H, Ohmido N, Ohtsubo H, Ohtsubo E, Fukui K (1997). Repetitive sequences: cause for variation in genome size and chromosome morphology in the genus *Oryza*. Plant Mol Biol, 35 (6): 791–799

Vaughan D A (1994). The Wild Relatives of Rice. A Genetic Resources Hand book. Manila: IRRI

Vaughan D A, Morishima H, Kadowaki K (2003). Diversity in the *Oryza* genus. Curr Opin Plant Biol, 6(2): 139–146

Wang Z X, Kurata N, Saji S, Katayose Y, Minobe Y (1995). A chromosome 5-specific repetitive DNA sequence in rice (*Oryza sativa* L.). Theor Appl Genet, 90(7–8): 907–913

Werner J E, Endo T R, Gill B S (1992). Toward a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci U S A, 89 (23): 11307–11311

Yan H H, Jin W W, Nagaki K, Tian S L, Ouyang S, Buell C R, Talbert P B, Henikoff S, Jiang J M (2005). Transcription and histone modifications in the recombination-free region spanning a rice centromere. Plant Cell, 17(12): 3227–3238

Yan H H, Liu G Q, Cheng Z K, Li X B, Liu G Z, Min S K, Zhu L H (2002). A genome-specific repetitive DNA sequence from *Oryza eichingeri*: characterization, localization, and introgression to *O. sativa*. Theor Appl Genet, 104(2–3): 177–183

Zhao Q, Zhang Y, Cheng Z K, Chen M S, Wang S Y, Feng Q, Huang Y C, Li Y, Tang Y S, Zhou B, Chen Z, Yu S, Zhu J, Hu X, Mu J, Ying K, Hao P, Zhang L, Lu Y, Zhang L S, Liu Y, Yu Z, Fan D, Weng Q, Chen L, Lu T, Liu X, Jia P, Sun T, Wu Y, Zhang Y, Lu Y, Li C, Wang R, Lei H, Li T, Hu H, Wu M, Zhang R, Guan J, Zhu J, Fu G, Gu M, Hong G, Xue Y, Wing R, Jiang J, Han B (2002). A fine physical map of the rice chromosome 4. Genome Res, 12(5): 817–823

Zwick M S, Hanson R E, Islam-Faridi M N, Stelly D M, Wing R A, Price H J, McKnight T D (1997). A rapid procedure for the isolation of C_0t-*1* DNA from plants. Genome, 40(1): 138–142

Zwick M S, Islam-Faridi M N, Zhang H B, Hodnett G L, Gomez M I, Kim J S, Price H J, Stelly D M (2000). Distribution and sequence analysis of the centromere-associated repetitive element CEN38 of sorghum bicolor (Poaceae). Am J Bot, 87(12): 1757–1764