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FISH Analysis of *Oryza latifolia* and *Oryza alta* Genomes with 45S rDNA Probes

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Abstract: Using Fluorescence in Situ Hybridization with 45S rDNA probes, we analyzed two wild rice species, Oryza alta and Oryza latifolia, both having CCDD genomes. The results showed that the hybridization signals of 45S rDNA in O.latifolia chromosome preparations were distributed on different chromosomes with the number of 10~16. The same signals in O.alta chromosome preparations were 6 and distributed on three pairs of homologous chromosomes: with two pairs in short chromosome arms and the other one pair in long chromosome arms. The number and positions of 45S rDNA signals are stable in O.alta genome, but there are some dynamic changes of signals in O.latifolia genome. Our observations indicated that there are distinct differences between the two CCDD genomes. Comparative analysis of the karyotype of O.alta and O.latifolia chromosomes based on the Fluorescence in Situ Hybridization pictures showed vast differences. We propose that the genome of O.alta may be differentiated earlier in evolution and tend to be stable, while O.latifolia may still be in its evolutionary process. Based on the imbalance of evolution and differences of genomic structure, we propose that O.alta and O.latifolia can be divided into two wild rice species, which may be better conforming with their evolution characteristics. The mechanism and characteristics of 45s rDNA's distribution in the chromosomes are discussed.

Keywords: 45S rDNA, Fluorescence in Situ Hybridization (FISH), *Oryza alta*, *Oryza latifolia*

1. Introduction

The genus Oryza is divided into sativa, officinalis, ridleyi, and meyeriana complex. In the officinalis complex, both the Oryza alta and Oryza latifolia are allopolyploids with CCDD genomes, and have excellent characteristics such as anti-insect, anti-disease, and anti-stress. But the classification status of these two species has always been controversial. Some researchers believed that the two kinds of wild rice are very similar in terms of geographical distribution and morphological characteristics, have no significant reproductive isolation, and their crossbreeding progenies' chromosomes display normal synapsis and pairing during meiosis. As a result, they inclined to classify O.alta and O.latifolia as one species [1]. On the other hands, other related studies in the field of molecular biology (studies using isoenzyme, AFLP markers, RFLP markers, repetitive sequences and ribosome ITS sequences, etc.) and cell biology (studies using cot-1 DNA hybridization, genomic hybridization, etc.) had revealed enormous differences between these two wild rice species, and demonstrated their divergence in the phylogenetic trees of the genus Oryza [2-4]. In order to better protect and utilize these wild rice genetic resources, more studies are needed to clarify the genetic relationship between them.

The 45S rDNA belongs to highly and moderately repetitive DNA sequences, and can be used to study the differentiation of species and the origin of genomes. As a result, it has been used to determine the original ancestry and establish the evolutionary tree between different species [5]. These are made possible by the fact that the 45S rDNA repetitive sequences contain less genes or genes with multi copies, and

their mutations are likely not to cause lethal effects. Therefore, the selection pressure in the process of evolution is relatively small, the variations in these regions can accumulate gradually and randomly, and can be used to differentiate between species within the genus. Mutations that usually occur in conserved sequences such as functional gene regions are more likely to cause lethal effects and less likely to be inherited or retained. As a result, compared with the single-copy and low-copy sequences that are more conservative in evolution, the medium and high repetitive sequences are more suitable for studying the relationship between the genomes of closely related species with small variation [6].

In studies of plant genetic resources, valuable information is often obtained using Fluorescent in Situ Hybridization (FISH) localization technology, which can be used to characterize about the structural changes of chromosomes, the phylogenetic relationship of proximal species and the origin of heteropolyploid [7-9]. The FISH analysis using 45S rDNA probes showed that the position of rDNA loci on chromosomes was usually stable among the related wheat species, in spite that of the primitive and derived polyploid species had undergone a long period of parallel evolution. Using this technique, it was found that the three basic diploids with A, B or C genomes in the brassica genus have 5, 3 and 2 pairs of the 45S rDNA loci, respectively [10]. When FISH technology was used to analyze 45S rDNA of different genomes of cultivated rice and wild rice, Shishido et al [11] found that there are some changes in the location and number of rDNA loci. For example, the numbers of rDNA sites of six diploid species in the rice genus fluctuate between 1 and 3 pairs, and there are also changes in the copy number of rDNA repeat units and rDNA loci. In addition, it was found that there is only 1 pair of 45S rDNA loci in O.Sativa ssp. Japonica, and 2 pairs of 45S rDNA loci in O.Sativa ssp. Indica [12].

In this study, we performed FISH and karyotype analysis on O.alta (CCDD) and O.latifolia (CCDD) using 45S rDNA probes, and compared the numbers and locations of 45S rDNA loci on the chromosomes of the two wild rice species. The role of 45S rDNA in the evolution of heteropolyploids, and the characteristics or mechanism of evolution of allotetraploid wild rice are discussed. By comparing and analyzing the differences in the number, size and location of 45S rDNA in the heterotetraploid genomes of O.alta (CCDD) and O.latifolia (CCDD), we provide relevant evidences for various classification status of the two genomes.

2. Materials and Methods

2.1. Materials

Wild rice accessions IRW41 (O.alta) and IRW6 (O.latifolia) were provided by Prof. Lu Yonggen of South China Agricultural University. The 45S rDNA plasmid was provided by Dr. Xiong Zhiyong of Huazhong University of Science and Technology. Wild rice materials were planted in porcelain vats by conventional methods. Well-grown plants were used to cut root tips for chromosome preparation.

2.2. Preparation of Chromosome Specimens

The chromosome preparations of wild rice materials were carried out by using a slightly modified method of Wang e t al [13]. The root tips were washed and cut for 1~2 mm, treated with 0.075 mmol/L KCl solution for 30 min. The root tip tissues were fixed overnight with methanol/glacial acetic acid (3:1) solution, and then washed 3~5 times for 5 min each. The cleaned root tip tissues were digested with an enzyme mixture solution of 2% pectinase and 2% cellulase for 3~4 hours in a 28°C incubator. After washing, chromosome slides were prepared by flame drying method. The slides were examined by the microscope and good slides were sealed and stored in -20°C.

2.3. Fluorescence in Situ Hybridization

In the pre-treatment process, the chromosome slides were dried in a 60°C oven for 1h, digested with 10μg/ml RNase A solution at 37°C for 1h, and fixed with 4 % paraformaldehyde for 10 min at room temperature. In the denaturation process, the slides were washed twice with 2×SSC solution at room temperature for 5 min each, denatured in 70% deionized formamide solution at 70°C for 3.5~5 min, and quickly dehydrated in an ethanol series (70%, 95% and 100%) at -20°C for 5 min each, and air-dried at room temperature by placing in a super-clean bench. In the hybridization process, the freshly prepared hybridization solution (contain with 100ng biotin labeled 45S rDNA probe, 50% deionized formamide, 2μg salmon sperm DNA, 10% 20×SSC, 0.1%SDS) was denatured in a 90°C oven for 10 min, then quickly placed on ice for >20 minutes. After adding 50μL hybridization solution on the chromosome film, the slides were covered with coverslip and put into a moisturizing box. The box was heated in a 80°C oven for 10 min, and then incubated in a 37°C incubator for 24h.

2.4. Signal Detection

The slides were washed with 20% formamide solution, 2×SSC solution and 0.2×SSC solution at 42°C for 10 min each; and then with 1×PBS solution at room temperature for 10 min before air-dried. Each slide was added 50μL 0.008 mg/mL fresh streptavidin-Cy3 solution, covered with coverslip, and incubated at 37°C for 1h. In dark conditions and at room temperature, the slides were washed with 1×PBS solution 3 times for 5 min each. Every air-dried slide was added with 50μL 0.008 mg/mL fresh Biotinylated Streptavidin solution, and incubated at 37°C for 1h. The slides were washed with 1×PBS solution for 3 times, added 50μL 0.008 mg/mL fresh streptavidin-Cy3 solution, and incubated at 37°C for 1h again. Then each slide was washed with 1×PBS solution for 3 times and counterstained with 50μL 10μg/mL DAPI solution. Finally, the fluorescence signals were observed under a microscope (OLYMPUS BX61), and the targeted pictures were obtained using the Cool-1300QSCCD (VDS, Germany) camera system controlled by Case Data Manager Expo2.1.1 image system, and the FISH View EXPO2.0 and Photoshop 8.0 were used to fine-tune images.

3. Result and Analysis

3.1. FISH Analysis of Metaphase Chromosomes

Using biotin labeled 45S rDNA as probes, FISH was performed on the metaphase chromosomes of the O.alta and O.latifolia (Figure 1). The 45S rDNA probes were detected as red signals by streptomycin-Cy3, and the chromosomes were shown to be blue by DAPI counterstain.

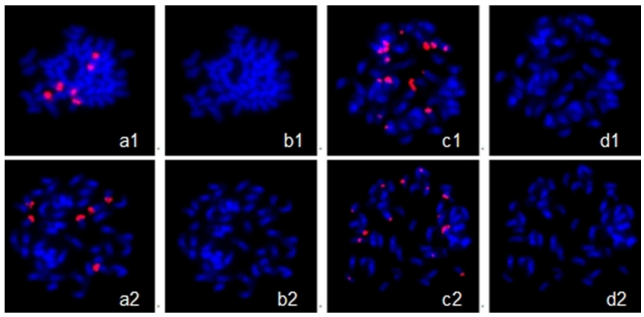


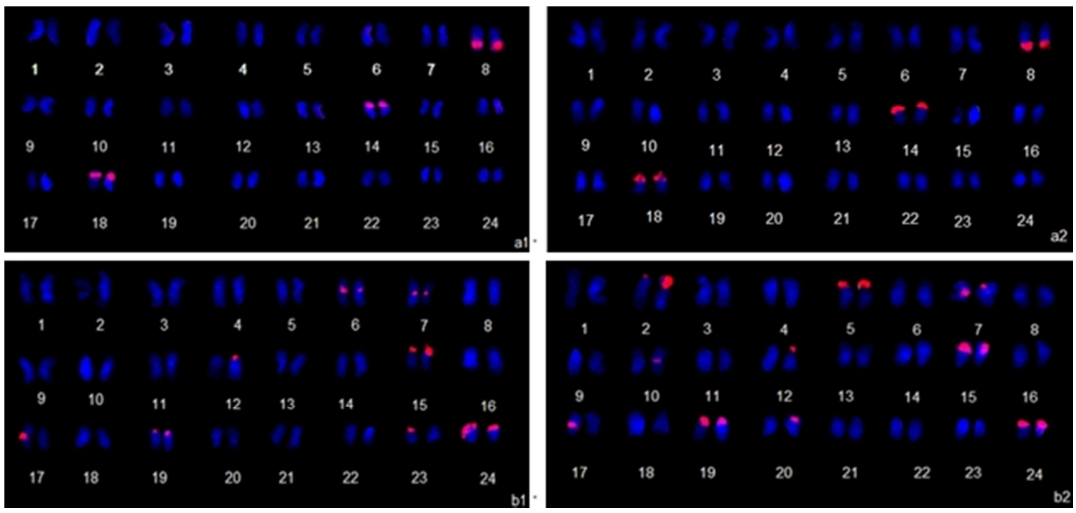
Figure 1. *FISH analysis with 45S rDNA on the chromosomes preparations of O.alta and O.latifolia.*

a1, a2 show the hybrid signals of 45S rDNA on the O.alta chromosomes; b1, b2 show the DAPI counterstained O.alta chromosomes; c1, c2 show the hybrid signals of 45S rDNA on the O.latifolia chromosomes; d1, d2 show the DAPI counterstained O.latifolia chromosomes.

There were 5 pieces of good slides of each plant were selected for FISH analysis, and in each slide at least 10 cells with clear metaphase chromosomes were used to count the chromosome and 45S rDNA signal numbers. The results showed that both the O.alta and O.latifolia chromosomes numbers were 48 (Figure 1). However, there were significant differences in the numbers of 45S rDNA signals between the two sets of chromosomes. All of the O.alta chromosome preparations (from different individual plants) had 6 hybridized signals (Figure 1-a1, a2), while the number of 45S rDNA signals were variable in the O.latifolia chromosome preparations from different individual plants. For example, there were 16 signals in Figure 1-c1 and 13 signals in Figure 1-c2. Through the analysis of 12 different plants of O.latifolia, we found that the minimum number of signal points was 10, and the maximum number was 16, among which 13 and 14 signal points appeared more times, that is, 4 plants had 13 signal points and 3 plants had 14 signal points. The statistical results showed that the number of 45S rDNA signal points on the O.latifolia chromosomes varied between 10 and 16.

3.2. Distribution Characteristics of 45S rDNA Signals

On the FISH images, the chromosomes were separated one by one using FISHView EXPO2.0 software, and the length of chromosomes were measured using curve measurement method of SPOT advanced software. Photoshop was used to fine-tune the images. Combined chromosome morphology with the statistical data, the chromosomes of O.alta and O.latifolia were paired and sorted from the longest to the shortest.



a1, a2 are karyogram of O. alta, b1, b2 are karyogram of O.latifolia.

Figure 2. *Karyogram of O.alta and O.latifolia with 45S rDNA signals.*

Figure 2 shows that the red 45S rDNA signals on the chromosomes of O.alta and O.latifolia are mainly distributed in the centromere, proximal centromere and telomere regions. By comparing the karyotype maps of the two wild rice species, we found that the distribution patterns of 45S rDNA on chromosomes are different, and each has a specific band type. The O.alta steadily had 6 signal points and they were mainly distributed near telomere. The 6 signal points belonged to 3 pairs of homologous chromosomes, with 2 pairs of signals points in the short arms and 1 pair in the long arms. On the other hand, the O.latifolia had more signal points (10~16) and signal bands were found in the chromosomes' short arms, long arms, centromeres or their vicinity areas. The end of the short arm was the most likely distribution region, in which one can find about 9 signal bands. Secondly, in both the centromere and near centromere regions we can found 2 or 3 signal bands, but there was none 45S rDNA signal found in the long chromosome arms. Moreover, the 45S rDNA signals of

O.latifolia were not all pairwise, with some chromosome pairs had only one hybridization signal, or some chromosome pairs had inconsistencies in the hybridization signal strength, indicating that 45S rDNAs of O.latifolia on homologous chromosomes were not evenly distributed. The differences of 45S rDNA signals distribution between O.alta and O.latifolia confirmed that the distributions of the middle and high repetitive sequences of these two species were different.

3.3. Chromosomal Karyotype Pattern Analysis

On the basis of data obtained by Photoshop software and SPOT advanced software, we used the Excel software to analyze the relative length of chromosome arms and ratio of arms of each chromosome (Table 1). By comparing the relative chromosome length, the corresponding chromosomes (with the same chromosome number) of these two sets of genomes were different from each other. Generally, the relative length of the O.latifolia chromosomes were longer than that of O.alta, with the difference value range from 0.63 to 6.88. For example, the relative length of chromosome 24 of O.alta was 25.37, and that of O.latifolia was 26.00, with a minimum difference value of 0.63.The relative length of chromosome 15 of O. alta was 31.62, and that of O.latifolia was 38.5, with a maximum difference value of 6.88. Analysis of Ratio of Arms (AR) of the both genomes showed that the centromeres of the O.alta chromosomes almost all concentrated on the middle, and the chromosomes had less variation of AR (1.08~1.37); while the O.latifolia chromosomes had larger variation of AR (1.18~1.69), that is, their centromeres dispersed in larger regions.

Table 1. *Karyotype analysis of O.alta and O.latifolia.*

Chr. No.	Oryza alta				Oryza latifolia			
	LRL±SD	SRL±SD	TRL±SD	AR±SD	LRL±SD	SRL±SD	TRL±SD	AR±SD
1	29.00±3.42	23.37±2.77	52.37±5.71	1.24±0.12	30.50±4.60	24.37±4.47	54.87±7.68	1.25±0.24
2	25.75±1.58	22.62±2.13	48.37±2.56	1.13±0.15	31.62±8.31	21.25±6.02	52.87±7.22	1.48±1.02
3	25.50±2.33	19.87±3.27	45.37±2.92	1.28±0.39	27.62±4.00	20.25±4.13	47.87±5.06	1.36±0.38
4	24.25±1.49	19.62±2.97	43.87±3.72	1.23±0.19	29.25±4.77	17.25±4.13	46.50±6.05	1.69±0.54
5	23.62±2.77	19.50±3.25	43.12±3.70	1.21±0.34	25.25±4.27	20.25±2.92	45.50±6.00	1.24±0.23
6	22.37±1.60	19.00±2.62	41.37±3.62	1.17±0.14	25.75±3.45	19.62±4.41	45.37±6.44	1.31±0.29
7	22.87±3.36	16.75±2.71	39.62±4.31	1.36±0.35	25.50±4.14	19.37±3.85	44.87±3.76	1.31±0.53
8	22.75±2.66	16.75±3.65	39.50±2.10	1.35±0.56	25.12±1.25	18.87±4.29	44.00±4.69	1.33±0.47
9	20.25±2.66	16.62±1.69	36.87±2.83	1.21±0.28	24.75±3.77	17.87±3.36	42.62±3.93	1.38±0.48
10	19.62±1.92	15.37±2.62	35.00±2.03	1.27±0.37	23.37±4.37	18.62±2.97	42.00±3.85	1.25±0.51
11	19.75±1.28	15.00±1.77	34.75±1.75	1.31±0.21	22.87±1.73	17.50±1.93	40.37±2.26	1.30±0.21
12	19.62±1.60	14.25±1.28	33.87±2.03	1.37±0.16	21.75±2.66	17.50±2.20	39.25±3.77	1.24±0.20
13	17.75±1.39	15.37±1.85	33.12±2.70	1.15±0.14	21.50±2.27	17.50±2.98	39.00±4.84	1.22±0.16
14	18.37±3.02	14.37±0.74	32.75±3.45	1.27±0.18	22.25±2.82	16.50±2.07	38.75±3.62	1.34±0.27
15	17.00±1.77	14.62±1.91	31.62±2.66	1.16±0.26	21.75±3011	16.75±3.01	38.50±4.47	1.29±0.31
16	17.62±1.77	13.87±1.60	31.50±2.97	1.27±0.12	20.87±2.53	17.62±1.77	38.50±3.96	1.18±0.11
17	18.00±2.33	13.75±2.17	31.75±2.56	1.30±0.36	20.62±2.88	16.75±2.25	37.37±4.37	1.23±0.18
18	15.62±1.77	14.37±2.42	30.00±3.11	1.08±0.33	21.50±3.89	14.75±1.75	36.25±3.73	1.45±0.35
19	16.50±1.85	13.87±1.51	30.37±3.25	1.18±0.06	20.00±2.56	15.62±2.07	35.62±3.54	1.28±0.23
20	17.00±2.39	12.75±2.12	29.75±2.12	1.33±0.39	20.50±4.34	14.37±4.44	34.87±3.76	1.42±0.97
21	16.50±2.20	12.75±2.49	29.25±3.41	1.29±0.33	18.75±2.38	15.25±1.75	34.00±3.38	1.22±0.17
22	15.87±2.53	12.37±2.50	28.25±3.45	1.28±0.38	17.62±2.50	14.87±1.36	32.50±3.59	1.18±0.12
23	15.00±1.51	11.25±1.98	26.25±2.76	1.33±0.25	17.62±2.39	13.00±1.60	30.62±2.92	1.35±0.24
24	13.75±0.71	11.62±2.07	25.37±2.45	1.18±0.25	14.62±2.20	11.37±2.07	26.00±3.30	1.28±0.30

Chr. No., chromosomes numbers; LRL, Long arm relative length; SD, standard deviation; SRL, short arm relative length; TRL, total arm relative length; AR, ratio of arms (long arm/short arm).

Using the Excel software, we constructed the karyogram mode of both O.alta and O.latifolia. The paired chromosomes were sequenced from the longest to the shortest according to the length and the shape. Of the equal-length chromosomes, those with short arms were arranged before. The 45S rDNA signal distributions in the chromosomes were based on the FISH analysis pictures.

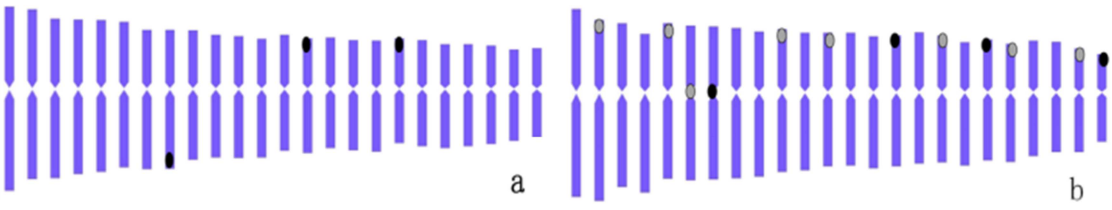


Figure 3. *Karyogram mode of O.alta and O.latifolia with 45S rDNA signals.*

a, is 45S rDNA on the karyogram mode of O.alta; b, is 45S rDNA on the karyogram mode of O.latifolia; the chromosomes from left to right are numbered chromosome 1 to 24; black ovals represent stable 45S rDNA signal points, gray ovals represent unstable 45S rDNA signal points.

Figure 3-a shows that the 3 pairs of 45S rDNA are located in the long arm of chromosome 8, the short arm of chromosome 14 and the short arm of chromosome 18 of O.alta, and these chromosome's relative lengths are 39.50, 32.75 and 30, respectively (Table 1). The distributions of 45S rDNAs in the O.latifolia chromosomes are more diversified. As can be seen from Figure 3-b, there are 4 pairs of 45S rDNA (black ovals) of O.latifolia stablely distributing on four chromosome pairs. They are located in the centromere of chromosome 7, and in the short arms of chromosome 15, 19 and 24, respectively. Some unstable 45s rDNA signals (gray ovals) appear in pairs (i.e. on homologous chromosomes), such as signals in centromere region of the 6th chromosome pair (shown in Figure 2-b1, 3b), signals in telomeres region of the 2nd and 5th chromosome pairs (shown in Figure 2-b2, 3b). There are also some unstable signals (gray ovals, Figure 3b) appear individually, such as only one signal in each the chromosome pairs of the 12th, 15th, 23rd chromosomes in Figure 2-b1, and only one signal in each the chromosome pairs of the 10th, 12th, 17th, 20th chromosome in Figure 2-b2. The results show that the signal locations of different plants are different, indicating vast variability in the repetitive sequences.

4. Discussion

Both O. alta and O.latifolia are heterotetraploid with CCDD genomes, belong to the officinalis complex of Oryza genus, and originally distributed in Latin America. Some believed that these two wild rice species should be classified to one species due to their overlapping geographical locations, extremely similar morphology, and the hybrids' normal reproductive capacity [1]. However, many related researches indicated that O. alta and O.latifolia were significantly different in terms of evolution [2-4]. Bao et al [14] studied the chloroplast DNA of these two wild rice and revealed difference from each other. In addition, Lan et al [15] performed FISH analysis on O. alta and O.latifolia using cot-1 DNA of O. officinalis (CC genome) as probes, and verified differences between their CCDD genomes, especially more differences among chromosomes from DD genomes. Using the cot-1 DNA of O. alta (CCDD genome) as the probes, Wang Debin et al [16] conducted FISH analysis on chromosomes of O. alta and O.latifolia, and also found obvious differences in hybridization signals' distribution. In the current study, we analyzed the differences in the number and distribution of 45S rDNA probe signals in the chromosomes of the two wild rice species, and further demonstrated the genetic differences and evolutionary relationship between the two species.

45S rDNA are moderately repetitive sequences in the genome and have high homology in different species, but the numbers and loci of 45S rDNA are variable. The results of different published research using FISH technology to study the rDNA sites of different species indicated that most diploid species had two or more 45S rDNA sites. For example, there were differences in the number of 45S rDNA loci in different subspecies of cultivated rice, 2 loci in indica and 4 in japonica [12]. Furthermore, there was a significant variation in the number of 45S rDNA loci in different wild rice species, with a variety range of 4~14 sites [11]. Shishido et al [17] reported that the rDNA number of tetraploid species is not a simple doubling of the rDNA number of diploid species. On the other hand, Wu et al [18] demonstrated that distribution of repeated sequences in the allopolyploid genomes are unstable. In addition, Dong et al [19] detected about 10-14 of 45S rDNA loci in chromosomes of O.latifolia and analyzed their distribution polymorphism. In this study, we analyzed 45S rDNA loci in two tetraploids. We demonstrated that not only the number but also the sites of 45S rDNA were stable in O. alta, i.e., 6 of 45S rDNA loci were always detected on 3 certain chromosome pairs of O. alta. The 45S rDNA number of O.latifolia were as many as 10~16, and their sites were more complex than expected. There were only 8 of 45S rDNA loci were steadily detected on 4 certain chromosome pairs; some other chromosome pairs may be detected 0, 1 or 2 loci and their distributions would varied among different individual plant. The analysis of locations of 45S rDNA showed that about 9 loci located in the ends of short arm of chromosomes, 2~3 in the centromere regions, and 2~3 in the near centromere regions. Although both O. alta and O.latifolia had CCDD genome types, they were significantly different in the 45S rDNA loci, indicating their differences in the internal composition and structure of genomes.

Previous studies have shown that the number of 45S rDNA sites in polyploid species generally tends to decrease during evolution [20]. In this study, we found that O.latifolia has a larger average chromosome length, and more 45S rDNA loci but unstable; on the other hands, the O. alta has smaller average chromosome length, and fewer 45S rDNA loci but stable. Therefore, we propose that the formation of O. alta is earlier than that of O. latifolia. The 45S rDNA loci of O.latifolia may be in the process of abatement, and may gradually become stable in the long future. Although O. alta and O.latifolia have similar morphological and chromosomal behaviors, due to their large differences in genomic structure and their imbalance in evolution, we believe that the division of them into different wild rice species is more consistent with their evolutionary characteristics.

It can be seen from figure 3-a and 3-b that, although the numbers of 45S rDNA loci were different between O. alta and O.latifolia, their locations on chromosomes were mostly the same, that is, most of them are located at the proximal end of chromosomes short arms. Wende et al [21] and Cronn et al [22] considered that the location of 45S rDNA on chromosomes had a close influence on the evolution rate of 45S rDNA, because the unequal exchange in the middle of a chromosome is more likely to result in genetic imbalance or the generation of inanimate gametes than that either in the end or in the proximal end of the chromosomes. Therefore, the 45S rDNA locating at the middle of the chromosome has higher variability than that locating at the proximal end. The 45S rDNA signal tends to be distributed at the end of the short arm

of the chromosome, which may be related to its specific function and number changes [23, 24]. Most of the 45S rDNA loci of O.latifolia are located at the end or proximal end of the chromosomes and the signal points are not distributed equally in chromosome pairs. Some chromosome pairs have only one hybridization signal, and some chromosome pairs have inconsistencies in the hybridization signal strength, possibly due to the asymmetric exchange during meiosis. These results further suggest that O.latifolia is still in the process of evolution, far from reaching a stable state.

Chromosome rearrangement, unequal exchange (deletion, insertion), and amplification of concealed rDNA copy caused by transposons all lead to changes in chromosome structure. Altinkut et al [25] found that En/Spm transposons existed in 45srDNA of rice. The transposons caused change may be one of the reasons for the changes of 45S rDNA number and loci position. The nucleolus organizer regions (NOR) of most species were found locating on the short arms of chromosomes, forming the secondary constriction of chromosomes. As a result, we speculate that the 45S rDNAs are involved in the composition of nucleolus organizer regions. Fransz et al [26] found that both euchromatin and heterochromatin have specific spatial structure in the interphase nucleus, and this specific structure can make the regulation of gene expressions more smoothly. Whether 45S rDNA is restricted by a specific spatial structure and tends to be distributed at the ends of chromosomes need to be further studied. The reasons of unsymmetry of 45S rDNA evolution and the heterozygosity of repeat sequence in O. alta and O.latifolia remained to be further investigated.

5. Conclusion

Both O.alta and O.latifolia are heteroploid plants with CCDD genomes. Because of the similarities, some researchers considered them to be just one wild rice species. However, in this study we found that there were many differences between these two species. We conducted FISH analysis with 45S rDNA as probes on both chromosome preparations of O.alta and O.latifolia, and carried out karyotype analysis of the two kinds of CCDD genomes. The results showed that O.alta had relatively shorter chromosomes, the number and distribution sites of its 45S rDNA loci tend to be stable. But O.latifolia had relatively longer chromosomes and more 45S rDNA loci, some distribution sites of its 45S rDNA loci were fixed and the other were alterable. From our new results, we concluded that the evolution of O.alta tend to be stable, while the O.latifolia has not reached the stable state and is still in the changing period of evolution. Based on these evolutionarily unbalanced characteristics, we suggest that it is more appropriate to define the two types of wild rice as different species, and it is beneficial to preserve and protect the resources of the two kinds of wild rice.

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References

[1] Bao Y, Ge S. Historical retrospect and the perplexity on the studies of the Oryza polyploids [J]. Acta Phytotaxonomica Sinica, 2008, 46(1): 3-12.

[2] Bao Y, Ge S. Rapid communication-identification of Oryza species with the CD genome based on RFLP analysis of nuclear ribosomal ITS sequences [J]. Acta bot sin, 2003, 4 5(7): 762-765.

[3] Yamaki S, Ohyanagi H, Yamasaki M, et al. Development of INDEL markers to discriminate all genome types rapidly in the genus Oryza [J]. Breeding science, 2013, 63(3): 246.

[4] Tang L, Zou X, Achoundong G, et al. Phylogeny and biogeography of the rice tribe (Oryzeae): evidence from combined analysis of 20 chloroplast fragments [J]. Molecular phylogenetics and evolution, 2010, 54(1): 266-277.

[5] Sousa, Aretuza & Cusimano, Natalie & Renner, Susanne. Combining FISH and model-based predictions to understand chromosome evolution in Typhonium (Araceae) [J]. Annals of botany. 2014, 113(4):741-752.

[6] Kang Lei, Du Xuezhu, Zhou Yingying, Zhu Bin, Ge Xianhong, Li Zaiyun. Development of a complete set of monosomic alien addition lines between Brassica napus and Isatis indigotica (Chinese woad) [J]. Plant cell reports. 2014, 33:1355–1364.

[7] Zhang D, Sang T. Chromosomal structural rearrangement of P.brownii and P.californica revealed by fluorescence in situ hybridization [J]. Genome, 1998, 41(6): 848-853.

[8] Kitamura S, Inoue M, Shikazono N, et al. Relationships among Nicotiana species revealed by the 5S rDNA spacer sequence and fluorescence in situ hybridization [J]. Theoretical and Applied Genetics, 2001, 103(5):678-686.

[9] Thomas H M, Harper J A, Morgan W G. Gross chromosome rearrangements are occurring in an accession of the grass Lolium rigidum [J]. Chromosome Research, 2001, 9(7): 585-590.

[10] Fukui K, Nakayama S, Ohmido N, et al. Quantitative karyotyping of three diploid Brassica species by imaging methods and localization of 45S rDNA loci on the identified chromosomes [J]. Theoretical and Applied Genetics, 1998, 96(3-4): 325-330.

[11] Shishido R, Sano Y, Fukui K. The third 45S rDNA locus in O. eichingeri (CC) newly detected by an improved FISH method [J]. Rice Genet Newslett, 1996, 13: 84-85.

[12] Shishido R, Sano Y, Fukui K. Ribosomal DNAs: an exception to the conservation of gene order in rice genomes [J]. Molecular and General Genetics MGG, 2000, 263(4): 586-591.

[13] Wang C, Wang K, Song G, Li M et al. Protocol of cotton FISH of somatic chromosomes with rDNA as probes [J]. J Cotton Sci. 2001, 13: 75-77.

[14] Bao Y, Ge S. Origin and phylogeny of Oryza species with the CD genome based on multiple-gene sequence data [J]. Plant Systematics and Evolution, 2004, 249(1-2): 55-66.

[15] Lan Weizhen, Qin Rui, Li Gang, He Guancun Comparative analysis of A, B, C and D genomes in the genus Oryza with C0t-1 DNA of C genome [J]. Chinese Science Bulletin. 2006, 51(14):1710-1720.

[16] Wang Debin, Wang Yang , Zhao Houming , LiI Gang , Qin Rui , Liu Hong. Comparative analysis of genomes of O. alta and O.latifolia by using C0t-1 DNA [J]. Chinese Journal of Rice Science, 2010, 24(2): 131-136.

[17] Shishido R, Sano Y, Fukui K. The third 45S rDNA locus in O. eichingeri (CC) newly detected by an improved FISH method [J]. Rice Genet Newslett, 1996, 13: 84-85.

[18] Wu Qi, Qin Rui, Li Gang, Liu Hong. Comparative Analysis on CCDD Genome of O. latifolia with CC C0t-1 DNA from O. officinalis [J]. Journal of Plant Genetic Resources, 2010, 11(5): 589-592.

[19] Dong Zhenwei. Comparative analysis of several wild rice genomes using repeated sequences [D]. South-Central University For Nationalities, 2008.

[20] Roa, F. & Guerra. Distribution of 45S rDNA sites in chromosomes of plants: Structural and evolutionary implications. BMC Evol Biol [J]. 2012, 12(1): 1-13.

[21] Gan Yimei, Liu Fang, Chen Dan, Wu Qiong, Qin Qin, Wang Chunying, Li Shaohui, Zhang Xiangdi, Wang Yuhong, Wang Kunbo. Chromosomal Locations of 5S and 45S rDNA in Gossypium Genus and Its Phylogenetic Implications Revealed by FISH [J]. PloS one [J]. 2013, 8(6). e68207:1-12.

[22] Morales A. G., Aguiar-Perecin M. L. R., Mondin M. Karyotype characterization reveals an up and down of 45S and 5S rDNA sites in Crotalaria (Leguminosae-Papilionoideae) species of the section Hedriocarpae subsection Macrostachyae [J]. Genet Resour Crop Evol, 2012, 59(2): 277–288.

[23] Mantovani M, Abel S, Moreira-Filho O. Conserved 5S and variable 45S rDNA chromosomal localisation revealed by FISH in Astyanax scabripinnis (Pisces, Characidae) [J]. Genetica, 2005, 123(3): 211-216.

[24] Lim KB, Wennekes J, de Jong J H, et al. Karyotype analysis of Lilium longiflorum and Lilium rubellum by chromosome banding and fluorescence in situ hybridisation [J]. Genome, 2001, 44(5): 911-918.

[25] Altinkut, Raskina O, Nevo E, et al. En/Spm-like transposons in Poaceae species: transposase sequence variability and chromosomal distribution [J]. Cellular & molecular biology letters, 2006, 11(2): 214-229.

[26] Fransz P, De Jong J H, Lysak M, et al. Interphase chromosomes in Arabidopsis are organized as well defined chromocenters from which euchromatin loops emanate [J]. Proceedings of the National Academy of Sciences, 2002, 99(22): 14584-14589.